


Pleistocene glacial cycles drive isolation, gene flow and speciation in the high-elevation Andes

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Summary

- Mountain ranges are amongst the most species-rich habitats, with many large and rapid evolutionary radiations. The tempo and mode of diversification in these systems are key unanswered questions in evolutionary biology. Here we study the Andean *Lupinus* radiation to understand the processes driving very rapid diversification in montane systems.
- We use genomic and transcriptomic data of multiple species and populations, and apply phylogenomic and demographic analyses to test whether diversification proceeded without interspecific gene flow – as expected if Andean orogeny and geographic isolation were the main drivers of diversification – or if diversification was accompanied by gene flow, in which case other processes were probably involved.
- We uncover several episodes of gene flow between species, including very recent events likely to have been prompted by changes in habitat connectivity during Pleistocene glacial cycles. Furthermore, we find that gene flow between species was heterogeneously distributed across the genome.
- We argue that exceptionally fast diversification of Andean *Lupinus* was partly a result of Late Pleistocene glacial cycles, with associated cycles of expansion and contraction driving geographic isolation or secondary contact of species. Furthermore, heterogeneous gene flow across the genome suggests a role for selection and ecological speciation in rapid diversification in this system.

Introduction

Rapid evolutionary radiations are ubiquitous across the Tree of Life, but there is still little understanding about the tempo and mode of speciation or the evolutionary mechanisms responsible for rapid diversification. This is especially true for very recent evolutionary radiations confined to the Pleistocene, exemplified by the many radiations of high-elevation plants in mountains across the globe (Linder, 2008; Hughes & Atchison, 2015). Rapid diversification in high-elevation montane habitats has often been assumed to result from increased ecological opportunities owing to the high physiographical heterogeneity in mountains prompting frequent episodes of geographic isolation and allopatric speciation (Simpson, 1964; Hughes & Eastwood, 2006). However, these highly heterogeneous montane habitats may also promote adaptive divergence via ecological speciation (Chapman *et al.*, 2013; Ruiz-Sanchez & Specht, 2014; Contreras-Ortiz *et al.*, 2018). Furthermore, patterns of geographic isolation in mountain ranges have changed dramatically over time

– most notably during Pleistocene glacial cycles (Flantua & Hooghiemstra, 2018).

The Andes are one of the richest biodiversity hotspots in the world (Myers *et al.*, 2000; Olson & Dinerstein, 2002; Mittermeier *et al.*, 2005; Orme *et al.*, 2005), and the high-elevation Andean grasslands of the Páramo and Puna, in particular, present an excellent system for investigating the processes driving rapid speciation and recent evolutionary radiations for several reasons. First, they harbour a remarkable number of large plant radiations exhibiting very high rates of species diversification and encompassing 50–100+ species (Madrinán *et al.*, 2013; Luebert & Weigend, 2014; Hughes & Atchison, 2015). Second, the fact that these large plant radiations are monophyletic and consist predominantly of endemic species suggests that they evolved via *in situ* diversification – contrary to other mountain systems where migration and colonization apparently played a more dominant role (Hughes & Atchison, 2015; Hughes, 2017; Kadereit, 2017; Xing & Ree, 2017). And third, high-elevation Andean plant radiations are typically very recent, all of them within the last 5 million yr (Myr), most of them < 2.5 Myr, and with the majority of species diversity arising in the Pleistocene (Madrinán *et al.*, 2013; Luebert & Weigend, 2014), in line with the recency of the final

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uplift of the Andes (Kroonenberg *et al.*, 1990; Ghosh *et al.*, 2006).

These high-elevation Andean plant radiations have been attributed to increased opportunity for geographic isolation and allopatric speciation, driven by the emergence of new and unoccupied island-like habitats following the final uplift of the Andes 2–5 Myr ago (Kroonenberg *et al.*, 1990; Ghosh *et al.*, 2006), coupled with extremely high physiographic heterogeneity and extended multidimensional environmental gradients found in the Andes (Hughes & Eastwood, 2006; Drummond *et al.*, 2012). While these scenarios of allopatric and clinal speciation are clearly relevant for explaining the remarkable concentration of young and fast radiations in the high-elevation Andes, they largely ignore the impacts of cyclical fragmentation and connectivity of high-elevation grassland habitats through the Pleistocene (Hooghiemstra & Van der Hammen, 2004; Flantua *et al.*, 2014; Flantua & Hooghiemstra, 2018). Pleistocene glacial cycles have strongly affected habitat connectivity in the high-elevation Andes, although in different ways compared with north temperate regions: while glacial cycles in the latter regions resulted in long-distance horizontal displacement of habitats (e.g. up to 2000 km shift in the treeline in Europe) (Hewitt, 1996, 2004; Gavin *et al.*, 2014), in the high-elevation Andes, glaciations were primarily associated with vertical and only minimal horizontal displacement of vegetation zones, with, for example, a downward shift of 1200–1400 m in the treeline at the Last Glacial Maximum (van der Hammen & Cleef, 1986; Hooghiemstra & Van der Hammen, 2004; Hooghiemstra *et al.*, 2006; Graham, 2009; Jomelli *et al.*, 2014). These vertical displacements resulted in expansion and contraction of available habitat areas and changes in the degree of connectivity (Simpson, 1974; Flantua *et al.*, 2014; Flantua & Hooghiemstra, 2018), presenting a highly dynamic and rapidly flickering connectivity landscape over the last 1 Myr (Flantua & Hooghiemstra, 2018). In general, glacial periods correspond to lowering of vegetation belts throughout the Andes and expansion of distribution ranges of many high-elevation species, while warmer interglacial periods resulted in population fragmentation, isolation and reduced available area of high-elevation grassland habitats (Simpson, 1975; van der Hammen & Cleef, 1986; Hooghiemstra *et al.*, 2006; Sklenář *et al.*, 2011). However, the inverse scenario probably occurred in the Altiplano, the high-elevation plateau covering part of the south-central Andes (Simpson, 1975), and timing of glacial advances and retreats during glacial cycles may have been somewhat different in different parts of the Andes (Zech *et al.*, 2008), reflecting the variable and complex mountain topography across the Andes.

The relative contributions of Andean orogeny and more recent Pleistocene glacial cycles in driving diversification of high-elevation Andean plants remain unclear, partly because of the difficulties associated with reconstructing the evolutionary history of rapidly diversifying clades. However, ‘next generation’ sequencing (NGS) datasets for high-elevation Andean plants are providing phylogenetic resolution in these groups for the first time (Nevado *et al.*, 2016; Uribe-Convers *et al.*, 2016; Vargas *et al.*, 2017; Contreras-Ortiz *et al.*, 2018), and recent studies suggest that interspecific gene flow may have occurred in some Andean

radiations (Kolar *et al.*, 2016; Duskova *et al.*, 2017; Vargas *et al.*, 2017; Pouchon *et al.*, 2018). While these reports are at odds with a strict allopatric speciation scenario driven by Andean orogeny, thus far the timing of species divergence and hybridization, and the effect of gene flow between species at a genome-wide scale have not been studied. In this work, we address this gap by focusing on one of the most prominent high-elevation Andean plant radiations, for which the largest genome-scale datasets are available – the legume genus *Lupinus* (Hughes & Eastwood, 2006; Drummond *et al.*, 2012).

The Andean clade of *Lupinus* comprises *c.* 85 species with an estimated crown age of 1.19–3.5 Myr (Drummond, 2008; Drummond *et al.*, 2012) and presents one of the highest documented rates of net species diversification in plants (Hughes & Eastwood, 2006; Drummond *et al.*, 2012). The majority of Andean *Lupinus* species diversity occurs in the high-elevation grasslands above 3200 m from Venezuela to northern Argentina, with only a few species found at lower elevations. The substantial variation in plant growth forms (Hughes & Eastwood, 2006; Hughes & Atchison, 2015; Contreras-Ortiz *et al.*, 2018) shows a clear correlation with elevation, with annuals at lower elevations below 3000 m, and a wide range of perennials mainly above 3000 m – including woody shrubs of various dimensions, small treelets to 6 m height, and dwarf prostrate mat-forming and acaulescent or stem rosette species. The Andean *Lupinus* species have a uniform $2n=48$ chromosome number (Conterato & Schifino-Wittmann, 2006), with no evidence that polyploidy played any role in the diversification of species in this clade. We have previously shown that adaptive evolution in this clade is widespread (Nevado *et al.*, 2016), and presented evidence to suggest both geographical and adaptive components of this radiation (Contreras-Ortiz *et al.*, 2018). However, the relative importance of purely geographical (i.e. nonadaptive) vs adaptive axes in the rapid diversification of Andean lupins, has yet to be resolved (Givnish, 2015; Nevado *et al.*, 2016; Contreras-Ortiz *et al.*, 2018).

To investigate the evolutionary processes underpinning Andean *Lupinus* diversification, we analyse the patterns of genetic diversity and divergence of multiple species and populations using genomic and transcriptomic datasets. We test whether diversification in this system proceeded without gene flow between species – as expected if geographic isolation is the main driver of diversification – or if diversification was accompanied by gene flow – in which case processes other than geographic isolation and allopatric speciation would be inferred. Our results support several episodes of gene flow between different *Lupinus* species, including very recent events of gene flow likely to have been prompted by changes to habitat connectivity during Late Pleistocene glacial cycles. Importantly, gene flow between species occurs heterogeneously across the genome – with gene flow restricted to some regions of the genome – which implies ecological divergence as an important process driving (or maintaining) species diversity. Taken together, these results suggest that rapid diversification in Andean *Lupinus* is at least partly a result of ecological speciation, and that Pleistocene glacial cycles played an important role in diversification in this system.

Materials and Methods

Analysis of phylogenetic incongruence

To infer to what extent gene flow between species has occurred during the diversification of Andean *Lupinus* we analysed the 6013 orthologous genes previously identified by Nevado *et al.* (2016). These genes were obtained by RNAseq and a clustering and phylogeny-based orthology inference approach as detailed in Nevado *et al.* (2016). We focused on the wild Andean species – that is, excluding the domesticated *Lupinus mutabilis* – and sampled 25 species, and used the Mexican lupin *Lupinus campestris* as an outgroup (Supporting Information Table S1). As positive selection was shown to be widespread in this clade (Nevado *et al.*, 2016), we considered only fourfold degenerate sites from each gene, and reduced the dataset by excluding genes with fewer than four phylogenetically informative sites and <10 species sequenced.

This dataset was used to infer whether a phylogenetic tree (without hybridization events) or a phylogenetic network (with one or several hybridization events) better describe the evolutionary history of Andean *Lupinus* using Species Networks applying Quartets (SNaQ) (Solís-Lemus & Ané, 2016) implemented in the software PHYLONETWORKS (Solís-Lemus *et al.*, 2017). In this approach, phylogenetic trees from multiple loci are used to estimate quartet concordance factors (CFs), that is the proportion of genes that support each possible relationship between each set of four species. These CFs are then used to reconstruct phylogenetic networks under incomplete lineage sorting (ILS) and a different number of hybridization events and to calculate their respective pseudolikelihoods. We used RAXML v.8 (Stamatakis, 2014) to obtain gene trees with bootstrap support for each gene (with the GTR+GAMMA model and 100 bootstrap replicates), and used these trees to generate a species tree with ASTRAL (Mirarab & Warnow, 2015), and estimate CFs with PHYLONETWORKS (function readTrees2CF). Using the phylogeny obtained with ASTRAL as a starting tree, we then estimated the best phylogenetic network with varying number of hybridization events allowed (h between 0 and 4) using the function snaq! in PHYLONETWORKS. To ensure convergence we performed 12 independent runs under each value of h . To infer the support for the best network identified, we analysed 100 bootstrap replicates, each performing 10 independent searches starting from the best network identified previously, and summarized the results with PHYLONETWORKS.

As the SNaQ approach estimates pseudolikelihoods, we cannot readily compare the fit of models with different number of hybridization events. Instead, we used the log pseudolikelihood profile with increasing number of hybridization events: pseudolikelihood is expected to increase sharply with h until it reaches the optimal value, and then to increase more slowly with increasing h (Solís-Lemus & Ané, 2016). In addition, we compared the fit of the observed CFs with those expected under a phylogenetic tree without hybridization (obtained with ASTRAL) and those expected under the best phylogenetic network with a single hybridization event (obtained with SNaQ), using the TCR test (Stenz *et al.*, 2015) with the function 'test.one.species.tree' from

the R package PHYLOM (Ho & Ané, 2014). This test calculates, for each quartet of species, a P -value that expresses how unlikely it is that the CFs observed have arisen under a model allowing for ILS alone. The P -values calculated for each quartet are then binned using four categories ($P < 0.01$, $0.01–0.05$, $0.05–0.1$, $0.1–1$), and this binned distribution is compared with the expected values of 0.01, 0.04, 0.05 and 0.9 using a chi-squared test with 3 df.

Demographic modelling of divergence events

To infer the demographic history of divergence between species, we analysed nextera-tagmented reductively-amplified DNA (nextRAD) data from two pairs of robustly supported sister lineages from the northern Andes, obtained with the nextRAD approach as detailed in Contreras-Ortiz *et al.* (2018). First, we analysed two high-elevation acaulescent rosette-forming sister species with disjunct distributions: *Lupinus alopecuroides*, restricted to the central cordillera of Colombia and the contiguous ranges in north-central Ecuador, between 3600 and 4700 m elevation; and *Lupinus triananus*, which is endemic to the southern páramos of the eastern Cordillera of Colombia, between 3300 and 3700 m (Fig. 1). These two species are geographically isolated by the deep Magdalena Valley separating these two Cordilleras. Second, we analysed two robustly supported geographically disjunct sister lineages (here referred to as *Ocetá* and *Pisba/Cocuy*), within an as yet undescribed acaulescent rosette-forming species of *Lupinus* (Contreras-Ortiz *et al.*, 2018) from the geographically adjacent páramos of Pisba/Cocuy and Ocetá in the northern part of the eastern Cordillera of Colombia (Contreras-Ortiz *et al.*, 2018). This dataset included between 14 and 17 individuals from each lineage, sequenced from between two and four locations in the Andes in Colombia and Northern Ecuador (Fig. 1).

The raw Illumina sequences from Contreras-Ortiz *et al.* (2018) were trimmed with CUTADAPT v.1.8 (Martin, 2011) to remove adaptors and low-quality ends (Phred score below 20), and the resulting trimmed reads were checked with FASTQC v.0.11 (Andrews, 2010). Trimmed reads were mapped to a draft genome assembly of the Andean crop lupin, *L. mutabilis* (B. Nevado *et al.*, unpublished) using BWA v.0.7 (Li & Durbin, 2010) with the mem algorithm and default settings. Reads with mapping quality below 20 were excluded, and the package STACKS v.1.42 (Catchen *et al.*, 2013) was used to identify nextRAD loci along the genome. Individual genotypes with a minimum stack depth of 10 per individual ($-m$ 10) and a minimum of two species represented at each locus ($-p$ 2) were inferred in STACKS. For subsequent analyses, we kept a single representative locus for each overlapping site ($-ordered_export$) and a single random single nucleotide polymorphism (SNP) from each locus ($-write_random_snp$). Population structure within each species was investigated with FASTSTRUCTURE v.1.0 (Raj *et al.*, 2014) assuming a varying number of clusters (1–5), while an analysis of molecular variance within each species was performed in ARLEQUIN v.3.5 (Excoffier & Lischer, 2010).

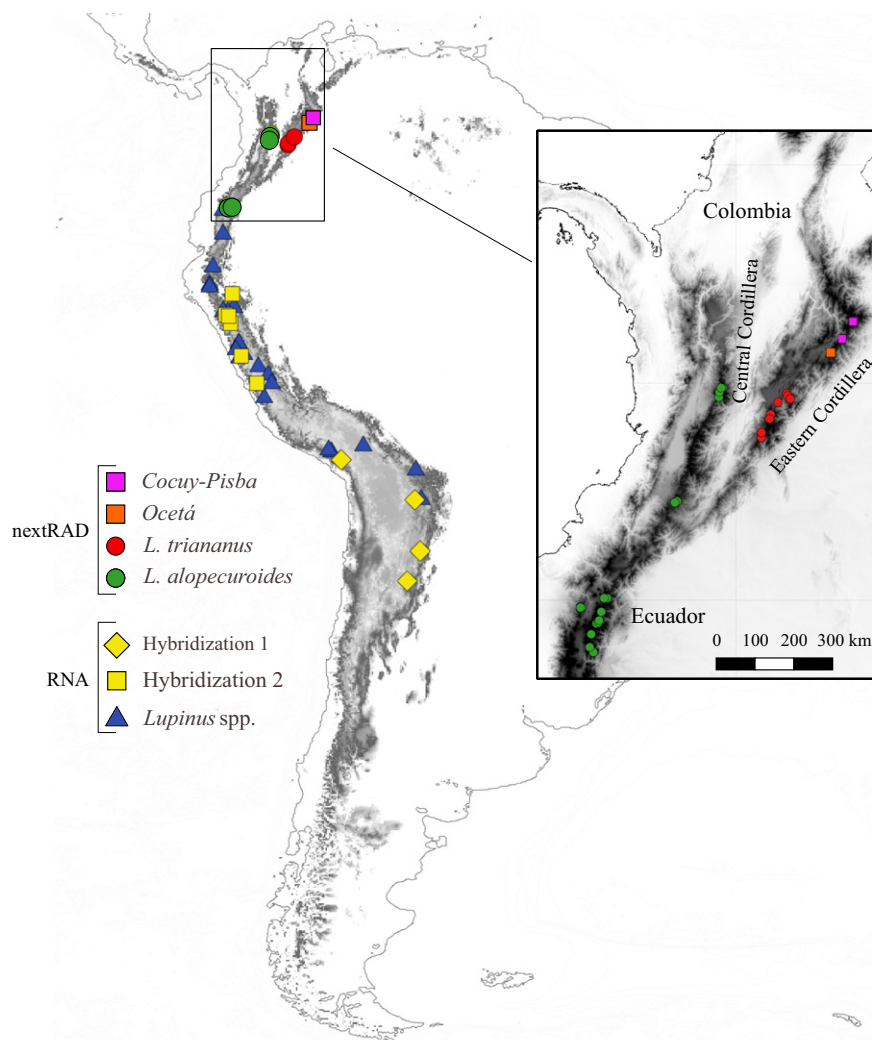


Fig. 1 Map of South America showing the sampling locations of the *Lupinus* specimens used in this study. For the RNA dataset, species putatively involved in the two hybridization events detected (see the Results section) are denoted with yellow diamonds and squares, and the remaining species with blue triangles. Species putatively involved in hybridization event 1: the ancestor of *L. sp. bangii* S and *L. subacaulis*; *L. misticola* and *L. sp. GA13*. Species putatively involved in hybridization event 2: the ancestor of *L. semperflorens* and *L. ramosissimus*; *L. ballianus*; the ancestor of *L. mantaroensis*, *L. sp. bangii* N and *L. sp. CEH3079A1*. Map produced with R package MARMAP. Inset: map of the northern Andes showing the geographically isolated distributions of *L. alopecuroides* and *L. triananus* at high elevations on the Eastern and Central Cordilleras, and the geographically more proximal Páramos of Cocuy-Pisba and Ocetá in Colombia for which detailed demographic analyses of gene flow were carried out. Data points are georeferenced herbarium specimen records. The map was produced with QGIS.

To infer the demographic history of divergence of each lineage pair, we analysed their joint site-frequency-spectrum (SFS) under several models of isolation with migration (IM), including previously published (Tine *et al.*, 2014; Filatov *et al.*, 2016) and newly implemented models (Table 3), in the program $\partial A\partial I$ v.1.7 (Gutenkunst *et al.*, 2009). Given the large geographic distance between sampling localities of *L. alopecuroides* (Fig. 1) and that only two individuals of these species were sampled from the two most southern localities, we excluded these two individuals from demographic inference. In the analysis, we used only SNPs that showed no sign of being under selection. To identify nextRAD loci putatively under selection, we used BAYESCAN v.2.1 (Foll & Gaggiotti, 2008; Fischer *et al.*, 2011) running 50 000 iterations as burn-in and 50 000 iterations thereafter. The demographic history reconstruction was based on the so-called 'unfolded' SFS – that is, only the SNPs with known ancestral states were used. The ancestral state of each SNP was reconstructed in a comparison with up to six outgroups, all of them New World lupin species (*L. albifrons*, *L. huaronensis*, *L. luteolus*, *L. mutabilis*, *L. nanus* and *L. nevadensis*), for which low-coverage genomic data are available (B. Nevado *et al.*, unpublished). Only SNPs with alleles scored in

at least two outgroup species, and where all outgroup species were homozygous for one of the two alleles seen in the nextRAD loci, were considered further. To deal with missing data we 'projected' the SFS down to 16 alleles (for 'Ocetá' – 'Pisba/Cocuy' analysis) and to 14 alleles (*L. alopecuroides* – *L. triananus*). This means that all SNPs with < 14 (or 16) alleles scored in each species were removed, and SNPs with > 14 (or 16) alleles were subsampled to this number of alleles. To confirm the convergence of analyses in $\partial A\partial I$, for each species pair we performed 20 runs from random starting points using large search ranges for all parameters. After confirming that most runs returned similar results for all parameters, we performed another set of 20 runs for each pairwise comparison with shorter search ranges around the optimal values identified before. Python code of the IM models used in this study is available from <https://github.com/brunonevado/IM-models>.

Results

To test whether rapid diversification of the Andean *Lupinus* has been primarily driven by geographic isolation, and to understand

the possible impacts of Pleistocene climatic oscillations on diversification in this group, we used two complementary analyses: analysis of phylogenetic incongruence across the phylogeny of Andean lupins; and demographic modelling of divergence events.

Analysis of phylogenetic incongruence

Analysis of phylogenetic incongruence relies on comparison of phylogenetic trees from multiple loci across the genome. To obtain such trees we analysed the 6013 orthologous genes previously identified (Nevado *et al.*, 2016). After extracting all fourfold degenerate sites from each orthologous gene, and excluding genes with <10 Andean species and <four phylogenetically informative sites, we retained 867 genes for analysis of phylogenetic incongruence. The CFs estimated from phylogenetic trees obtained from these genes were used to estimate phylogenetic networks with different numbers of hybridization events (h), and to calculate their respective pseudolikelihood. The pseudolikelihood increased sharply from $h=0$ to $h=2$, while increasing the number of hybridization events past two resulted in smaller improvements in pseudolikelihood values (Fig. 2). This suggests that the best-fitting phylogenetic model involved two hybridization events.

Analysis of the resulting phylogenetic network with $h=2$ (Fig. 3) showed that this network is similar to the coalescent-based phylogeny reported previously (Nevado *et al.*, 2016), with the following differences: the sister-group relationship between the three most derived clades changed (the sister clade to clade6 is now clade4, instead of clade5 as before), species relationships between members of clade 4 changed, and the second accession of *L. misticola*, previously resolved within clade 4, is now resolved as basal in clade 6. In addition, the phylogenetic network obtained herein received lower bootstrap support than our previous phylogenetic reconstruction (Nevado *et al.*, 2016). This is

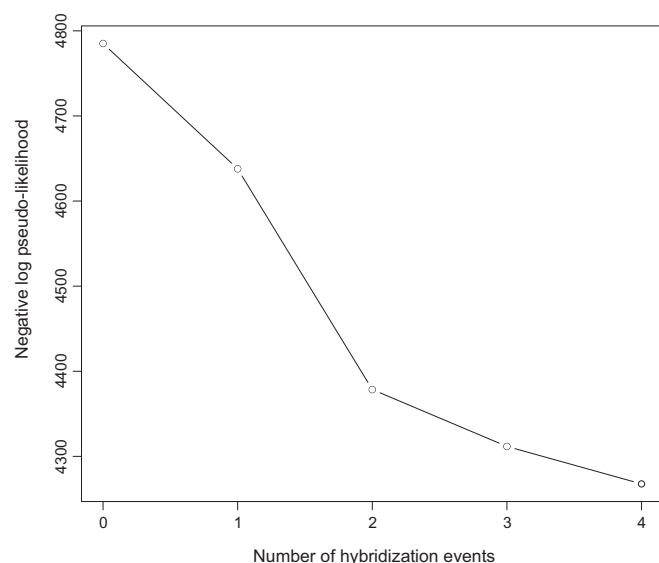


Fig. 2 Pseudolikelihood profile with increasing number of hybridization events allowed, obtained with the Species Networks applying Quartets (SNAQ) pipeline.

probably a result of inclusion in this study of only fourfold degenerate sites from 867 genes, while our previous work used the entire coding sequences of all 6013 genes. Reconstructing the phylogenetic history of very young and rapidly diversifying species is notoriously difficult, yet the high degree of congruence between the phylogenies obtained here and in our previous work supports our phylogenetic reconstruction, and suggests strong phylogenetic signal is present in the dataset.

One of the hybridization events detected occurred within clade 5, suggesting that *c.* 9% of genetic material in the ancestor of *L. bangii* S and *L. subacaulis* originated from the lineage leading to *L. misticola* (Fig. 3). This event was well supported in the bootstrap analysis, with 85% of bootstrap replicates identifying the same donor and recipient clades, with a mean contribution of genetic material into the ancestor of *L. bangii* and *L. subacaulis* of 6.7% (max = 18.7, min = 3.8, SD = 2.1).

The second hybridization event that was detected involved members of clades 3 and 4, with the ancestor of *L. semperflorens* and *L. ramosissimus* resolved as a hybrid clade with approximately equal contributions from both lineages (Fig. 3). This hybridization event was not well supported in the bootstrap analysis, with only 8% of bootstraps recovering the same two clades as being involved in the hybridization event. Despite this uncertainty over the exact placement of the hybridization event of the tree, the clade consisting of *L. semperflorens* and *L. ramosissimus* was identified as of hybrid origin in 69% of the bootstrap replicates, although the contributing lineages differed between replicates: the most frequently found clades involved in this hybridization were *L. ballianus* (44% of replicates), the ancestor of *L. ballianus* and *L. praestabilis* (20% of replicates), *Lupinus* sp. CEH3079A1 (17%) and the ancestor of *Lupinus* sp. CEH3079A1, *L. bangii* N and *L. mantaroensis* (16%).

To further test whether the evolutionary history of Andean lupins is better represented by a phylogenetic network or a phylogenetic tree, we used the Tree Incongruence Checking in R (TICR) method. This test uses the CFs observed across the entire Andean clade, and revealed a significant excess of outlier quartets with highly significant values ($P=1.316508\text{e-}08$), showing that the observed CFs are very unlikely to have arisen under ILS alone.

Demographic modelling of divergence events

To reconstruct demographic history of speciation in the Andean *Lupinus* we compiled population-level nextRAD datasets for two pairs of sister lineages from the northern Andes (Contreras-Ortiz *et al.*, 2018). Preprocessing of raw illumina reads resulted in a very low percentage of reads lost as a result of low quality (average 0.6%, maximum 1.37%). Using the draft genome of *L. mutabilis* as the reference, the percentage of reads mapped averaged 88%, with only two samples having <80% of reads mapped. The STACKS pipeline identified 22 936 variable loci present in at least two species, and 5435–11 689 polymorphic sites in each lineage analysed (Table 1). Nucleotide diversity per site within each lineage, estimated by STACKS across all loci sequenced (Hohenlohe *et al.*, 2010), was between 0.0004 and 0.0007 (Table 1).

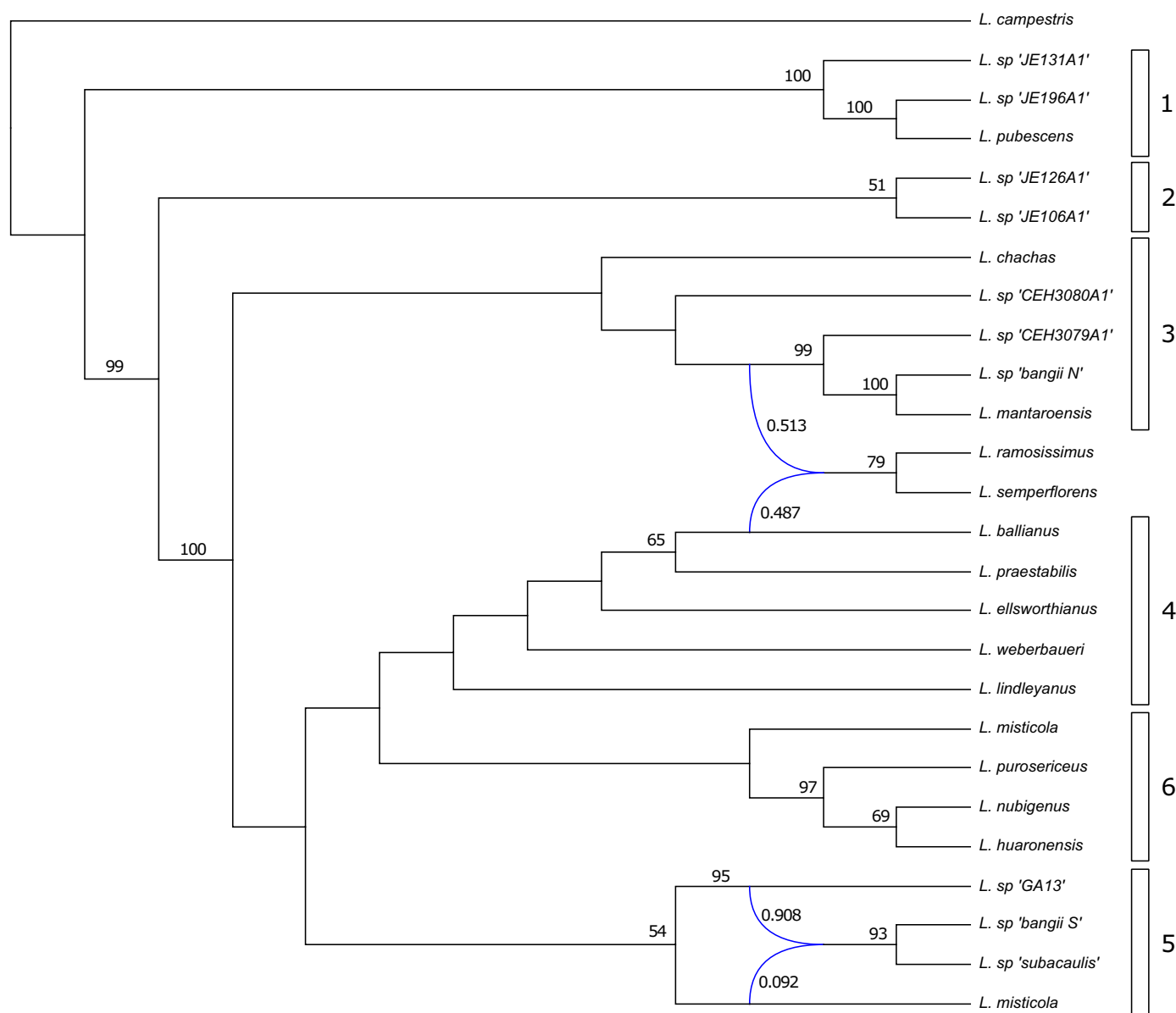


Fig. 3 Phylogenetic network estimated with the Species Networks applying Quartets (SNaQ) pipeline with two hybridization events. The blue edges denote the hybridization events identified, with numbers next to the edges denoting the proportion of genes that were transferred from each lineage. Bootstrap support for nonhybrid edges are plotted above corresponding edges (not shown when support is below 50). Clades are numbered as mentioned in the text.

The analysis of population structure with FASTSTRUCTURE revealed that the number of clusters that maximized the marginal likelihood was one for *Pisba/Cocuy* and *Ocetá*, two for *L. triananus* and three for *L. alopecuroides* (Fig. S1). Analysis of molecular variance (AMOVA) accordingly revealed that the percentage of variation between localities was higher in *L. alopecuroides* and *L. triananus* than in *Pisba/Cocuy* and *Ocetá* (Table 2). Using low-coverage resequencing data from six other Andean lupins, we confirmed the ancestral allele in 15 869 of the 22 936 SNPs obtained with STACKS. BAYESCAN analysis of selection identified 60 loci with significant traces of selection (FDR $q < 0.05$), with all loci exhibiting low F_{st} and negative alpha parameter, indicative of purifying or balancing selection. We removed these 60 loci before demographic modelling in $\partial A\partial I$.

To infer the most likely demographic history of divergence for each lineage pair we investigated 12 models (Table 3; Fig. 4) chosen to represent a range of plausible and relevant demographic scenarios underpinning divergence in Andean *Lupinus*, and particularly the extent and timing of gene flow between diverging lineages. All scenarios are based on the IM model, in which two populations split at some point in the past (T) after which gene flow between populations (M_{12} and M_{21}) was restricted. The key difference between the scenarios is how migration between diverging populations is modelled. For instance, in the simplest model (isolation) gene flow between diverging species does not occur after splitting ($M_{12} = M_{21} = 0$). More complex models allow for asymmetric migration between populations ($M_{12} \neq M_{21}$, e.g. IM or IMpre models), symmetric but time-dependent migration

Table 1 Number of polymorphic sites (*S*), and nucleotide diversity per site (*n*) estimated in STACKS using all nextRAD loci identified in each species

Population ID	<i>N</i>	<i>S</i>	<i>n</i>
<i>Lupinus alopecuroides</i>	14	5435	0.0004
<i>Lupinus osetá</i>	15	11 689	0.0007
<i>Lupinus pisba-cocuy</i>	15	11 082	0.0006
<i>Lupinus trianaus</i>	17	10 871	0.0007

Table 2 Analysis of molecular variance showing the distribution of variance within each species

Source of variation	df	Sum of squares	Variance components	Percentage of variation
<i>Lupinus alopecuroides</i> between localities	3	1133.075	52.150 96	20.77
<i>L. alopecuroides</i> within localities	24	4773.318	198.888 26	79.23
<i>Lupinus osetá</i> between localities	1	315.124	4.016 17	1.32
<i>L. osetá</i> within localities	28	8403.643	300.130 10	98.68
<i>Lupinus pisba-cocuy</i> between localities	1	364.933	15.202 17	4.70
<i>L. pisba-cocuy</i> within localities	28	8629.000	308.178 57	95.30
<i>Lupinus trianaus</i> between localities	3	1833.478	60.289 13	17.00
<i>L. trianaus</i> within localities	30	8830.375	294.345 83	83.00

between populations (e.g. splitExpMig, eSplitExpMig), migration occurring at different rates in different loci (M_A and M_B , e.g. IM2M) or times (e.g. ancestral migration (AM), secondary contact (SC)), or a combination of these (Table 3; Fig. 4). In addition, some models allow for population size changes during and after splitting (e.g. IM, IMpre), while others assume constant population size throughout (e.g. AM, SC).

Maximum-likelihood parameter estimates for each model, and their respective likelihoods, are given in Table 4. Because not all models are nested, we first used the Akaike's information criterion (AIC) to rank the different models. This approach assumes that the different SNPs are unlinked, which is a reasonable assumption given that we used a single SNP per nextRAD locus. The best-fitting demographic model for the split between 'Osetá' and 'Pisba/Cocuy' was the IM2MSC, and for *L. alopecuroides* and *L. trianaus* it was the IM2M (Table 4). In both cases all alternative models received considerably less support ($\Delta\text{AIC} > 3$). The best-fitting models resulted in a reasonable fit to the observed two-dimensional SFS in both lineage pairs (Fig. 5), although there was a slight deficit of high-frequency variants in *L. trianaus* and medium- to high-frequency variants in *L. alopecuroides*. For both divergence events investigated, demographic modelling supports a scenario of postdivergence gene flow with two classes of sites having very different amounts of gene flow (Table 4).

For the split between *Osetá* and *Pisba/Cocuy*, the best-fitting model was the IM2MSC, in which gene flow occurs after an

initial period of complete isolation. To formally test that a period of secondary contact results in a significantly better fit than a model with constant gene flow after initial divergence, we performed a likelihood ratio test (LRT) between the full IM2MSC model and a simpler, nested model. In the simpler model, the time period following divergence, during which there is no gene flow (T_A in Fig. 4), was fixed at 0, and LRT between models was performed assuming 0.5 degrees of freedom. Given that $\partial\text{A}\partial\text{I}$ uses composite likelihoods to approximate the full likelihood of each model, LRT *P*-values were adjusted using the Godambe function (Coffman *et al.*, 2016), with the more complex IM2MSC being significantly better supported ($P = 0.023$). Similarly, a simpler nested model in which migration occurred homogeneously across the genome (i.e. with M_A fixed to 0; Fig. 4) could also be rejected in favour of the full IM2MSC model ($P = 0.022$).

For the split between *L. alopecuroides* and *L. trianaus*, the best-fitting model was IM2M. This scenario implies gene flow between species after the initial split, but without an initial period of complete isolation. Nevertheless, demographic models including periods of secondary contact usually provided a better fit than comparable models assuming older migration, in particular: SC vs AM models ($\log\text{Lik} = -497.80$ vs -505.78 , respectively) and IM2MSC vs IM2MAM ($\log\text{Lik} = -312.73$ vs -327.52). An LRT between the full IM2M model and a simpler model assuming a single migration value for all sites (M_A fixed to 0, performed as described earlier) provided significant support for the more complex model ($P = 0.004$), confirming that gene flow between species was heterogeneous across the genome.

Having found the best-fitting demographic model for each split, it is also possible to estimate the absolute times of divergence in each case. The split times estimated in $\partial\text{A}\partial\text{I}$ are in units of $2 \times N_A$ generations, with N_A representing the effective population size of the ancestral population before the split. We also know, from the parameter estimates of the best-fitting model in each species, the relative sizes of current populations compared with their ancestral population sizes (N_1 and N_2 in Table 4). Thus, to obtain absolute estimates for speciation times, we need estimates of current population sizes and generation times of each species. If we use a mutation rate of 7.0×10^{-9} per generation per site, which is similar to the available estimates in plants (*Arabidopsis thaliana* (Ossowski *et al.*, 2010) and *Prunus* (Xie *et al.*, 2016)), to convert the genome-wide estimates of nucleotide diversity of each species (Table 1) into effective population sizes, we get estimates of current $N_e = 14\,285$ (for *L. alopecuroides*), $N_e = 21\,428$ (for *Pisba/Cocuy*) and $N_e = 25\,000$ (for *Osetá* and *L. trianaus*). From these current population sizes, we can estimate the ancestral size of each species pair using the relative population size parameters (N_1 and N_2 in Table 4), which gives average values of $N_A = 15\,458$ (for *L. alopecuroides* and *L. trianaus*) and $N_A = 7673$ (for *Osetá* and *Pisba/Cocuy*). We can now convert the split times (Table 4) into number of generations using the estimated ancestral population sizes: for *L. alopecuroides* and *L. trianaus* this would be $2 \times N_A \times T = 2 \times 15\,458 \times 1.725 = 53\,330$ generations ago; and for *Osetá* and *Pisba/Cocuy* it would be $2 \times N_A \times (T_A + T_S) = 2 \times$

Table 3 The demographic models investigated in this study

Model	Description	Source
Isolation	Model without migration after population split	Default model in $\partial A\partial I$
IM	Isolation-with-migration model with asymmetric migrations and exponential population growth	Default model in $\partial A\partial I$
IMpre	IM model with a size change before split	Default model in $\partial A\partial I$
splitExpMigr	Constant population size and exponentially changing symmetric migration after split	Filatov <i>et al.</i> (2016)
eSplitExpMigr	Exponential population growth with exponentially changing symmetric migration after split	This study, modified from Filatov <i>et al.</i> (2016)
AM	Ancient migration model, asymmetric migration persists for some time after initial split, constant population sizes after split	Tine <i>et al.</i> (2014)
SC	Secondary contact model, no migration immediately after split but includes a recent period of asymmetric migration. Constant population sizes after split	Tine <i>et al.</i> (2014)
AM2M	Ancient migration model with two classes of loci experiencing different and asymmetric migration rates. Constant population sizes after split	Tine <i>et al.</i> (2014)
SC2M	Secondary contact model with two classes of loci experiencing different and asymmetric migration rates. Constant population size after split	Tine <i>et al.</i> (2014)
IM2M	The isolation-with-migration model with two classes of sites experiencing different and symmetric migration rates. Exponential population growth after split	This study, modified from Tine <i>et al.</i> (2014)
IM2MSC	The isolation-with-migration model with two classes of sites experiencing different and symmetric migration rates and a period of no migration after split. Exponential population growth after split	This study, modified from Tine <i>et al.</i> (2014)
IM2MAM	The isolation-with-migration model with two classes of sites experiencing different and symmetric migration rates, migration persists for some time after split. Exponential population growth after split	This study, modified from Tine <i>et al.</i> (2014)

$7673 \times (0.72 + 0.95) = 25\,628$ generations ago. Definitive information about generation times for these species is lacking, but these species are certainly not annuals and are likely to start flowering only in the second year (N. Contreras, pers. obs.). Using a 2 yr generation time we can thus estimate that *L. alopecuroides* and *L. trianaus* started to diverge *c.* 106 000 yr ago (ka), and *Oceta* and *Pisba/Cocuy* *c.* 51 ka. With the same assumptions, secondary contact between *Oceta* and *Pisba/Cocuy* can be dated to *c.* 29 ka.

Discussion

While it has been suggested that geographic isolation may be the predominant driver of rapid diversification in Andean *Lupinus* (Givnish, 2015), our results suggest that gene flow after speciation may be common in this system. We show that interspecific gene flow resulted in clades of hybrid origin, that gene flow occurred in the recent evolutionary past (following recent divergence events *c.* 51–106 ka), and that gene flow has occurred at disparate localities across the Andes (Fig. 1). Furthermore, our results are minimum estimates for the number of hybridization events occurring during diversification of Andean *Lupinus*. In fact, our taxonomic sampling included only 25 species, that is <30% of the estimated 85 species of Andean lupins. Increasing taxonomic sampling may lead to detection of additional cases of hybridization. Also, the analysis of phylogenetic incongruence with SNaQ can only detect a restricted subset of hybridization events (Solís-Lemus & Ané, 2016): hybridization events between sister lineages can only be detected if both lineages have at least two descendant species;

and only level-1 networks are considered – that is, each hybrid node can only be part of a single hybridization event. As such, the two hybridization events identified by analysis of phylogenetic incongruence are most likely an underestimate of the true amount of gene flow among Andean lupins. In this regard, it is noteworthy that our demographic modelling detected recent episodes of postsplit gene flow associated with both lineage pairs analysed. These results are in line with recent studies suggesting that gene flow may have occurred between currently geographically isolated Andean cordilleras (Kolar *et al.*, 2016) and that hybridization may have played an important role in other Andean plant radiations (Duskova *et al.*, 2017; Vargava *et al.*, 2017; Pouchon *et al.*, 2018).

Hybridization in Andean *Lupinus*

The finding that hybridization might be common in Andean *Lupinus* is surprising because it is likely that both pollen and seed long-distance dispersal are limited. Although there are no data on seed dispersal and few observations of pollinators for Andean *Lupinus*, the dispersal mechanism in almost all species in the genus is ballistic dispersal – the seed pods dry and explosively dehisce, ejecting the seeds at most a few metres away from the parent plants (e.g. 0–4 m for the North American species *L. texensis*; Turner *et al.*, 2017). Furthermore, the seeds of wild Andean lupins are relatively large and smooth and not easily transported by wind, while pollination is typically dependent on bees (Langridge & Goodman, 1977), which are also infrequent in the high-elevation Andes.

In addition to low dispersal ability, many Andean lupins are narrow endemics, or exhibit larger distribution ranges but consist

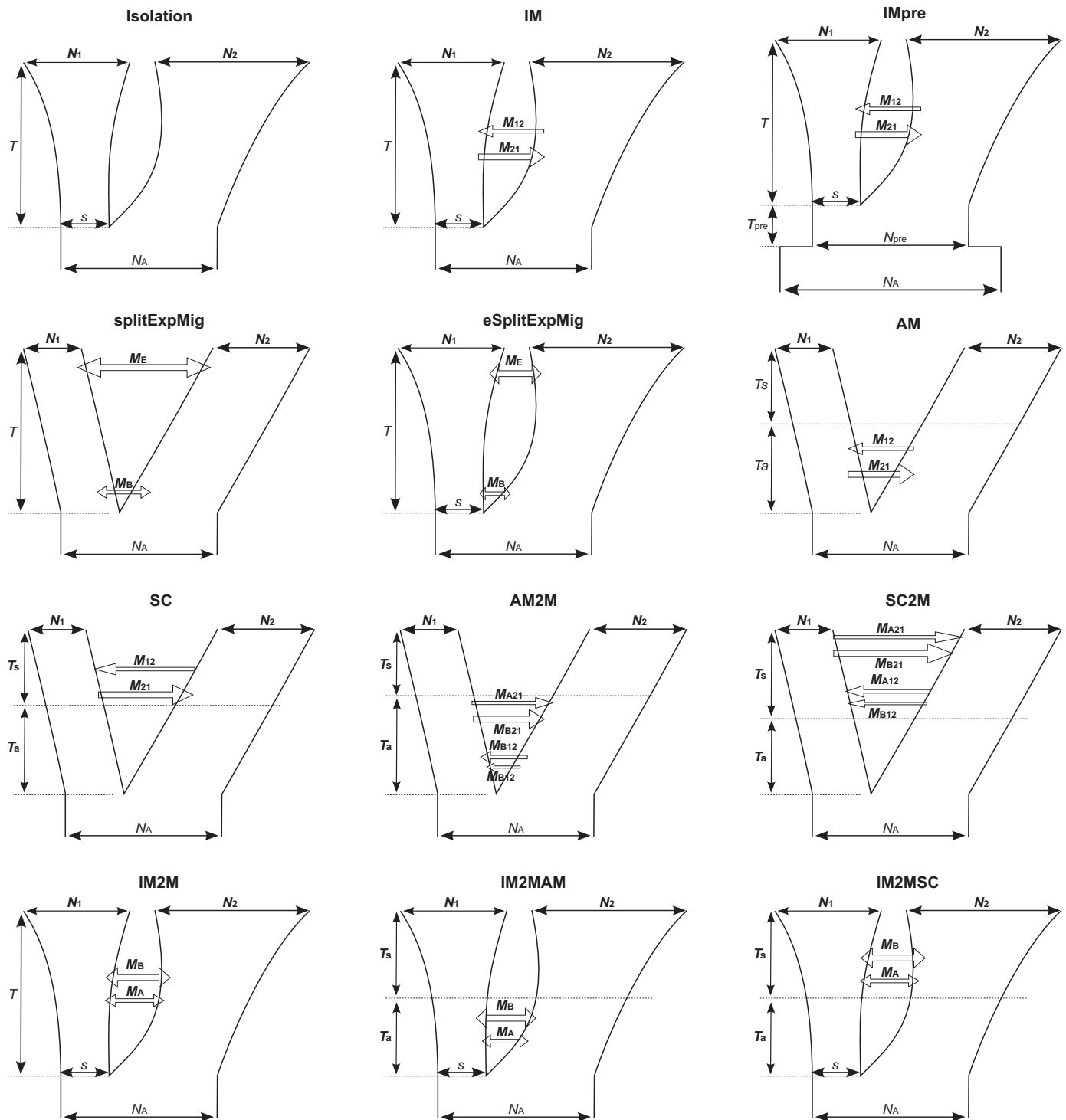


Fig. 4 Schematic representation of the demographic models investigated in this study. Models are named as in Table 3, and the free parameters in each model are depicted in bold in the respective figures. In each diagram, the top shows the two current population sizes (N_1 and N_2), and moving down (which is backwards in time) the assumed demographic history since the split. Models that allow for exponential population size changes since the split have curved lines and include the parameter s , which is the relative size of population 1 just after the split (relative size of population 2 just after the split is $1 - s$). Models that assume constant population size since the split are represented by straight lines, and if the sum of the two current population sizes ($N_1 + N_2$) is different from the ancestral size (N_A), then an instant population size change is assumed to occur during the split. For all models, N_A is the ancestral population size before the split, and is not a free parameter. All population sizes (N_1 , N_2 and N_{pre}) are expressed in units of N_A . All parameters related to time are given in units of $2 \times N_A$ generations. All migration parameters (M) are represented by horizontal arrows, and expressed in units of $2 \times N_A \times m$, where m is the proportion of the receiving population consisting of immigrants in each generation. Models AM2M, SC2M, IM2M, IM2MAM and IM2MSC assume two classes of sites which experience different migration rates (M_A and M_B), and include an extra parameter (P) not shown in the figures, and denoting the proportion of sites that experience a migration rate of M_A (proportion of sites that experience M_B is $1 - P$).

Table 4 The likelihood of each demographic model investigated in $\partial A\partial I$ for each species pair, their respective parameter estimates, Akaike information criterion (AIC) scores, Δ -AIC scores (relative to best model), and the relative likelihood of each model compared with the best model

Model	df	logLik	Parameter maximum-likelihood estimates	AIC	Δ -AIC	$L(\text{Mij})$
<i>Lupinus osetá</i> vs <i>Lupinus pisba-cocuy</i>						
Isolation	4	−639.98	$s = 0.674, N_1 = 0.899, N_2 = 0.607, T = 0.581$	1287.97	530.62	0.00
IM	6	−477.00	$s = 0.911, N_1 = 2.110, N_2 = 1.417, T = 3.074, M_{12} = 0.054, M_{21} = 0.096$	965.99	208.65	0.00
splitExpMig	5	−500.22	$N_1 = 1.563, N_2 = 0.923, T = 2.845, M_B = 0.027, M_E = 0.085$	1010.45	253.10	0.00
eSplitExpMig	6	−479.91	$s = 0.874, N_1 = 2.828, N_2 = 2.012, T = 4.960, M_B = 0.073, M_E = 0.050$	971.83	214.48	0.00
IMpre	8	−477.00	$N_{PRE} = 0.025, T_{PRE} = 0.160, s = 0.901, N_1 = 0.054, N_2 = 0.035, T = 0.076, M_{12} = 2.14, M_{21} = 3.77$	969.99	212.65	0.00
AM	6	−498.95	$N_1 = 2.344, N_2 = 1.319, M_{12} = 0.047, M_{21} = 0.060, T_A = 4.744, T_S = 0.006$	1009.90	252.55	0.00
SC	6	−497.87	$N_1 = 1.652, N_2 = 0.922, M_{12} = 0.071, M_{21} = 0.092, T_A = 1.633, T_S = 1.161$	1007.74	250.40	0.00
AM2M	9	−411.66	$N_1 = 1.191, N_2 = 0.817, M_{A12} = 0.051, M_{A21} = 2.4e-10, M_{B12} = 5.822, M_{B21} = 3.5e-5, T_A = 2.104, T_S = 0.009, P = 0.657$	841.32	83.98	0.00
SC2M	9	−390.61	$N_1 = 1.994, N_2 = 1.209, M_{A12} = 0.031, M_{A21} = 0.022, M_{B12} = 5.466, M_{B21} = 6.0e-7, T_A = 0.201, T_S = 4.010, P = 0.680$	799.22	41.88	0.00
IM2M	7	−374.26	$s = 0.911, N_1 = 1.871, N_2 = 1.362, T = 2.841, M_A = 2.958, M_B = 0.042, P = 0.196$	762.52	5.17	0.08
IM2MSC	8	−370.67	$s = 0.66, N_1 = 3.99, N_2 = 2.36, T_A = 0.72, T_S = 0.95, M_A = 0.04, M_B = 8.25, P = 0.86$	757.34	0.00	1.00
IM2MAM	8	−414.522	$s = 0.661, N_1 = 1.297, N_2 = 0.775, T_A = 1.108, T_B = 1.04e-11, M_A = 8.89e-54, M_B = 3.104, P = 0.807$	845.04	87.70	0.00
<i>Lupinus alopecuroides</i> vs <i>Lupinus triananus</i>						
Isolation	4	−610.32	$s = 0.053, N_1 = 1.280, N_2 = 1.294, T = 0.271$	1228.63	597.20	0.00
IM	6	−460.66	$s = 2.6e-4, N_1 = 1.283, N_2 = 2.912, T = 2.106, M_{12} = 0.205, M_{21} = 0.089$	933.32	301.89	0.00
splitExpMig	5	−500.64	$N_1 = 0.605, N_2 = 2.298, T = 2.215, M_B = 0.010, M_E = 0.191$	1011.28	379.85	0.00
eSplitExpMig	6	−470.29	$s = 1.471, N_1 = 3.631, N_2 = 3.534, T = 0.100, M_B = 0.111, M_E = 0.002$	952.57	321.15	0.00
IMpre	8	−460.95	$N_{PRE} = 1.945, T_{PRE} = 1.776, s = 0.001, N_1 = 1.769, N_2 = 4.162, T = 3.007, M_{12} = 0.126, M_{21} = 0.056$	937.90	306.47	0.00
AM	6	−505.78	$N_1 = 0.743, N_2 = 2.859, M_{12} = 0.116, M_{21} = 0.094, T_A = 3.341, T_S = 4.2e-4$	1023.56	392.13	0.00
SC	6	−497.80	$N_1 = 0.579, N_2 = 2.279, M_{12} = 0.179, M_{21} = 0.172, T_A = 1.542, T_S = 0.561$	1007.59	376.16	0.00
AM2M	9	−350.19	$N_1 = 1.266, N_2 = 4.845, M_{A12} = 0.007, M_{A21} = 0.035, M_{B12} = 3.6e-5, M_{B21} = 3.299, T_A = 8.610, T_S = 2.8e-4, P = 0.408$	718.38	86.95	0.00
SC2M	9	−350.46	$N_1 = 1.445, N_2 = 5.486, M_{A12} = 0.007, M_{A21} = 0.033, M_{B12} = 1.0e-13, M_{B21} = 2.551, T_A = 4.670, T_S = 5.269, P = 0.397$	718.92	87.49	0.00
IM2M	7	−308.71	$s = 1.0e-4, N_1 = 0.790, N_2 = 1.948, T = 1.725, M_A = 0.081, M_B = 5.978, P = 0.573$	631.43	0.00	1.00
IM2MSC	8	−312.73	$s = 0.005, N_1 = 2.389, N_2 = 2.535, T_A = 0.300, T_S = 1.036, M_A = 9.819, M_B = 0.077, P = 0.475$	641.46	10.03	0.01
IM2MAM	8	−327.52	$s = 0.054, N_1 = 0.578, N_2 = 2.593, T_A = 1.540, T_S = 2.7e-5, M_A = 0.061, M_B = 9.581, P = 0.639$	671.03	39.60	0.00

Best-fitting models (according to the AIC score) are denoted in bold. $L(\text{Mij})$, relative likelihood of model M_i compared to the best model; N_1 and N_2 , two current population sizes; N_A , ancestral population size before the split; N_{PRE} , size after first population size change; s , relative size of population 1 just after the split; M , migration parameter; P , proportion of sites that experience a migration rate of M_A ; T_A and T_S , duration of first and second periods after split.

of multiple small isolated populations. For instance, *L. alopecuroides* is known from only a handful of isolated mountain tops (mainly above 4000 m) in Colombia and Ecuador (Fig. 1), with microsatellite data showing a high degree of differentiation between populations (Vásquez *et al.*, 2016). Lower-altitude valleys separating these populations are probably effective barriers to gene flow. Yet, our demographic modelling shows that gene flow occurred between populations of *L. alopecuroides* and *L. triananus*, which are currently isolated on the central and eastern Cordilleras separated by the deep Magdalena valley in Colombia (Fig. 1).

Gene flow between species could result from speciation with ongoing gene flow, or secondary contact after allopatric speciation. Analysis of the fit of alternative demographic models supports the scenario of secondary contact for the divergence between *Ocetá* and *Pisba/Cocuy*, suggesting that divergence was originally driven by geographic isolation. For *L. alopecuroides* and *L. triananus*, the best-fitting model (IM2M) includes a single period of gene flow postspeciation, and although this suggests continuous gene flow since divergence, it could also reflect the difficulties in discerning the timing of gene flow after speciation.

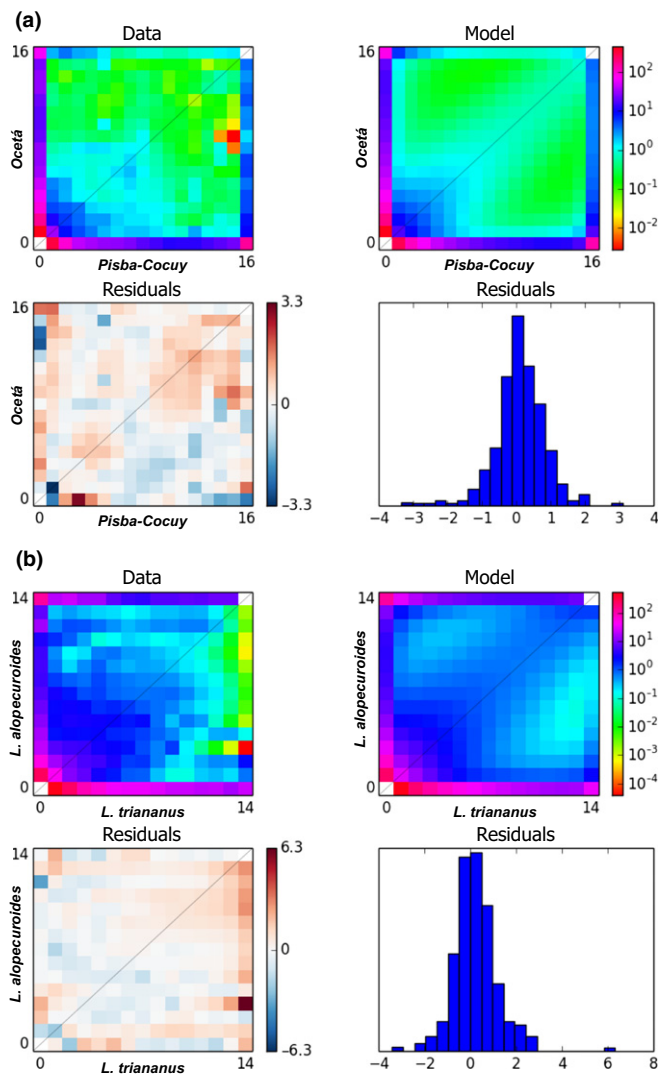


Fig. 5 Fit of the best-fitting demographic models for each species pair obtained with $\partial A\partial i$: (a) the IM2MSC model for *Pisba-Cocuy* vs *Ocetá*; and (b) the IM2M model for *Lupinus alopecuroides* vs *Lupinus triananus*. Within each panel, upper graphs show the two-dimensional site-frequency-spectrum observed (left) and expected under the best-fitting model (right); lower graphs show the residuals between the observed and the expected site-frequency-spectra.

A role for natural selection in diversification in Andean *Lupinus*

It has been suggested that the exceptionally high speciation rates estimated for recent evolutionary radiations is evidence of an ephemeral speciation model whereby speciation is very frequent, but most species rapidly become extinct or are reabsorbed into parental forms (Rosenblum *et al.*, 2012). This is a possible scenario for the high-elevation *Lupinus* species radiation, whereby many recognized extant species could be viewed as ephemeral. However, in the case of Andean *Lupinus*, our results suggest that even in the face of gene flow precipitated by secondary contact, species are not immediately reabsorbed into parental forms. Our results show that, in both pairs investigated, gene flow occurs at

two different rates, with some sites introgressing freely while others introgress at very low rates (Table 4). The proportion of sites that freely introgress between lineages is high in both cases: 43% of sites in *L. alopecuroides* and *L. triananus*, and 14% of sites in *Ocetá* and *Pisba/Cocuy*. Yet, a large part of the genome appears to be protected from introgression between species.

Heterogeneous gene flow across the genome can be explained in three different ways. First, regions that do not introgress may harbour genetic incompatibilities, which can cause hybrid inviability or sterility (Coyne & Orr, 2004). However, hybrid sterility between plant lineages accrues over much longer timescales than considered here, with most plant species studied to date showing little to no hybrid sterility within 1 Myr of divergence (Levin, 2012). Second, regions that experience no migration between species may harbour loci responsible for species-specific adaptations, with selection preventing gene flow in linked regions of the genome and leading to 'genomic islands of divergence' (Wu, 2001; Nosil *et al.*, 2009). This scenario has been extensively studied in recent years (Renaut *et al.*, 2013; Marques *et al.*, 2016; Han *et al.*, 2017; Larson *et al.*, 2017; Vijay *et al.*, 2017); however, it remains unclear in which cases the observed heterogeneity is a result of selection, drift, or variation of recombination rate across the genome (Noor & Bennett, 2009; Cruickshank & Hahn, 2014; Ravinet *et al.*, 2017). Third, heterogeneity in gene flow may result from selection acting on introgressed genes, possibly promoting introgression of advantageous genes (Staubach *et al.*, 2012; Dannemann *et al.*, 2016; Bechsgaard *et al.*, 2017).

Diversification of Andean *Lupinus* was previously found to be associated with increased rates of positive selection genome-wide (Nevado *et al.*, 2016). Our results suggest this widespread positive selection may play a role in the large species diversity of Andean *Lupinus*: without a sizeable number of genes evolving species-specific adaptations, gene flow between species would not be impeded, and the gene pools of both species could merge (Seehausen, 2006; Taylor *et al.*, 2006). Thus, while geographical isolation may initiate speciation, species differentiation in the face of changing environmental conditions is only achieved once positive selection drives fixation of different adaptive alleles in each species. This form of ecological speciation in geographic isolation is probably driven by the extreme heterogeneity of the Andes: in addition to the typical changes in temperature and insolation in mountains, the Andes are notable for dramatic differences also at smaller scales; for example, within the same mountain peak the slopes facing the inter-Andean valleys can be much drier than those facing the Amazonian forest or the Pacific Ocean (van der Hammen & Cleef, 1986). In the case of the species studied herein, *L. alopecuroides* and *L. triananus* occupy slightly different elevation ranges (3600–4700 m and 3300–3700 m, respectively), suggesting possible ecological divergence related to altitude. Morphologically, the floral bracts of *L. alopecuroides* are very long, strongly exerted and persistent such that they largely conceal the flowers even at full anthesis, while in *L. triananus* the bracts are smaller and wither quickly, and do not conceal the flowers at full anthesis. It is likely that the long bracts of *L. alopecuroides* insulate the flowers from the daily freeze–thaw regime that prevails at very high elevations in the Andes. Whether these habitat and

ecological differences are indeed responsible for divergence of these two species remains to be tested.

Isolation and gene flow driven by Pleistocene climatic oscillations

That *Lupinus* species were already present in the Andes by 2.25 Myr is evident from the pollen record (Hooghiemstra, 1983), with the age model of Torres *et al.* (2013) in line with molecular clock estimates of 1.19–3.5 Myr for the crown node of the Andean clade (Hughes & Eastwood, 2006; Drummond *et al.*, 2012). However, how many and which species were present at that time remain unknown. It is clear that many present-day species must have diverged much more recently, and in agreement with this scenario our demographic modelling suggests very young divergence times in both cases: *c.* 106 ka (*L. alopecuroides* and *L. trianatus*) and 51 kyr (*Oceta* and *Pisba/Cocuy*). While these ages must be interpreted with caution, given the large uncertainty involved, they suggest that speciation occurred long after the final uplift of the Andes and emergence of the Páramo, and is more likely to have resulted from climatic oscillations in the Late Pleistocene. In this case, the exceptionally fast speciation rates in Andean *Lupinus* might result, in part, from cyclic climatic oscillations that periodically change species' suitable habitat, promoting continuous cycles of expansion and contraction coupled with geographic isolation and secondary contact of species – a mechanism sometimes referred to as the 'species pump'.

Given their low dispersal abilities, gene flow between *Lupinus* species currently isolated by large areas of unsuitable habitat is likely to have been brought about by environmental changes that affected connectivity of the high-elevation Andean grassland habitats. In this regard, it is notable that the Last Glacial Maximum in the Andes occurred significantly earlier than in temperate zones, at *c.* 34 kyr (Smith *et al.*, 2005). At this time, with the treeline *c.* 2100 m, there would have been maximal connectivity across the north Andean high-elevation grassland habitats, including numerous closely adjacent stepping-stone areas of grasslands linking the central and eastern Cordilleras in Colombia (see fig. 12.3a in Flantua & Hooghiemstra, 2018), and potentially coinciding with the estimated period of secondary contact between *Oceta* and *Pisba/Cocuy* *c.* 29 ka.

While a role for Late Pleistocene glacial cycles in species diversification has long been suggested (Haffer, 1969; Simpson, 1974), the impacts of Pleistocene glacial cycles in different environments seems highly variable (Haffer, 1969; Hewitt, 1996; Klicka & Zink, 1997; Rull, 2011; Weir *et al.*, 2016). Diversification caused by Pleistocene sea-level fluctuations on oceanic island archipelagos appear to be mainly associated with infraspecific divergence (Papadopoulou & Knowles, 2015a,b). However, in other settings there is growing evidence for ice age or postglacial speciation across diverse organismal groups, including fish in postglacial lakes (Hudson *et al.*, 2011), birds in boreal North America (Weir & Schluter, 2004) and temperate coastal regions of New Zealand (Weir *et al.*, 2016), grasshoppers in western North American montane sky islands in the Rockies (Knowles, 2000), and plants in the high Arctic (Brochmann *et al.*, 2004).

Here we add to this growing tally of examples of recent Late Pleistocene speciation, and suggest an important role for Pleistocene glacial cycles in driving the very high rates of speciation associated with the Andean *Lupinus* radiation.

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Author contributions

B.N. and D.A.F. designed the research. B.N. and N.C-O. generated the data. B.N. carried out the research. B.N., C.H. and D.A.F. wrote the manuscript. All authors have read and agree with the manuscript. C.H. and D.A.F. are joint senior authors.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Result of clustering analysis of individuals of *L. triananus* and *L. alopecuroides*.

Table S1 Details of the *Lupinus* species used for phylogenetic analysis

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