ORIGINAL ARTICLE

Fire regimes and pollinator behaviour explain the genetic structure of *Puya hamata* (Bromeliaceae) rosette plants

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Abstract

Ecological interactions play a fundamental role in determining the genetic structure of plant species in time and space. The demography of the Andean *Puya hamata* has been linked to fire regimes and hummingbird behaviour, which might modify the plant's population genetic structure. Naturally, poor dispersal results in patches of genetically related plants, a pattern intensified further by burning which promotes seedling germination around parent plants. Later, when these plants flower, large patches are attractive to territorial hummingbirds which prevent visits by traplining hummingbird species, carrying pollen from likely unrelated plants. To explore this hypothesis, a genetic study of *P. hamata* using microsatellite markers was conducted with (i) isolated and grouped adults in two size categories of patches, and (ii) seeds collected from the same patches and isolated individuals. Isolated individual plants presented a higher observed heterozygosity with close to zero inbreeding. Adult plants from large patches showed a lower observed heterozygosity and higher inbreeding than plants from other spatial contexts. Seed genetic structure displayed a gradient of diversity: lower at patch centres but higher at patch edges, in small patches, and for isolated infructescences. The spatial context of these plants, especially the contrast between large patch centres and other situations, determines the genetic diversity of their seeds via hummingbird foraging behaviour. Territorial hummingbirds restrict gene flow in and out of large patches, but traplining hummingbirds maintain genetic diversity among isolated plants, small patches, and plants at the edges of large patches. Our study illustrates the need to consider interactions between land use, plants, and their pollinators when considering genetic diversity at the landscape scale.

Keywords Páramos · Hummingbirds · Conservation · Population genetics · Pollination · Burning

Introduction

The high-elevation tropical Andean páramo ecosystem is characterized by rich plant diversity and endemism (Madriñán et al. 2013; Sklenar et al. 2011). Additionally, páramos are hotspots for ecosystems services, including providing water reservoirs, carbon sequestration, irrigation, and rural livelihood improvement (Madriñán et al. 2013).

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However, páramo ecosystems face challenges like climate change (Aide et al. 2019; Carilla et al. 2018) and anthropogenic perturbations (Keating 2007; Vásquez et al. 2015) that threaten their ecological integrity. These challenges produce regional landscape transformations and ecosystem modifications (Aide et al. 2019), which strongly affect plant richness as well as biotic and abiotic interactions that shape the genetic structure of ecological communities (Gilman et al. 2010; Young and Leon 2007).

Pollination and seed dispersal are keystone interactions that shape plant species' genetic structure (Midgley et al. 1991; Valenta et al. 2017). Within the context of climate change, these species interactions could alter, modifying communities and creating new scenarios for species diversification (Buermann et al. 2011; Gilman et al. 2010). These new scenarios could lead to novel ecological connections that may reduce plants' genetic diversity and reproductive output through genetic drift and inbreeding (Chase et al. 1996; Fadrique et al. 2018). Thus, understanding the biotic

and abiotic interactions that shape current plant populations' genetic diversity in the páramo is important to be able to handle the unpredictable landscape transformations caused by climate change.

Puya hamata L.B. Sm. (Bromeliaceae) is a good biological model for exploring the impact of species interaction and environmental constraints on genetic diversity. *P. hamata* is a conspicuous giant basal rosette that forms dense local populations in humid páramos in Ecuador and Colombia (Schmidt Jabaily and Sytsma 2013) and has multiple ecological interactions with vertebrates (Garcia-Meneses and Ramsay 2014). Its seed dispersal is mainly by gravity and wind, and it has restricted seed mobility, which explains its aggregated distribution (Benzing 2000; Miller 1988). Additionally, *P. hamata* seeds have a higher germination rate, seedling recruitment, and survival in fire-prone páramos (Laegaard 1992). The plant is adapted to fire, with thermal insulation of its meristem, and seed germination occurs only at temperatures above 14 °C, which are often found in more open vegetation, often in recently burned tussock grassland (Garcia-Meneses and Ramsay 2014; Ramsay and Oxley 1996). These traits result in the formation of high-density patches of young plants derived from one or several parents in recently burned páramos (Garcia-Meneses and Ramsay 2014). These patches vary from isolated individuals to large patches with > 100 individuals, and this mosaic of patches covers dozens of square kilometers on the páramo. Thus, the páramo fires promote seed germination, but have only limited effects on the growth rate and survival of adult plants.

Moreover, *P. hamata* is a hummingbird-pollinated plant offering a concentrated nectar resource that attracts hummingbirds with a high metabolic rate (Altshuler and Dudley 2006; Woods and Ramsay 2001). Competition for nectar among pollinators often results in different foraging strategies (territorial or traplining; Rappole and Schuchmann 2003) that promote or inhibit the transfer of genetic material among the plants. This behaviour has been previously linked to the spatial distribution of nectar for *P. hamata* pollinators (Garcia-Meneses and Ramsay 2012; Woods and Ramsay 2001) and other high-elevation Andean species like *Oreocallis grandiflora* (Hazlehurst and Karubian 2016). Resource availability defines foraging behaviour that eventually structures plant population genetic diversity.

In summary, the fire response of *P. hamata* would be expected to drive spatial and genetic structure in adult plants, while hummingbird foraging behaviour, in response to the spatial pattern of flowering plants, would be expected to affect the genetic structure of seeds. However, *P. hamata*'s fine-scale genetic structure has not yet been explored; population genetic studies have focused mainly on *P. raimondii* (Hornung-Leoni et al. 2013; Sgorbati et al. 2004).

This study explores *P. hamata* populations' genetic diversity and structure in the northern Andes of Ecuador. We will test the following ecological hypotheses related to *P. hamata*'s dispersal and pollination using molecular markers, following ideas proposed by Garcia-Meneses and Ramsay (2012): (i) we expect individuals in patches to have low genetic diversity and high rates of inbreeding, due to the plant's limited seed dispersal and germination response to fire, while isolated individuals' genetic composition would be more diverse, because they come from many provenances; (ii) we expect that seeds from plants in the centre of large patches will exhibit lower genetic diversity and higher inbreeding (better defended by territorial hummingbirds) than those from patch edges, smaller patches, or isolated individuals (influenced more by traplining hummingbirds).

Materials and methods

Model system and study site

The genus *Puya* (Bromeliaceae) comprises conspicuous rosettes widely distributed on the páramos from Costa Rica to northern Argentina and Chile (Sklenar et al. 2011). *P. hamata* is a rosette that forms locally clumped populations in humid páramos between 3300 and 3700 m asl in Colombia, Ecuador and Peru (Miller and Silander 1991; Schmidt Jabaily and Sytsma 2013). *P. hamata* has a rosette reaching > 2 m in diameter and a 4-m tall inflorescence with around 1000 flowers (Garcia-Meneses and Ramsay 2012), attracting hummingbirds (Miller 1986).

The study area comprises the humid páramos on the southern slopes of Volcán Chiles on the Ecuador-Colombian border (Carchi Province, UTM coordinates: 18 N 173248 89318), between 3400 and 4200 m asl, with an annual precipitation of 1000–1500 mm and constant environmental humidity (Hofstede et al. 1998). This area is owned by Comuna La Esperanza farmers in Tufiño who, in the past, have used burning as an agricultural tool associated with livestock grazing, leading to a landscape mosaic of areas with different times since fire and patches of *P. hamata* of different ages (Garcia-Meneses and Ramsay 2014).

Sampling method

The experimental design comprised two surveys across a variety of spatial contexts for *P. hamata*: (i) leaf sampling to explore the genetic structure of adult individuals; and (ii) seed sampling to assess their genetic structure. Leaves were sampled in August 2013 from 20 isolated individuals (I), 35 individuals from three small patches of > 5 and < 20 individuals (S1 $n = 12$; S2 $n = 10$; S3 $n = 13$); and 39 individuals from three large patches, each > 100 individuals (L1 $n = 13$, L2 $n = 13$, L3 $n = 13$). In total, 94 individuals were sampled (Fig. 1).
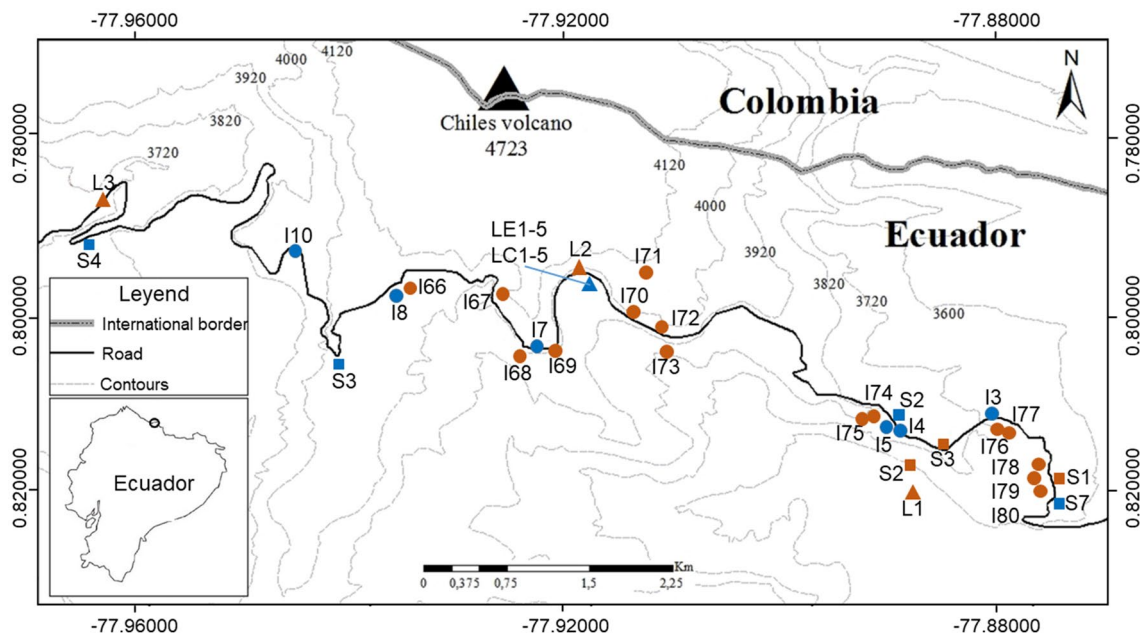
Fig. 1 Study site. The location of the 74 *P. hamata* adults (red color): 15 isolated plants (circles: I66–I80), small patches (squares: S1, S2, S3), and large patches (triangles: L1, L2, L3). The location of the 20 *P. hamata* infructescence (blue color): isolated plants (circles: I3, I4, I5, I7, I8, I10), small patches (squares: S2, S3, S4, S7), and large patches (triangle: LC1–LC5 + LE1–LE5)

Seed sampling occurred in February 2014 and comprised eight infructescences from isolated individuals (I), six infructescences from individuals in small patches (S), and 14 infructescences from individuals from a single large patch of which seven were from individuals on the patch edge (LE) and seven from individuals in the patch centre (LC). From each infructescence, we randomly selected 10 seeds from different sections of the infructescence. A total of 28 infructescences were collected (280 seeds).

DNA isolation, primer screening, and genotyping

DNA extraction from the leaf samples followed Doyle (1991)'s method. DNA extraction from seeds was done using the Wizard genomic DNA Purification Kit according to the manufacturer's instructions. A set of five microsatellite markers (simple sequence repeats or SSRs) of genera *Aechmea* (Bromeliaceae) and 15 SSRs of *Ananas* (Bromeliaceae) were chosen to be transferred to *P. hamata*. Polymerase chain reaction (PCR) amplifications followed Goetze et al. (2013) and Wöhrmann and Weising (2011). PCR products were separated by electrophoresis in 6% denatured polyacrylamide gels and silver nitrate staining. Samples without an amplification of at least four loci were not considered for the study.

Statistical analysis

Genetic data derived from adult individuals and infructescences were evaluated at two different levels: (i) genetic structure and (ii) patch arrangement. Genetic structure was evaluated with GenAlEx 6.5 (Peakall and Smouse 2006) using 10,000 permutations. The inbreeding coefficient (F_{is}), the number of private alleles (PA, as a measure of flow gene), average expected heterozygosity (H_e, genetic diversity), and observed heterozygosity (H_o) were calculated for adults and seeds. Allelic richness (AR) within each patch and isolated individuals was computed by the rarefaction method implemented in HP-RARE (Kalinowski, 2005). Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium for adults and seeds were computed using GENEPOP v4.2 with default parameters (Raymond and Rousset 1995). Statistical differences among the genetic indexes were computed with a Mann–Whitney U test using XLSTAT for Excel (Addinsoft Inc, New York, USA).

A genetic distance-based analysis (patch arrangement) was conducted to visualize the genetic composition among adults and seeds. A principal coordinate analysis (PCoA) was performed using Codom-Genotypic distance as implemented by GenAlEx 6.5. Seeds PCoA was conducted using a pooled data set of seeds for each infructescence. A second PCoA was conducted among *P. hamata* adults. A Bayesian clustering analysis was performed to determine

genetic similarity among adults and seeds using Structure v2.3 (Pritchard et al. 2000). An admixture mode and simulations were performed with a burn-in length of 1,000,000 repetitions followed by 2,000,000 Markov-Chain Monte Carlo replicates. The number of distinct genetic clusters (K) present in the data set was from 1 to 10 using 5 iterations per K. We used the ΔK method (Evanno et al. 2005) as implemented in STRUCTURE HARVESTER v.0.6.94 (Earl and von Holdt 2012) to detect the number of K that best fits the data.

Three distance matrices were created: a spatial matrix using distance (m) between pairs of plants, calculated with Esri ArcMapTM 10.2.0.3348 (Environmental Systems Research Institute, Redlands, CA, USA); a genetic matrix using Codom-Genotypic distance between plants; and an altitude matrix of altitudinal distance (m) between plants. A Mantel test analysis was conducted to examine relationships between the matrices independently for adults and seeds using GenAlEx 6.5 with 10,000 permutations.

Results

Transfer of molecular markers and DNA isolation

Six out of 20 SSRs' loci were successfully transferred to *P. hamata*: five loci of *Ananas comosus* and one SSR of *Aechmea caudata*. Among the loci transferred, Acom 64.22 was monomorphic, while the others were polymorphic ranging from 3 to 13 alleles (Table 1). We used the five polymorphic loci for the genetic analysis. Of the 94 adult individuals, only 74 gave a positive signal for DNA isolation (Table 2). *P. hamata* seeds are less than 2 mm in size and were, therefore, troublesome for the DNA isolation and PCR amplification. Of the 280 seeds sampled, only 187 seeds (grouped in 20 infructescences) gave a positive signal for DNA isolation (Table 3). Linkage disequilibrium was not significant ($P > 0.05$) for any pair of loci when tested within clusters of adults and seeds (Online Resource 1), supporting the assumption of loci independence. Significant deviations

Table 1 Set of positive microsatellite loci of the bromeliad genera *Aechmea* (*Ac*) and *Ananas* (*Acom*) successfully transferred to *P. hamata*

Locus	Repeat motif	Primer sequence (5′–3′)		Mean (bp)	A
Acom 64.22	$(TCC)_5$	F:	CTCCTCATCTACCGCACCTC	195	1
		R:	CCCTAGACGACGACGAAGAG		
Acom 78.4	$(GT)_{14}$	F:	GCAAATGAGGCCACAAACTT	171	3
		R:	GGGTGGTGTGGACTTTCTCT		
Acom 101.1	$(CGT)_{11}$	F:	CGAGAGAGATTGTGCGTTTG	183	5
		R:	GGGGGAACACACTGCTAAAG		
Acom 117.15	$(CT)_{20}$	F:	GCAACCCCAATACCCTAACC	182	13
		R:	GTACTCCGCCATTGTTGGTG		
Acom 119.1	$(CTTT)_7$	F:	TTCTGATCAATGAGTGGACACC	208	5
		R:	TCCTGAATCCAAAGGCAAAG		
Ac40	$(AG)_{18}$	F:	GCAGCACCAGAGACAGCA	198	11
		R:	GTGGGAGAGTGTGGAGAGGT		

Number of alleles (A) was computed with 261 *P. hamata* samples (74 individuals, and 187 seeds)

Table 2 Genetic diversity of six patches (with 59 individuals) and isolated individuals ($n = 15$) of *P. hamata* derived from five simple microsatellite loci

Patch	Altitude (m asl)	n	PA	AR	H_e	H_o	F_{is}
S1 (small)	3658	11	0	2.74	0.39	0.37	− 0.01**
S2 (small)	3654	3	0	1.4	0.12	0.07	0.45**
S3 (small)	3737	11	0	1.99	0.42	0.63	− 0.50**
I (isolated)	3800	15	2	2.74	0.54	0.57	− 0.08**
L1 (large)	3580	10	1	2.4	0.34	0.25	0.32**
L2 (large)	3979	12	2	1.75	0.19	0.12	0.17**
L3 (large)	3730	12	0	2.8	0.53	0.37	0.27**
Mean ± SD				2.4 ± 0.44	0.40 ± 0.13	0.38 ± 0.19	0.02 ± 0.30

Significant departures from HWE are indicated as **$P < 0.01$. Number of individuals (n), allelic richness (AR), number of private alleles (PA), expected heterozygosity (H_e), observed heterozygosity (H_o), and the inbreeding coefficient (F_{is}) are shown. S2 was excluded for calculations of mean and standard deviation (SD) due to its small-sample size

Table 3 Genetic diversity of 20 *P. hamata* infructescences (187 seeds) derived from five microsatellite loci

Infructescences	Altitude (m asl)	n	PA	AR	H_e	H_o	F_{is}
Isolated			Mean	2.38	0.45	0.54	− 0.23
I3	3718	10	1	2.39	0.53	0.73	− 0.40**
I4	3761	10	0	2.74	0.55	0.52	− 0.05**
I5	3768	10	3	2.84	0.50	0.55	− 0.18**
I7	4009	10	0	1.89	0.27	0.42	− 0.37**
I8	4054	10	1	2.12	0.41	0.36	0.14**
I10	3981	10	0	2.34	0.45	0.68	− 0.54**
Small patch			Mean	2.64	0.53	0.61	− 0.20
S2	3749	10	0	2.49	0.53	0.52	0.02**
S3	4042	10	0	2.56	0.50	0.70	− 0.39**
S4	3754	9	0	2.83	0.53	0.58	− 0.16**
S7	3647	10	0	2.7	0.57	0.66	− 0.26**
Edge of large patch			Mean	2.28	0.49	0.67	− 0.33
LE1	3979	10	0	2.54	0.53	0.73	− 0.39**
LE2	3979	10	0	2.07	0.42	0.56	− 0.23**
LE3	3979	10	0	2.1	0.49	0.66	− 0.31**
LE4	3979	10	0	2.44	0.54	0.72	− 0.40**
LE5	3979	4	0	1.8	0.32	0.55	− 0.62**
Centre of large patch			Mean	2.03	0.36	0.48	− 0.31
LC1	3979	10	0	2.14	0.39	0.50	− 0.29**
LC2	3979	10	0	1.81	0.32	0.54	− 0.56**
LC3	3979	10	0	1.99	0.33	0.48	− 0.38**
LC4	3979	6	0	1.93	0.34	0.40	− 0.16**
LC5	3979	8	0	2.29	0.42	0.48	− 0.18**
Overall mean				2.32	0.46	0.58	− 0.27

Significant departures from HWE are indicated as **$P < 0.01$. Number of individuals (n), number of private alleles (PA), allelic richness (AR), expected heterozygosity (H_e), observed heterozygosity (H_o), and the inbreeding coefficient (F_{is}) are shown. LE5 was excluded for calculations of mean and standard deviation due to its small-sample size

from HWE were computed within patches for adults and seeds ($P < 0.05$; Tables 2, 3).

Diversity and genetic structure

The allelic richness showed no evident pattern among patches. Meanwhile, the observed heterozygosity (H_o) was higher for the small patch S3 and for isolated individuals, and expected heterozygosity (H_e) was higher for isolated individuals. Private alleles were only present for isolated plants and ones from large patches. Plants in large patches tended to be more homozygous than the HWE expectation (range 0.17–0.32; Table 2).

PCoA analysis placed the 74 adult plants into three weakly defined clusters (A, B, C; Fig. 2), which correlated somewhat with the large patches in this study: A ($n = 32$) included 7/10 L1 individuals plus others; B ($n = 16$) included all L2 individuals plus four individuals from other patches; and C ($n = 21$) included 7/12 L3 individuals plus others. Five individuals from small patches and isolated individuals were placed outside these analysis clusters (Fig. 2a). By contrast,

Bayesian analysis recognized only two groups ($\Delta K = 2$, Fig. 2b): K1 ($n = 23$) corresponded to PCoA cluster B and K2 ($n = 51$) included the rest of the individuals (Online Resource 2). K1 mostly comprised individuals from altitudes ≥ 3980 masl, while K2 those from < 3980 masl (Fig. 2c).

The allelic richness in seeds decreased from those of small patches to those from plants in the centre of large patches (Table 3), with significant differences for S-LE (Mann–Whitney U test: $U = 15$; $P = 0.057$) and S-LC ($U = 20$; $P = 0.02$). Only isolated plants had private alleles. Statistically significant differences were detected for genetic diversity among S–LC ($U = 20$; $P = 0.02$) and LE–LC ($U = 19.5$; $P = 0.01$); and for observed heterozygosity between S–LC ($U = 19$; $P = 0.03$) and LC–LE ($U = 20$; $P = 0.02$).

Four PCoA clusters were derived from the 20 infructescences (W, X, Y, Z; Fig. 3). W comprised mostly isolated infructescences, X all small patches, Y mostly edges of large patches, and Z all centres of large patches (Fig. 3a). Bayesian analysis with 187 seeds from the 20 infructescences detected three groups ($\Delta K = 3$, Fig. 3b; Online Resource 3).
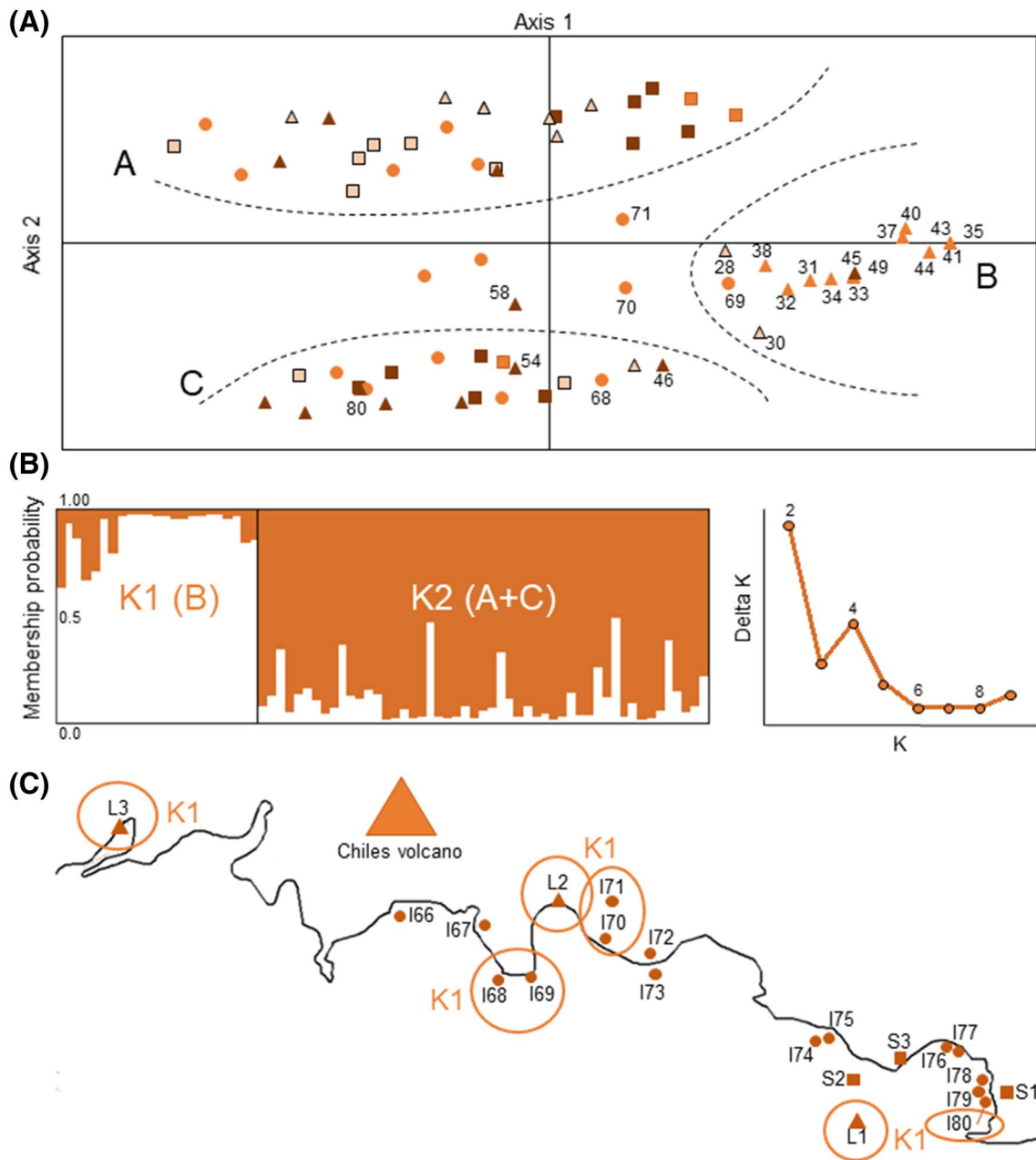
Fig. 2 a Principal coordinates analysis (PCoA) of 74 *P. hamata* adult individuals. The two PCoA axes explain 63.18% of the variance. Isolated plants (orange circles), small patches (squares: light orange S1, orange S2, dark orange S3), and large patches (triangles: light orange L1, orange L2, dark orange L3) are shown. Only the 23 individuals clustered by K1 are named in the PCoA. **b** Schematic representation of Bayesian analysis by STRUCTURE with the respective Delta *K*/*K* plot. **c** Geographical location of the *P. hamata* adult individuals based on the Bayesian analysis

This grouping pattern strongly matched those from PCoA, where K1 corresponded to W, K2 to Z + Y, and K3 to X. Unlike PCoA, Bayesian analysis did not detect genetic differentiation between infructescences from the large patches' centres and edges.

Nearby patches tended to be genetically more similar than expected by chance, and genetic differences increased linearly with spatial distances and altitude (Table 4).

Discussion

Effect of fire on the genetic structure of *P. hamata* individuals

Isolated adults and small patches had higher values for both expected and observed heterozygosity than larger patches. In addition, larger patches presented high values
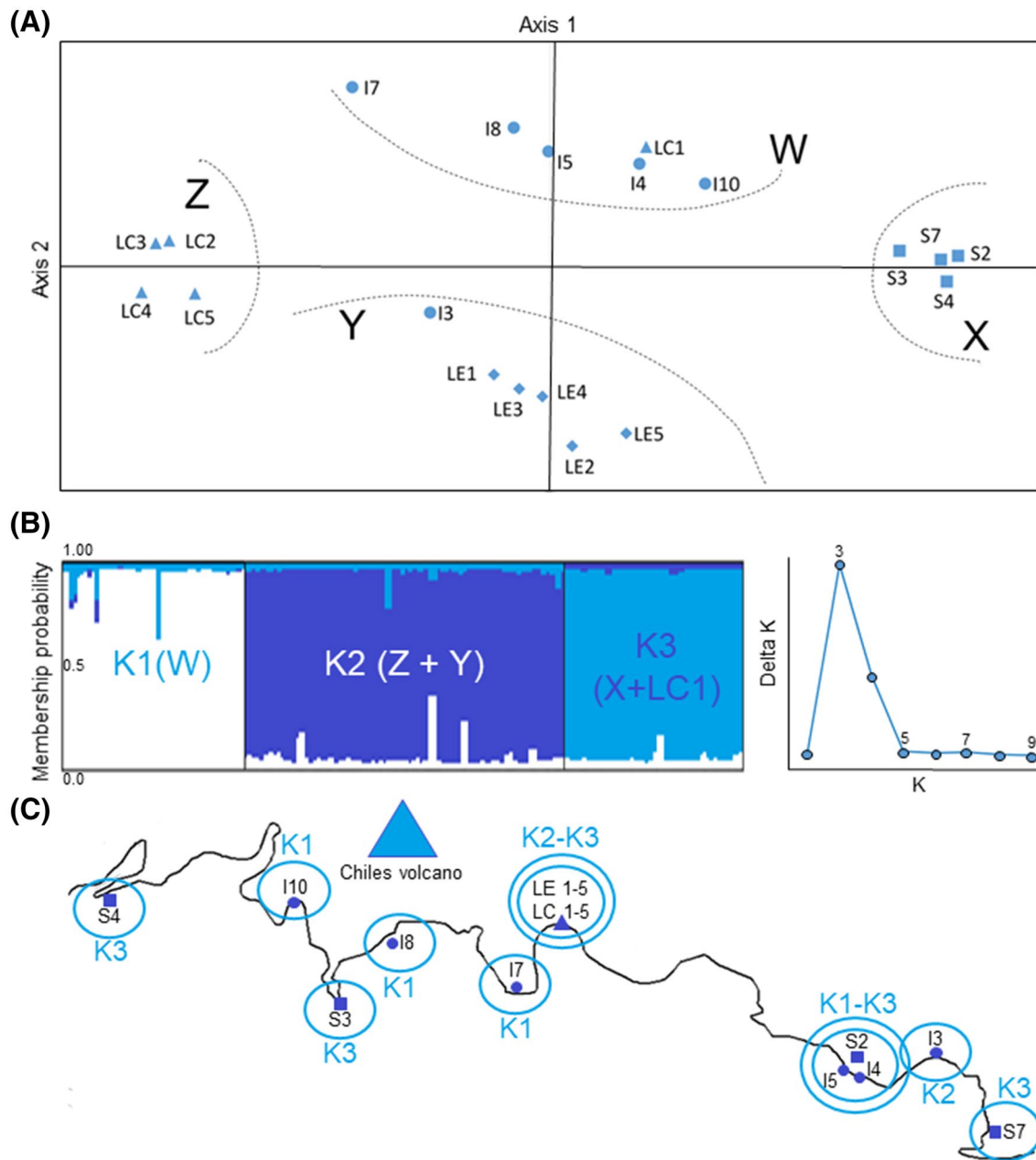
Fig. 3 **a** Principal coordinates analysis (PCoA) of 20 *P. hamata* seeds/infructescences. The two PCoA axes explain 62.03% of the variance. Isolated plants (blue circles), small patches (blue squares), centres of large patches (blue triangles), and edges of large patches (blue diamonds) are shown. **b** Schematic representation of Bayesian analysis by STRUCTURE: K1 ($n=50$), K2 ($n=88$) and K3 ($n=49$), with the respective Delta *K/K* plot. **c** Geographical location of the seeds/infructescences based on the Bayesian analysis

of inbreeding. Limited seed dispersal in *Puya* explains the higher inbreeding found in large patches, where most plants would be expected to derive from the same parent after fire had opened up the vegetation canopy (Garcia-Meneses and Ramsay 2014). By contrast, the higher genetic diversity among isolated plants and small patches can be explained by their probable origin as rare, unconnected seed dispersal events from distant parents. Thus,

the spatial context of adult *Puya* plants is linked to their heterozygosity, allelic richness and inbreeding levels.

The genetic diversity of *P. hamata* adults in this study was relatively high and corresponded to a wider genetic pool when compared with some other *Puya* species. For instance, Sgorbati et al. (2004), applying AFLP and cpSSRs markers, found a low and extremely uniform genetic diversity among *P. raimondii* populations distributed in Peru's central and

Table 4 Results of Mantel test (r^2) showing the significant correlations among spatial, genetic, and altitude distance matrices of *P. hamata* populations included in this study (adults and seeds)

	Seeds		
	Altitude	Genetic	Spatial
Adults			
Altitude	–	0.044**	0.541**
Genetic	0.087**	–	0.031**
Spatial	0.050**	0.052**	–

All correlations were statistically significant with a *P* value < 0.01

southern Andes, which suggests a single progenitor causes genetic uniformity among and within *P. raimondii* populations. Conversely, Hornung-Leoni et al. (2013), working with several *P. raimondii* populations from Peru's central Andes and using AFLP markers, reported a high level of genetic variation among populations. Geographical bias in sampling and limitations in technical tools could explain this significant difference (Hornung-Leoni et al. 2013). In particular, the nature of the genetic marker types needs to be interpreted carefully, because the unknown marker properties like mutation rate would affect the levels of variation. Our study revealed higher levels of genetic diversity at a finer spatial scale than the aforementioned studies. It would be possible to observe both high and low genetic diversity within the same *Puya* population, depending on whether samples were taken from isolated individuals or ones from the centres of large patches. The spatial context of the sampled plants should be accounted for in future studies, and samples of isolated individuals would provide better estimates of genetic diversity for an area than samples from within large patches of *P. hamata*.

The moderate levels of genetic diversity exhibited by *P. hamata* plants (mean $H_e = 0.46 \pm 0.07$ SD) are comparable with other Andean plant studies at similar spatial scale and with similar molecular markers. Almeida et al. (2013) reported higher genetic diversity values (range $H_e = 0.70–0.76$) for perennial evergreen *Lasiocephalus ovatus* populations, but Vásquez et al. (2016) found several populations of the long-lived semelparous giant rosette *Lupinus alopecuroides* had lower diversity (range $H_e = 0–0.51$).

Effect of pollinator behaviour on the genetic structure of seeds of *P. hamata*

A fine-scale genetic structure was detected among *P. hamata* seeds. This pattern can be explained by (i) the isolated or aggregated spatial context of infructescences, and (ii) the location of infructescences within larger patches (edge or centre). There was a diversity gradient from the centres of large patches (less diversity) to the edges of large patches,

small patches, and isolated plants (more diversity), consistent with the hypothesis of Garcia-Meneses and Ramsay (2012). Traplining hummingbirds, moving from plant to plant at a landscape scale, forage more frequently on *P. hamata* inflorescences in isolated, small-patch or patch-edge contexts, while the centres of larger patches are well defended by territorial hummingbirds against traplining competitors (Woods and Ramsay 2001). This favours outcrossing in isolated, small-patch or patch-edge contexts and boosts genetic diversity. The territorial defence of patch centres reduces the probability of incorporating new genetic material from other populations (Groom 1998; Woods and Ramsay 2001). Territory size for the *Aglaeactis cupripennis* hummingbird varied from 0.03 to 0.54 ha in Ecuadorian and Peruvian montane forest (Céspedes et al. 2019), but the concentrated nectar sources in large *P. hamata* patches are likely to result in smaller end of this range in the páramo. Nevertheless, territorial individuals are often unable to defend all plants in their territory simultaneously, allowing trapliners to enter territories for short feeding bouts, especially at the margins. The emergence of traplining or territoriality as foraging strategies in hummingbirds has been linked to ecological and evolutionary strategies to reduce competition between these pollinators (Rappole and Schuchmann 2003) and is likely to be robust in the face of changes in hummingbird composition from place to place. In places where *A. cupripennis* is absent, other hummingbird species have been observed to establish territories around *P. hamata* patches (Miller 1988), maintaining the barrier for outcrossing in patch centres.

Altitude partially explained the genetic relationships of adults and seeds, with some differentiation between individuals at higher versus lower altitudes. This was expected, since altitude controls the altitudinal movement of genotypes (Almeida et al. 2013) as well as hummingbird movements (Buermann et al. 2011; Ohsawa and Ide 2008; Rappole and Schuchmann 2003). However, seeds from small patches of *P. hamata* were defined as a single, discrete genetic cluster, despite the altitudinal range they occupied (3647–4042 m). Although spatially and altitudinally distant, genetic flux existed among these small patches, which resulted in similar genetic composition. It seems likely that traplining hummingbirds transfer genes among these patches over a wide altitudinal range. Traplining hummingbirds not only visit widely separated individuals (Gill 1988), but also fly over greater altitudinal ranges compared to territorial hummingbirds (Barbará et al. 2007; López-Segoviano et al. 2018). In our study, the territorial species, *A. cupripennis*, is more often found in cloud forest at lower altitudes, but forages in the lower reaches of páramo grasslands, especially during *Puya* flowering events.

There is some evidence in our study for different pollinator behaviour for small patches compared with isolated

plants. Seeds were genetically distinct for these categories (Fig. 3). We do not have an evidenced explanation for this pattern, but we speculate that different traplining species might partition their foraging, such that some species might specialise on small patches, while others focus on isolated plants. This would require confirmation with behavioural studies.

The incorporation of isolated plants and small patches into traplining by pollinators could move alleles among larger patches of *Puya*, with the isolated plants and small patches acting as stepping stones, in the manner suggested by Howe and Miriti (2004). In this regard, the ecological and genetic contribution of isolated individuals located between forest remnants has already been reported in the context of tropical forest ecology (Chase et al. 1996; Fuchs and Hamrick 2010; Guevara et al. 1992).

Conclusion

The spatial context of *P. hamata* plants at a landscape scale is driven by burning, common throughout the páramo grasslands of the Andes and Central America (Horn and Kappelle 2009). *P. hamata* patchiness in association with burning has been noted several times (Garcia-Meneses and Ramsay 2014; Laegaard 1992; Miller and Silander 1991), and in its absence, *Puya* species would be rarer and the population would consist mostly of isolated individuals or small patches. Only in the presence of burning would large patches of *Puya* be expected. Thus, the genetic patterns reported here are the outcome of the interaction of burning influences on patchiness of adult plants (driven by germination requirements and poor seed dispersal) and pollinator behaviour in response to patchiness of nectar resources (specifically territoriality).

Andean mountains are particularly at risk from climate change (Urrutia and Vuille 2009), with more rapid change expected at higher elevations (Pepin et al. 2015). The impact of this warming, alongside land-use changes, makes it difficult to predict species' responses (Buermann et al. 2011; Frei et al. 2010) and the influence of burning on future páramo landscapes. Temperature increases at the páramo-forest ecotone will favour more agricultural activity and, consequently, increase anthropogenic fires (Anderson et al. 2011). In addition, potentially drier conditions in the páramo zone could favour the rapid spread of fires forming larger patches (Ruiz Carrascal et al. 2011), changing the balance of large patches versus small patches and isolated plants. The dominance of larger patches in a population could mean a loss of genetic diversity in *P. hamata*. It is clear from our work that isolated and small-patch *Puya* plants are a significant genetic resource that should be taken into account when developing management strategies for these plants and their pollinators.

In conclusion, poorly dispersed *P. hamata* seeds with particular germination requirements lead to the formation of large patches of closely related plants in burned landscapes. Territorial hummingbirds restrict gene flow in and out of these large patches, but traplining hummingbirds maintain genetic diversity among small patches and isolated plants. These traplining foragers also introduce genetic diversity to plants at the edges of large patches. All of this is consistent with the findings of Garcia-Meneses and Ramsay (2012) on reproductive output and their predictions on gene flow. Our study illustrates the need to consider interactions between land use, plants, and their pollinators to maintain genetic diversity at the landscape scale.

Data availability The datasets generated during the current study are available at the DRYAD repository: https://doi.org/10.5061/dryad.g79cnp5kq.

Compliance with ethical standards

Conflict of interest Data and findings presented in this manuscript have not been published and are not under consideration for publication elsewhere. All the authors have approved this submission and all persons entitled to authorship have been named. The authors have no conflict of interest to declare.

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