



# Temporal drop of genetic diversity in *Bombus pauloensis*

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**Abstract** – Bumblebees are economically important insects which perform essential pollination tasks in natural and managed ecosystems. Recent research studying Neotropical bumblebee species in Brazil showed a clear decrease in genetic diversity over time in *Bombus pauloensis*. A new temporal assessment of genetic diversity is needed to know whether this was a location-specific result, or a more general phenomenon. This knowledge is essential to be able to prioritize conservation and management needs. Here, the genetic variability of *B. pauloensis* populations in Argentina was investigated over time using museum collection specimens from 1933 to 2016, and compared with reanalyzed data from Brazilian populations. Furthermore, specific time series were made for two Argentinean locations, Candelaria and La Plata, and compared with the time series of Porto Alegre (Brazil). All collected specimens were genotyped with 16 microsatellite loci to estimate genetic diversity parameters. Our results showed no drop in either allelic richness or expected heterozygosity over all Argentinean populations. However, a clear drop in genetic diversity was observed in two out of three location-specific time series. This loss of diversity will have negative impacts on population survival, especially over longer periods of time. Furthermore, the use and release of mass-reared specimens of *B. pauloensis*, which may be inbred and specifically selected for certain commercial but non-adaptive traits, could further diminish the genetic pool. Thus, our result implies the urgent need for regional conservation policies of *B. pauloensis* in South Brazil and North Argentina.

bee decline / genetic diversity / microsatellites / *Bombus pauloensis* / South America

## 1. INTRODUCTION

Bumblebees are economically important insects worldwide in natural ecosystems, and also in managed ecosystems. Around 26 bumblebee species are native to South America. Both Argentinean and Brazilian faunas harbor eight native species (Abrahamovich et al. 2001; 2007; Cameron et al.

2007; Santos Júnior et al. 2015; Françoso et al. 2016). In both countries, *Bombus (Thoracobombus) pauloensis*, formerly named *Bombus atratus* Franklin, 1913 (Moure and Melo 2012), generally is the most abundant and widely distributed species (Abrahamovich et al. 2007; Martins and Melo 2010). As with *Bombus terrestris* in Europe, *Bombus impatiens* in Northern America, and *Bombus ignitus* in China, *B. pauloensis* is commercially reared for the South American market to perform pollination services essential to improving plant production of diverse crops under cover (Cruz et al. 2007, 2008).

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In the Neotropical region, bumblebees are showing major population declines due to the invasion of non-native bumblebee species with hitchhiking parasites, and the ongoing and continuous destruction of suitable habitat (Freitas et al. 2009; Martins et al. 2013; Arbetman et al. 2013; Schmid-Hempel et al. 2014). Recent research shows temporal losses of genetic diversity in *Bombus morio* and *B. pauloensis* populations from Southern Brazil (Maebe et al. 2018). These results can have strong conservation implications as populations with lower genetic diversity levels are more susceptible to diseases (Cameron et al. 2007; Whitehorn et al. 2011; 2014), and have a more limited potential to adapt to, and thus survive, future environmental changes (Frankham 2005; Zayed 2009; Habel et al. 2014). However, whether the result of genetic pauperization was a location-specific result or a more general phenomenon is not yet known. A temporal assessment of genetic diversity is needed to gain knowledge which would enable us to prioritize conservation and management needs.

Here, the temporal stability of genetic diversity was investigated in the widespread South American bumblebee species *B. pauloensis*. Museum collection materials were selected from five locations, and 16 microsatellite (MS) loci were used to genotype those sampled specimens. South Brazilian *B. pauloensis* specimens described in Maebe et al. (2018), together with one additional population from São Francisco de Paula, were reanalyzed with the same 16 microsatellites to allow comparison of genetic diversity. As genetic diversity parameters, both allelic richness and expected heterozygosity were estimated. For two Argentinean locations (Candelaria and La Plata), time series were made from 1946 to 2012, and compared with the time series from Porto Alegre in Brazil (Maebe et al. 2018). Our results should bring more insight into the occurrence of temporal changes in genetic diversity, and gain knowledge for future conservation and management measures.

## 2. MATERIAL AND METHODS

### 2.1. Specimen collection

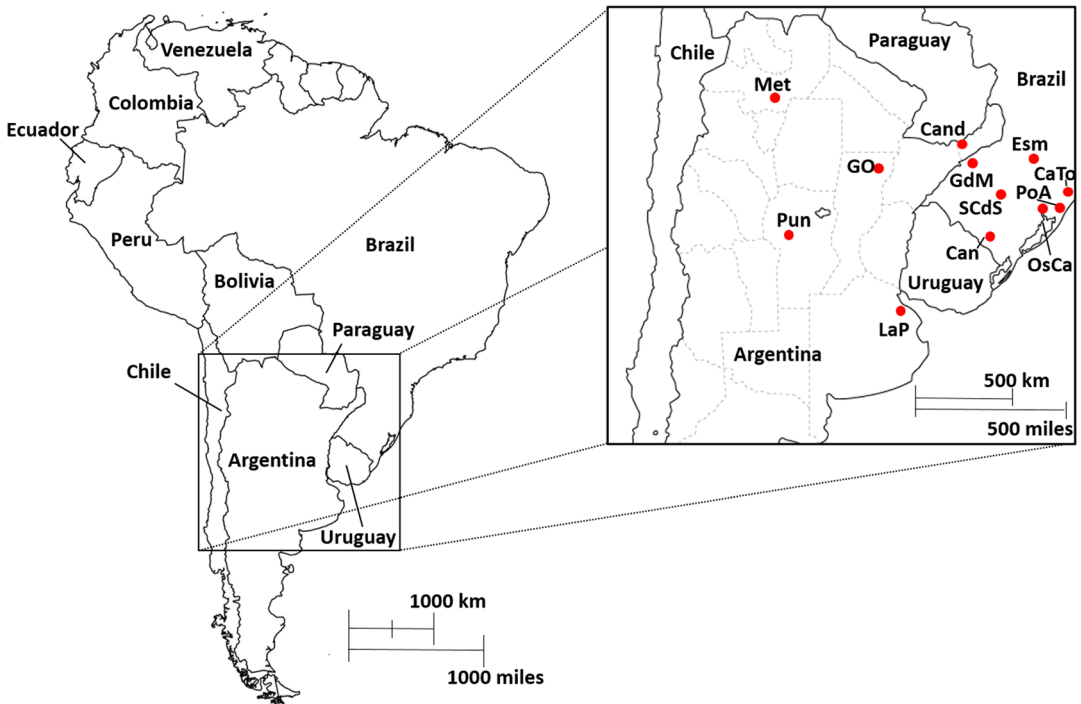
Historical *B. pauloensis* specimens were originally collected from five Argentinean locations

and were retrieved from the Museo de La Plata (MLP), Argentina, and the Centro de Estudios Parasitológicos y de Vectores (CEPAVE) (Figure 1). The data from these locations will be compared with the reanalyzed data obtained from a recent study (Maebe et al. 2018) in which *B. pauloensis* specimens from several locations in Brazil were genotyped with the same 16 MS (Figure 1), together with 25 extra specimens collected from an additional location (São Francisco de Paula, 1996–1997) from the Museum of Science and Technology at Pontificia Universidade Católica do Rio Grande de Sul (PUCRS). The data of Brazilian *B. pauloensis* had to be reanalyzed as the data described in Maebe et al. (2018) were based on only 14 MS loci. In the latter study, removal of 2 MS loci (BT08 and 0294) was necessary to be able to compare the obtained genetic diversity parameters for *B. pauloensis* with those obtained from another bumblebee species, *B. morio*, for which these two loci could not be reliably amplified.

Furthermore, time series were made for two Argentinean locations: two time periods for La Plata (LaP), 1985–1987 and 2016; and three time periods for Candelaria (Cand), 1946–1959, 1985–1986, and 2007–2012. These time series were compared with the time series of Porto Alegre (PA, Brazil), including four time periods: 1946–1959, 1991–1994, 1999–2004, and 2007–2012 (see Maebe et al. 2018).

### 2.2. DNA extraction and genotyping protocol

Individual DNA extractions were performed on one middle leg following a Chelex protocol as described in Maebe et al. (2013). Afterwards, each specimen was genotyped with 16 microsatellite loci which showed reliable signals in previous research with bumblebee collection material (Maebe et al. 2015, 2016, 2018; Table I). MS loci were amplified by multiplex PCR in 10 µl using the Type-it QIAGEN PCR kit. Per sample, four multiplex reactions were performed each containing 1.33-µl template DNA, Type-it Multiplex PCR Master Mix (2×, Qiagen), and four forward and reverse primers (Maebe et al. 2016, 2018;



**Figure 1.** Overview of the sampling locations of *B. pauloensis* in Argentina and South Brazil. Argentinean sampling locations were La Plata (LaP), Candelaria (Cand), Metan (Met), General Obligado (GO), and Punilla (Pun). Brazilian sampling locations were Esmeralda (Esm), Candiota (Can), Guarani das Missões (GdM), São Francisco de Paula (SFdP), Santa Cruz do Sul (SCdS), Cambará do Sul and Torres (CaTo), Osório and Capão da Canoa (OsCa), and Porto Alegre (POA).

Table 1). PCR protocol, capillary electrophoresis, and fragment scoring were done as described in Maebe et al. (2013).

### 2.3. Data validation and genetic diversity

Before data analysis, several genotyped individuals which could not be scored in a reliable manner for at least 12 loci, and all detected sisters using Colony 2.0 (Wang 2004) with 5% genotyping error correction per locus, and Kinalyzer (Ashley et al. 2009) using the 2-allele algorithm and consensus method, were excluded. Furthermore, genotypic linkage disequilibrium, deviations from Hardy-Weinberg (HW) equilibrium, and presence of null alleles were tested using Fstat 2.9.3 (Goudet 2001), GenAlEx 6.5 (Peakall and Smouse 2006), and Microchecker (Van Oosterhout et al. 2004), respectively.

Estimations of Nei's unbiased expected heterozygosity ( $H_E$ , Nei 1978) and the sample size-corrected private allelic richness ( $A_R$ ) normalized to 10 diploid specimens were performed with GenAlEx 6.5 (Peakall and Smouse 2006) and Hp-Rare 1.1 (Kalinowski 2002), respectively. Linear mixed models (LMMs) were performed for both genetic diversity parameters ( $A_R$  and  $H_E$ ) in RStudio (R Development Core Team 2008) with R package lme4 version 1.1-10 (Bates et al. 2015). Models started with location and time period as fixed factors and microsatellite loci as random factor to account for inter-locus variability (Soro et al. 2017). The best-fitting model was selected based on Akaike's information criterion (AIC) obtained by using the dredge command within the MUMIn package (Barton 2015; Maebe et al. 2016). Selected LMMs were run, main effects determined, and marginal and conditional coefficients of determination

**Table I.** List of microsatellite primers and multiplexes used for characterization of Brazilian and Argentinean *B. pauloensis*

Locus	Dye	Forward primer	Reverse primer
Multiplex 1 ( $T_a = 52^\circ\text{C}$ )			
BL13	PET	CGAATGTTGGGATTTTCGTG	GCGAGTACGTGTACGTGTTCTATG
BT02	NED	AGGAACCGAGCGATAGAACCAC	GCTTTGCCCTTTCCATCTTGCTG
BT23	FAM	GCAACAGAAAATCGTCGGTAGTG	GCGGCAATAAAGCAATCGG
BT24	VIC	TCTTTCGGTTTTCCCCCTG	CACCCACTTACATACATACACGCTC
Multiplex 2 ( $T_a = 52^\circ\text{C}$ )			
BL02	NED	GAACAGTGAGAGCGAGGAACAGAG	TTGCCACGTATATCCGAGCGAACC
BT04	FAM	GAGAGAGATCGAATGGTGAGAGC	TGAGCACGTTCTTTTCGTTTAC
BT08	PET	AGAACCTCCGTATCCCTTCG	AGCCTACCCAGTGCTGAAAC
BT10	VIC	TCTTGCTATCCACCACCCGC	GGACAGAAGCATAGACGCACCG
Multiplex 3 ( $T_a = 49^\circ\text{C}$ )			
B100	FAM	CGTCCTCGTATCGGGCTAAC	CGTGGAAACGTCGTGACG
B11	NED	GCAACGAAAACGAAATCG	GTTCATCCAAGTTTCATCCG
B126	PET	GCTTGCTGGTGAATTGTGC	CGATTCTCTCGTGTACTCC
B1232	VIC	GAAATTCGTGCGGAGGG	CAGAGAACTACCTAGTGCTACGC
Multiplex 4 ( $T_a = 52^\circ\text{C}$ )			
0294	FAM	AGTACGATAAAGCCAGGAAAAG	TGTATGCCTATTGTACGAGTGT
0304	NED	GTATGAGTGAGTGATGTGCAAG	CCCTTCATCTCTGAACAATATC
0810	PET	TTAACAAATCCGAATTTAAAGG	GATAGTGGTTGCTTGTCATCTT
BT05	VIC	TTTCCTATGCCGAACGTCACC	CCCAGATAAAAAGACCGCTCTAGTC

computed (Nakagawa and Schielzeth 2013; Soro et al. 2017). Temporal stability of genetic diversity was investigated by Tukey HSD post hoc comparisons using the R package multcomp (Hothorn et al. 2008) as described in Soro et al. (2017).

**Data availability** The genotype of each specimen, based on our set of 16 microsatellite loci, will be archived at DRYAD: doi:<https://doi.org/10.5061/dryad.3sc3c6p>.

### 3. RESULTS

#### 3.1. Data analysis

Of the 291 specimens in total, 129 were collected from the Argentinean museum collection and 162 from Brazilian museum collections (see also Maebe et al. 2018), which were analyzed and reanalyzed with 16 microsatellite loci,

respectively. In total, 107 specimens were removed from further analysis (Table II), which consisted of 83 full-sibs detected with Colony 2.0 and Kalyzer, and 24 specimens with too much missing data (for 5 loci or more). For the remaining 74 Argentinean and 110 Brazilian specimens (Table II), all 16 loci could be scored reliably and no significant linkage disequilibrium was found. Heterozygote deficits and excesses were detected in Hardy–Weinberg equilibrium (HWE) tests. These deviations may be due to the presence of low frequencies of null alleles (< 5% for all loci), the low sample sizes, or the fact that populations consisted of samples collected over several years and thus over multiple generations.

#### 3.2. Genetic diversity estimations

The allelic richness ( $A_R$ ) ranged from 2.113 ( $SE \pm 0.252$ ) to 3.175 ( $SE \pm 0.511$ ), with a mean  $A_R$  of 2.685 ( $SE \pm 0.117$ ; Table III) for the

**Table II.** Number of *B. pauloensis* specimens genotyped in the analysis categorized per country. With  $N$  the total number of specimens genotyped, *Missing data* number of not amplifiable specimens, *Sisters* number of removed full sibs, and  $N_{final}$  actual number of workers used in all further analyses. Argentinean sampling locations were *LaP* La Plata, *Cand* Candelaria, *Met* Metan, *GO* General Obligado, *Pun* Punilla. Brazilian sampling locations were *Esm* Esmeralda, *Can* Candiota, *GdM* Guarani das Missões, *SFdP* São Francisco de Paula, *SCdS* Santa Cruz do Sul, *CaTo* Cambará do Sul and Torres, *OsCa* Osório and Capão da Canoa, *POA* Porto Alegre

Country	Code	Time period	$N$	Sisters	Missing data	$N_{final}$
Argentina	Met	1933	9	3	0	6
	Cand	1946–1959	10	4	0	6
	GO	1946–1959	39	24	3	12
	Pun	1985–1987	11	5	0	6
	LaP	1985–1987	30	9	0	21
	Cand	1985–1987	10	3	0	7
	Cand	2007	10	2	0	8
	LaP	2016	10	2	0	8
	Subtotal		129	52	3	74
Brazil	PoA	1946–1959	13	0	8	5
	PoA	1991–1994	8	1	1	6
	CaTo	1991–1994	6	0	1	5
	OsCa	1991–1994	9	0	4	5
	SFdP	1996–1997	25	7	3	15
	Can	1999–2004	19	13	0	6
	PoA	1999–2004	6	0	0	6
	SCdS	1999–2004	8	0	2	6
	Esm	2010–2012	22	4	1	17
	GdM	2010–2012	33	4	1	28
	PoA	2010–2012	13	2	0	11
	Subtotal		162	31	21	110
	Total		291	83	24	184

Argentinean populations. Per population, the expected heterozygosity ( $H_E$ ) ranged from 0.300 (SE  $\pm 0.071$ ) to 0.492 (SE  $\pm 0.083$ ; Table III). Mean genetic diversity parameters in the Brazilian populations using 16 MS were higher than those described within Maebe et al. (2018) based on “only” 14 MS ( $A_R = 3.213$  and  $H_E = 0.474$  versus  $A_R = 2.313$  and  $H_E = 0.458$ , respectively). A comparison between Argentinean and Brazilian populations revealed significantly lower mean  $A_R$  estimations in Argentinean *B. pauloensis* populations (Student  $t$  tests,  $df = 17$ ,  $t = 2.944$ ,  $p = 0.009$ ) but not for mean  $H_E$  (Student  $t$  tests,  $t = 0.990$ ,  $p = 0.336$ ;  $A_R$  and  $H_E$ , respectively; Table III; Figure 2).

### 3.3. Temporal shifts in genetic diversity of *B. pauloensis* populations

The best-fitting LMMs for  $A_R$  and  $H_E$  based on the AIC score included “time period” as a fixed factor in the Brazilian *B. pauloensis* populations (delta  $> 2$ ; Table IV). The importance of time period within the model could also be seen by comparing the model with and without time period as a factor (LRT,  $A_R$ ,  $\chi^2 = 10.995$ ,  $df = 4$ ,  $p = 0.027$ ; and  $H_E$ ,  $\chi^2 = 28.527$ ,  $df = 4$ ,  $p < 0.001$ , respectively). As expected, genetic diversity dropped over time (see Maebe et al. 2018, Table II; Figure 2). However, another pattern was observed within the Argentinean

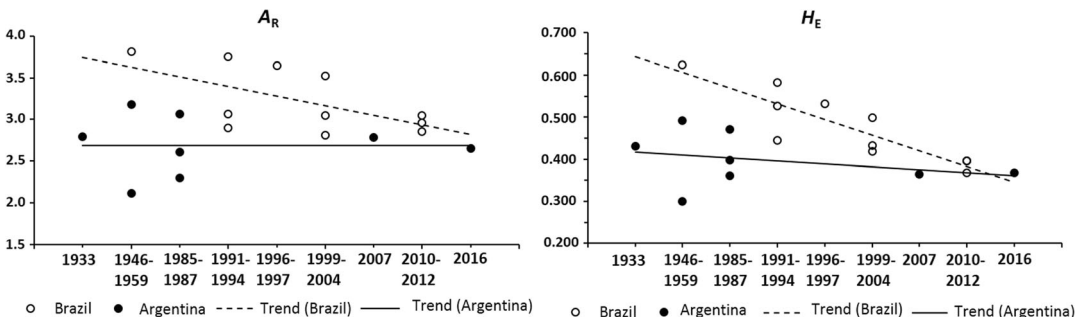
**Table III.** Genetic diversity estimated for each *B. pauloensis* population. The mean values (and SE) of the expected heterozygosity ( $H_E$ ), the observed heterozygosity ( $H_O$ ), and the allelic richness ( $A_R$ ) over all microsatellite loci are given for each population. With  $N$  the number of specimens per population

Species	Location	Period	$N$	$A_R^*$	SE	$H_O$	SE	$H_E$	SE	
Argentina	Met	1933	6	2.791	0.378	0.302	0.068	0.430	0.078	
	Cand	1946–1959	6	3.175	0.511	0.479	0.103	0.492	0.083	
	GO	1946–1959	12	2.113	0.252	0.246	0.066	0.300	0.061	
	Pun	1985–1987	6	2.299	0.277	0.465	0.098	0.397	0.071	
	LaP	1985–1987	21	2.609	0.429	0.154	0.039	0.361	0.087	
	Cand	1985–1987	7	3.059	0.456	0.363	0.067	0.471	0.078	
	Cand	2007	8	2.778	0.438	0.320	0.085	0.365	0.088	
	LaP	2016	8	2.653	0.492	0.375	0.098	0.368	0.091	
		Mean		9.25	2.685	0.117	0.338	0.036	0.398	0.021
Brazil	PoA	1946–1959	5	3.063	0.438	0.392	0.075	0.625	0.078	
	PoA	1991–1994	6	2.903	0.393	0.439	0.095	0.444	0.082	
	CaTo	1991–1994	5	3.063	0.359	0.274	0.039	0.526	0.072	
	OsCa	1991–1994	5	3.750	0.534	0.531	0.083	0.583	0.079	
	SFdP	1996–1997	15	3.647	0.430	0.429	0.066	0.532	0.072	
	Can	1999–2004	6	3.525	0.532	0.406	0.087	0.499	0.077	
	PoA	1999–2004	6	3.046	0.442	0.365	0.091	0.432	0.078	
	SCdS	1999–2004	6	2.811	0.401	0.219	0.058	0.418	0.083	
	Esm	2010–2012	17	2.956	0.469	0.411	0.102	0.396	0.089	
	GdM	2010–2012	28	3.047	0.522	0.359	0.087	0.395	0.091	
	PoA	2010–2012	11	2.846	0.556	0.369	0.098	0.368	0.095	
		Mean		10.00	3.213	0.107	0.381	0.024	0.474	0.024

\*Allelic richness calculated based on 10 diploid specimens

*B. pauloensis* populations (Figure 2). Indeed, for both  $A_R$  and  $H_E$ , best-fitting LMMs were with “location” as a fixed factor but without time period as a fixed factor (delta > 2; Table IV). That time period has no effect on

the best model can be seen in comparing the model with and without time period as a factor (LRT,  $A_R$ ,  $\chi^2 = 0.406$ ,  $df = 4$ ,  $p = 0.982$ ; and  $H_E$ ,  $\chi^2 = 1.367$ ,  $df = 4$ ,  $p = 0.850$ , respectively). So, overall, in Argentinean populations,



**Figure 2.** Comparison of temporal shifts in mean  $A_R$  and  $H_E$  between Argentinean and Brazilian *B. pauloensis* populations.

**Table IV.** Best-fitting model explaining the patterns in genetic diversity parameters  $A_R$  and  $H_E$ . Based on their high (negative or positive) Akaike’s information criterion (AIC), only the best models fitting linear mixed effect models (with a delta  $< 2 + 1$ ) are given using only the Brazilian (A) and Argentinean populations’ (B) and (C) the population-specific time series. With + parameters included in the model, NA not included parameters, and best models in italic.

		(Intercept)	Location	Period	Location:period	df	logLik	AIC	Delta	Weight
A	$A_R$									
	<i>M2</i>	3.813	NA	+	NA	7	-283.930	582.5	0.00	0.553
	M1	3.219	NA	NA	NA	3	-289.407	585.0	2.43	0.164
	$H_E$	(Intercept)	Location	Period	Location:period	df	logLik	AIC	Delta	Weight
	<i>M2</i>	0.625	NA	+	NA	7	35.111	-55.6	0.00	0.890
	M4	0.687	+	+	NA	13	39.116	-50.0	5.57	0.055
	M8	0.687	+	+	+	13	39.116	-50.0	5.57	0.055
B	$A_R$	(Intercept)	Location	Period	Location:period	df	logLik	AIC	Delta	Weight
	<i>M3</i>	2.789	+	NA	NA	7	-199.857	414.6	0.00	0.797
	M1	2.684	NA	NA	NA	3	-206.303	418.8	4.15	0.100
	$H_E$	(Intercept)	Location	Period	Location:period	df	logLik	AIC	Delta	Weight
	<i>M1</i>	0.398	NA	NA	NA	3	2.590	1.0	0.00	0.540
	<i>M3</i>	0.430	+	NA	NA	7	6.397	2.1	1.13	0.308
	M4	0.430	+	+	NA	10	8.378	5.1	4.11	0.069
	M8	0.430	+	+	+	10	8.378	5.1	4.11	0.069
C	$A_R$	(Intercept)	Location	Period	Location:period	df	logLik	AIC	Delta	Weight
	<i>M2</i>	3.494	NA	+	NA	6	-231.793	476.2	0.00	0.595
	M4	3.228	+	+	NA	8	-230.644	478.3	2.15	0.203
	M3	2.631	+	NA	NA	5	-234.538	479.5	3.31	0.114
	$H_E$	(Intercept)	Location	Period	Location:period	df	logLik	AIC	Delta	Weight
	<i>M2</i>	0.558	NA	+	NA	6	19.256	-25.9	0.00	0.637
	<i>M4</i>	0.511	+	+	NA	8	20.520	-24.0	1.92	0.244
	M8	0.625	+	+	NA	11	23.255	-22.5	3.38	0.118

genetic diversity remained fairly stable over time. However, location played a significant role, as Candelaria (Cand) had a significantly higher genetic diversity in comparison with General Obligado (GO) and La Plata (LaP) ( $A_R$  and  $H_E$ ; Tukey HSD post hoc test comparisons,  $p < 0.05$ ), and for  $A_R$  also with Punilla (Pun) (Tukey HSD post hoc test comparisons,  $p < 0.05$ ; Table V).

### 3.4. Temporal shifts in genetic diversity present in the three time series?

As location can play a significant role in patterns of genetic diversity (see Table IVB and Table V), we specifically investigated genetic diversity over time in only those locations having clear time series. In these location-

specific time series, the factor time period actually played an important role in the model (delta  $> 2$ ; Table IV and  $A_R$  and  $H_E$ ; LRT,  $\chi^2 = 10.293$ ,  $df = 3$ ,  $p = 0.016$  and  $\chi^2 = 19.159$ ,  $df = 3$ ,  $p < 0.001$ , respectively). The drop of  $A_R$  and  $H_E$  over time in these populations is visualized in Figure 3. Multiple comparisons of time periods showed significantly lower levels of genetic diversity, especially those in comparison with time period 1946–1959 (Tukey HSD post hoc test comparisons,  $p < 0.05$ ; Table V).

## 4. DISCUSSION

Recent research described the loss of genetic diversity over time within Brazilian populations of *B. pauloensis* (Maebe et al. 2018).

**Table V.** Output of the different factors in the linear mixed models (LMMs) on  $A_R$  and  $H_E$ . (A) in only the Argentinian *B. pauloensis* populations; and (B) in the location-specific time series. After  $t$  tests and for each factor or interaction, the estimate, standard error (SE), and  $p$  value are shown. Significant factors in italics

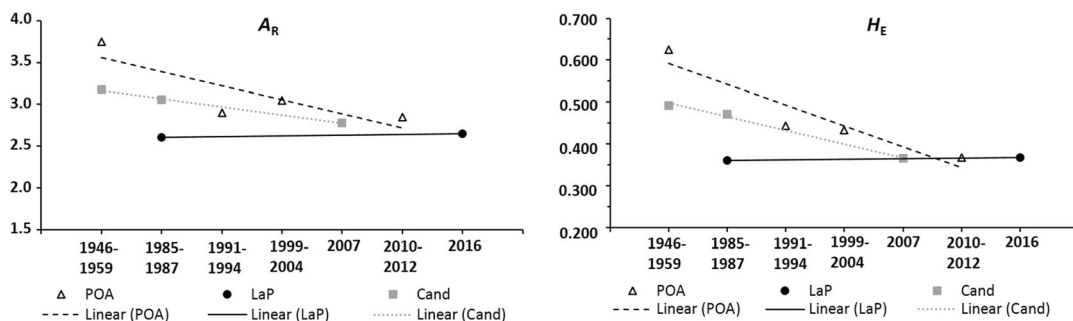
A. Argentina				
$A_R$	Estimate	SE	$t$ value	$p$
Met vs Pun	-0.447	0.481	-0.929	0.353
Met vs GO	-0.748	0.589	-1.271	0.204
Met vs LaP	-0.136	0.340	-0.401	0.689
Met vs Cand	0.314	0.481	0.654	0.513
Pun vs GO	-0.186	0.361	-0.514	0.607
Pun vs LaP	0.333	0.313	1.064	0.287
Pun vs Cand	<i>0.706</i>	<i>0.295</i>	<i>2.394</i>	<i>0.017</i>
GO vs LaP	0.518	0.289	1.794	0.073
GO vs Cand	<i>0.892</i>	<i>0.272</i>	<i>3.272</i>	<i>0.001</i>
LaP vs Cand	<i>0.373</i>	<i>0.164</i>	<i>2.269</i>	<i>0.023</i>
$H_E$	Estimate	SE	$t$ value	$p$
Met vs Pun	-0.026	0.096	-0.274	0.784
Met vs GO	-0.144	0.118	-1.224	0.221
Met vs LAP	-0.062	0.069	-0.917	0.359
Met vs Cand	0.048	0.096	0.496	0.620
Pun vs GO	-0.097	0.073	-1.335	0.182
Pun vs LaP	-0.033	0.063	-0.518	0.604
Pun vs Cand	0.045	0.059	0.763	0.445
GO vs LaP	0.065	0.059	1.088	0.276
GO vs Cand	<i>0.143</i>	<i>0.056</i>	<i>2.551</i>	<i>0.011</i>
LaP vs Cand	<i>0.078</i>	<i>0.034</i>	<i>2.301</i>	<i>0.021</i>
B. Location specific				
$A_R$	Estimate	SE	$t$ value	$p$
(1946–1959) vs (1985–1994)	-0.636	0.230	-2.767	0.006
(1946–1959) vs (1999–2004)	-0.422	0.303	-1.392	0.164
(1946–1959) vs (2007–2016)	-0.735	0.230	-3.195	0.001
(1985–1994) vs (1999–2004)	0.210	0.219	0.960	0.337
(1985–1994) vs (2007–2016)	-0.098	0.158	-0.623	0.533
(1999–2004) vs (2007–2016)	-0.298	0.194	-1.538	0.124
$H_E$	Estimate	SE	$t$ value	$p$
(1946–1959) vs (1985–1994)	-0.133	0.041	-3.263	0.001
(1946–1959) vs (1999–2004)	-0.116	0.054	-2.161	0.031
(1946–1959) vs (2007–2016)	-0.191	0.041	-4.706	< 0.001
(1985–1994) vs (1999–2004)	0.017	0.039	0.436	0.663
(1985–1994) vs (2007–2016)	-0.056	0.028	-2.106	0.035
(1999–2004) vs (2007–2016)	-0.072	0.035	-2.085	0.037

The authors found a decrease of  $A_R$  (26.65%) and  $H_E$  (40.14%) from 1946 until 2012 (see also Figure 2). However, it remained unclear if

the temporal drop in genetic diversity was a location-specific result, or if all populations of this species undergo such dramatic losses.



### Temporal genetic variability shifts



**Figure 3.** Temporal shift in mean  $A_R$  and  $H_E$  between location-specific time series. Argentinean locations: La Plata (LaP) and Candelaria (Cand). Brazilian location: Porto Alegre (POA).

Additional research is needed to make the distinction between both possibilities, which in turn is essential to prioritize more local conservation and management needs or specific measurements spanning over the species' full distribution range. Therefore, we performed in this study a temporal assessment of genetic diversity of *B. pauloensis* in Argentina and compared it with the reanalyzed Brazilian data (Maebe et al. 2018). Firstly, the reanalyzed data of the Brazilian populations confirmed the drop in genetic diversity over time (Table IVA). However, when analyzing the genetic variability in the Argentinean populations of *B. pauloensis*, no such pattern was observed (Figure 2). Indeed, as time period was not significantly affecting the model (Table IVB), our data showed that genetic diversity remained temporally stable within the Argentinean *B. pauloensis* populations. Hence, location was significantly affecting the model. Therefore, we searched for the temporal effect of genetic diversity in location-specific time periods. Our results showed a clear drop in genetic diversity observed over time in Candelaria (Argentina) and Porto Alegre (Brazil) (Figure 3). Although for La Plata no drop could be detected, the lack of old pre-1980 specimens could be a possible explanation for the latter result. Additional time points could bring more light to this question.

In general, our results showed a location-specific loss of genetic diversity over time in South American *B. pauloensis* populations. This will have negative impacts on population survival of this supposed stable species (Martins and Melo 2010). Indeed, populations

with low genetic diversity can become more vulnerable to diseases and pathogens (Whitehorn et al. 2011; Cameron et al. 2011). If these populations become small, genetic diversity may decrease further due to an increased impact of genetic drift. Furthermore, due to an increased change of brother-sister mating, inbreeding and inbreeding depression may worsen the situation (Frankham 2005; Zayed 2009; Habel et al. 2014). If these populations also become isolated, gene flow will decrease, and this will cause a further loss of genetic variability. Furthermore, the use and release of mass-reared specimens of *B. pauloensis*, which may be inbred and specifically selected for certain non-adaptive traits, could also contribute to the genetic pauperization of the local *B. pauloensis* populations. In general, these all will have a negative impact on population viability and these populations' potential to adapt to current and future changes in the environment (Frankham 2005; Zayed 2009, Habel et al. 2014).

What could have caused the loss of genetic diversity in Brazilian *B. pauloensis* populations and why it is only present at specific locations in Argentina are not known. Land use changes in South Brazil and Northeast Argentina, with strong agricultural expansions and increases in urban regions, could have caused the reduction in genetic diversity as they have led to the population decline of another Neotropical bumblebee species, *B. bellicosus* (Martins et al. 2013). Temporal differences in the impact of these drivers may explain the local differences in detection of a

reduction over time between Argentinean and Brazilian *B. pauloensis* populations. As genetic diversity was significantly lower in Argentinean populations, maybe the major losses in genetic diversity happened earlier in time, while this phenomenon is only recently present in Brazil. Hence, Maebe et al. (2018) mentioned recent deforestation events as a possible explanation for the reduced genetic diversity levels in South Brazil (Hansen et al. 2013), especially as this was the major difference between South Brazil, where losses of genetic diversity were observed, and mainland Europe where no drop in genetic diversity has been observed over the last century (Maebe et al. 2016). Differences in deforestation rates and history between locations could maybe also explain the local differences between South American *B. pauloensis* populations. Further research is needed to find if recent deforestation events are indeed causing shifts in genetic diversity levels in *B. pauloensis* populations and bumblebees in general, especially now that bees also face an additional stressor—climate change. The latter could cause shifts in suitable climatic conditions, as is already been shown for *B. bellicosus* within the same region as our study (Martins et al. 2015). This additional driver may play a role in the further genetic pauperization of these already-vulnerable populations. More studies are necessary to broaden our knowledge on how climate change can and will affect local bumblebee populations. In general, our result implies the urgent need for conservation of *B. pauloensis* and Neotropical bumblebees in general.

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## AUTHORS' CONTRIBUTION

MH collected all Argentinean specimens. ML and JLA curated and helped collect the collection specimens. MH performed the experiments. KM and MH designed the study. KM performed the analysis, drafted the manuscript, and designed the figures. All authors contributed to the writing of the paper. Funding information

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**Baisse temporelle de la diversité génétique de *Bombus pauloensis***

**Déclin des abeilles / diversité génétique / microsatellites / *Bombus pauloensis***

**Amérique du Sud Zeitweiliger Verlust der genetischen Diversität bei *Bombus pauloensis***

**Rückgang von Bienen / genetische Diversität / Mikrosatelliten / *Bombus pauloensis* / Südamerika**

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