ORIGINAL RESEARCH

A population genetics perspective on the evolutionary histories of three clonal, endemic, and dominant grass species of the Qinghai–Tibet Plateau: *Orinus* (Poaceae)

Yuping Liu[1,2,3] | AJ Harris[4] 🔘 | Qingbo Gao[5] | Xu Su[1,2,3] | Zhumei Ren[6]

[1]Key Laboratory of Medicinal Plant and Animal Resources of the Qinghai-Tibet Plateau in Qinghai Province, School of Life Science, Qinghai Normal University, Xining, China

[2]Key Laboratory of Physical Geography and Environmental Process in Qinghai Province, School of Life Science, Qinghai Normal University, Xining, China

[3]Key Laboratory of Education Ministry of Environments and Resources in the Qinghai-Tibet Plateau, School of Life Science, Qinghai Normal University, Xining, China

[4]Department of Biology, Oberlin College and Conservatory, Oberlin, Ohio

[5]Qinghai Provincial Key Laboratory of Crop Molecular Breeding, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China

[6]School of Life Science, Shanxi University, Taiyuan, China

Correspondence
Xu Su, Key Laboratory of Medicinal Plant and Animal Resources of the Qinghai-Tibet Plateau in Qinghai Province, School of Life Science, Qinghai Normal University, No. 38 Wusixi Road, Xining 810008, China.
Email: xusu8527972@126.com
and
Zhumei Ren, School of Life Science, Shanxi University, No. 92 Wucheng Road, Taiyuan 030006, China.
Email: zmren@sxu.edu.cn

Funding information
Open Project of Qinghai Provincial Key Laboratory of Crop Molecular Breeding, Grant/Award Number: 2017-ZJ-Y14; Natural Science Foundation of Qinghai Province, Grant/Award Number: 2017-ZJ-904; High-end Innovative Talents Thousands of People Plan; National Natural Science Foundation of China, Grant/Award Number: 31260052 and 41761009; 135 High-level Personnel Training Project

Abstract

We performed analyses of amplified fragment length polymorphism (AFLP) in order to characterize the evolutionary history of *Orinus* according to its population genetic structure, as well as to investigate putative hybrid origins of *O. intermedius* and to provide additional insights into relationships among species. The genus *Orinus* comprises three clonal grasses that are dominant species within xeric alpine grasslands of the Qinghai–Tibet Plateau (QTP). Here, we used eight selectively obtained primer pairs of *Eco*RI/*Mse*I to perform amplifications in 231 individuals of *Orinus* representing 48 populations and all three species. We compared our resulting data to genetic models of hybridization using a Bayesian algorithm within NewHybrids software. We determined that genetic variation in *Orinus* was 56.65% within populations while the among-species component was 30.04% using standard population genetics statistics. Nevertheless, we detected that species of *Orinus* were clustered into three highly distinct genetic groups corresponding to classic species identities. Our results suggest that there is some introgression among species. Thus, we tested explicit models of hybridization using a Bayesian approach within NewHybrids software. However, *O. intermedius* likely derives from a common ancestor with *O. kokonoricus* and is probably not the result of hybrid speciation between *O. kokonoricus* and *O. thoroldii*. We suspect that recent isolation of species of *Orinus* in allopatry via vicariance may explain the patterns in diversity that we observed, and this is corroborated

by a Mantel test that showed significant positive correlation between geographic and genetic distance ($r = 0.05$, $p < 0.05$). Recent isolation may explain why *Orinus* differs from many other clonal species by exhibiting the highest diversity within populations rather than among them.

KEYWORDS

alpine grassland, amplified fragment length polymorphism, genetic variation, hybridization, population biology

1 | INTRODUCTION

Genetic diversity is a particularly significant factor in the long-term stability of plant populations (Hedrick, 2001; Jump, Marchant, & Peñuelas, 2009; Rahimmalek, Tabatabaei, Arzani, & Etemadi, 2009; Wang et al., 2007). For example, low genetic diversity of a population may both represent critical local adaptation and, simultaneously, limit overall evolutionary potential in the face of environmental disturbances (Cortés et al., 2014; Jump et al., 2009; Sedlacek et al., 2016, 2015). Therefore, knowledge of population genetic diversity is extremely important for recognizing conservation needs and developing sustainable strategies (Gordon, Sloop, Davis, & Cushman, 2012; Kaljund & Jaaska, 2010). Conservation of species is an urgent global issue, especially within biodiversity hotspots, such as the Qinghai–Tibet Plateau (QTP) and surrounding mountainous areas, which represent some of the highest priorities within temperate zones for conservation research and implementations (Beger et al., 2015; Maréchaux, Rodrigues, & Charpentier, 2016; Myers, Mittermeier, Mittermeier, Da Fonseca, & Kent, 2000).

The biodiversity of the QTP appears to be correlated with its complex, recent history of environmental change and its present-day heterogeneous landscape. Environmental change and landscape heterogeneity are well-known drivers of biodiversity according to classic ecological theory (Risser, 1987). In the present, the QTP exhibits substantial landscape heterogeneity; for example, its elevation range is from 3,000 to 5,000 m and represents a steep ecological gradient comprising diverse niches for a rich composition of species (Feng et al., 2017; Feng et al., 2017; Liu, Luo, Li, & Gao, 2017). With respect to environmental change, the QTP has undergone extreme ecological disturbances on an evolutionary timescale, especially rapid uplifts since the Miocene–Pliocene or Miocene–Quaternary epochs and subsequent climatic oscillations in the Quaternary (Liu, 2004; Liu, Gao, Chen, & Lu, 2002; Liu, Wang, Geng, et al., 2006; Liu, Wang, Wang, Hideaki, & Abbott, 2006; Liu et al., 2018, 2015; Shi, 2002; Shi, Li, & Li, 1998; Wen, Zhang, Nie, Zhong, & Sun, 2014; Zheng & Nat, 1998). The biodiversity within the QTP is reflected within its flora, which harbors ca. 9,000 vascular plant species of which more than 18% are endemic (Wu, 2008), including at least 20 endemic genera (Wu, Yang, & Fei, 1995).

Many recent studies have sought to address evolutionary diversification of plant species within the QTP and have especially used population genetics methods to elucidate patterns of diversity and distributions and better understand the underlying mechanisms (Liu, Wang, Geng, et al., 2006; Ren, Conti, & Salamin, 2015; Wen et al., 2014). Recently, Wen et al. (2014) reviewed current evidence of mechanisms of speciation on the QTP using exemplar species within diverse vascular plant families, especially of Asteraceae, Crassulaceae, Ericaceae, Orobanchaceae, and Papaveraceae. However, the mechanisms of speciation within alpine areas of the QTP (and beyond) remain poorly understood. These mechanisms likely include allopatric processes and, possibly, rapid genetic isolation due to increased mutation rates under high levels of ultraviolet light exposure (Davies, Savolainen, Chase, Moat, & Barraclough, 2004; Madriñán, Cortés, & Richardson, 2013; Willis, Bennett, & Birks, 2009). Within the QTP, studies of many plant species are needed to serve as models for diversification and speciation patterns and processes, especially to represent the numerous habits, life histories, environmental preferences, and other features of the rich botanical diversity of the region. Such studies are particularly urgent for regions, such as the alpine grasslands (Bowman, 2000; Li et al., 2014; Yi et al., 2011), that have become imperiled during the Anthropocene (Crutzen & Stoermer, 2000) especially due to climate change and pressures from intensive grazing by livestock (Han, Brierley, Cullum, & Li, 2016; Wilcox, Sorice, & Young, 2011).

Within the alpine grasslands of the QTP, the dominant vascular plants are three endemic species comprising the entirety of the genus, *Orinus* Hitchcock (Figure 1; Poaceae; Liu et al., 2018; Su, Wu, Li, & Liu, 2015). *Orinus* consists of clonal grasses and was established in 1933 by Hitchcock based on the type species *O. arenicola* Hitchc. [=*O. thoroldii* (Stapf ex Hemsl.) Bor] collected in the Kashmir region. The genus is sister to *Cleistogenes* Keng in subtribe Orininae P. M. Peterson, Romasch. & Y. Herrera from the QTP (Peterson, Romaschenko, & Arrieta, 2016; Soreng et al., 2017). The species of *Orinus* occur especially in high-elevation, xeric areas of the QTP. Among the three species, *Orinus thoroldii* is primarily distributed in the western QTP, *O. kokonoricus* (K. S. Hao) Tzvelev occurs in the eastern QTP, and *O. intermedius* X. Su & J. Quan Liu is native to the southeastern QTP.

Orinus is especially characterized by long scaly rhizomes with numerous nodes, which serve as the basis for its clonal reproduction. It also reproduces sexually via seeds borne on sparse panicles within pedicelled and laterally compressed spikelets that have 3- to 5-veined lemmas with short awns (Su, Liu, Wu, Luo, & Liu, 2017). *Orinus thoroldii* is distinguished from *O. kokonoricus* by having pubescent leaf

FIGURE 1 Photographs showing the species of *Orinus* in their habitats: (a) *O. kokonoricus*, (b) *O. intermedius*, and (c) *O. thoroldii*

blades and dark brown or purple spikelets with two to six flowers (Su et al., 2017). Leaf blades in *O. kokonoricus* are glabrous and spikelets are yellow or white and bear one to three flowers (Su et al., 2017). In a recent taxonomic revision of the genus, Su et al. (2017) described *O. intermedius*, a new species, as most similar to *O. kokonoricus* but bearing intermediate features between *O. kokonoricus* and *O. thoroldii*, such as having caryopses and stamen of intermediate lengths. Su et al. (2017), Su et al. (2015) recognized *O. intermedius* as distinct on account of its rhizomes bearing sparse small scales compared to *O. kokonoricus* and *O. thoroldii*, which have many larger scales. However, Su et al. (2015) suspected that *O. intermedius* may have a hybrid origin with the other two species as progenitors. Nevertheless, *O. intermedius* appeared more likely to be an incompletely isolated sister of *O. kokonoricus* than a hybrid based on a population-level phylogenetic study comprising chloroplast and nuclear ribosomal internal transcribed spacer (ITS; Liu et al., 2018). At present, the putative hybrid status of *O. intermedius* remains incompletely resolved.

Orinus represents an important model for evolution and biodiversity of vascular plants within the grasslands of the QTP for several reasons. As the dominant vascular plant species within the xeric, alpine grasslands, *Orinus* can provide a representative first glimpse into evolutionary diversification and diversity within this threatened habitat type (Ma et al., 2017; Sedlacek et al., 2016; Yang et al., 2004). Moreover, few population genetics studies have targeted clonal species, which may exhibit different patterns of diversification than species that most often reproduce sexually. Finally, *Orinus* possesses an extensive system of roots and rhizomes (Cai, 2004; Su et al., 2015; Su, Yue, & Liu, 2013) that limit soil loss within the wind-swept alpine grasslands of the QTP (Figure 1; Yang et al., 2004). Thus, the diversity and diversification of the genus can also yield insights into the timing, mechanisms, and ecological consequences of regional desertification (Guo et al., 2002; Han, Fang, & Berger, 2012; see also Liu et al., 2018).

In this report, we investigated diversity and diversification in *Orinus* using analyses of amplified fragment length polymorphism (AFLP) markers. We specifically sought to address the following questions: (a) Are there three distinct species of *Orinus*, and do these exhibit recent or ongoing gene flow? and (b) Does *O. intermedius* have a hybrid origin? Additionally, we used our data to compare patterns of diversity and diversification in *Orinus* to other clonal plants, especially of alpine regions.

2 | MATERIALS AND METHODS

2.1 | Taxonomic sampling strategy and obtaining AFLPs

The AFLPs analyzed in this study were previously published in Liu et al. (2018) where they were used in a distance-based phylogenetic analysis complementary to phylogenetic reconstructions based on chloroplast and nuclear gene sequences. Here, we analyzed the AFLPs for the first time using population genetics methods and applied them to perform the first explicit test of the hybrid origin hypothesis for *O. intermedius*. Below, we describe obtaining the AFLPs,
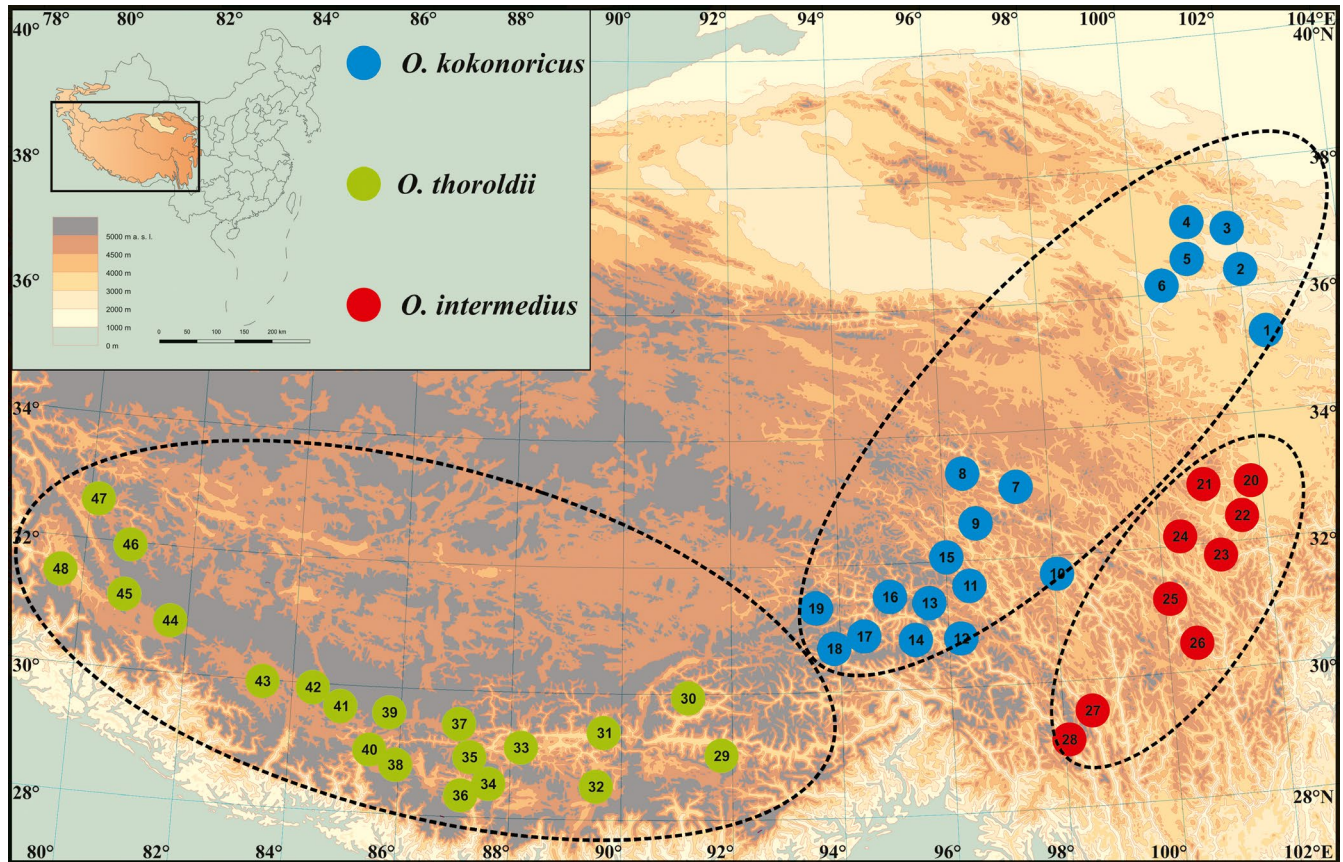
FIGURE 2 Localities of *O. thoroldii* (green), *O. kokonoricus* (blue), and *O. intermedius* (red) sampled in this study

including taxonomic sampling, in brief, and refer to our prior work for greater detail (Liu et al., 2018).

We sampled a total of 231 individuals of the genus *Orinus* from 48 natural populations from 28°21′51.0 to N and 79°48′9.0 to 102°30′59.7E representing the distributional ranges of the species and including the type localities of each (Figures 1 and 2, Table 1). As species of *Orinus* are dominant within the grasslands of the QTP, the boundaries among populations can be difficult to determine. Thus, we sampled from localities at least 30 km apart to ensure, to the best of our abilities, the genetic independence of the sampling localities except via dispersal of pollen, seeds, or propagules. Per population, we collected fresh leaf blades from three to five vegetative units spaced at least 20 m apart in order to try and sample genetically unique individuals of this clonal species. Our sampling protocol was designed to detect the diversity of genotypes within and among populations covering a vast region, especially to capture rare alleles (e.g., as in Pluess & Stöcklin, 2004), and, notably, our objectives do not include determining the abundance of clonal genotypes within populations at this time. Nevertheless, we regard our within-population sampling as preliminary and acknowledge that greater depth of sampling will yield deeper insights into some aspects of diversity and diversification in the genus in future studies. We dried the leaf samples in silica gel. For each population, voucher specimens and geolocations are reported in Liu et al. (2018).

For the AFLP analyses of all individuals, we performed DNA digestion with DNAs obtained using standard methods (Doyle & Doyle, 1987; see Liu et al., 2018) and the restriction enzymes *Pst*I and *Mse*I (40 U/µl; Beijing Dingguo Biotechnology Co., Ltd). We performed two rounds of PCR on the digestion products comprising preamplification and selective amplification (Table 2). We carried out selective amplification (Zuo, Wen, Ma, & Zhou, 2015) in 25 µl volume of reaction mixture containing of 2.0 µl *Pst*I/*Mse*I primer combinations (GAA/CAA, GAC/CAC, GAC/CAG, GAC/CTA, GAG/CAA, GAG/CAG, GAG/CTG, and GAT/CAG; Table 2). Subsequently, we separated and analyzed the fluorescently-labeled amplification products on an ABI PRISM 377 DNA Sequencer (Applied Biosystems) using GeneScan ROX-500 with an internal size standard. We scored the presence or absence of the resulting AFLP products (Figure 3) using GeneScan 3.1 (Applied Biosystems). We imported the scored data into Binthere (Garnhart, 2001) and MG (Zhou & Qian, 2003) to generate a presence/absence, or 0/1 binary, matrix (data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.403j5s4) for downstream analyses.

2.2 | Genetic diversity and population genetic structure

For each population, we calculated the average standard deviation among markers. Thus, a population with all 1s or 0s for a particular

TABLE 1 Localities for samples of *Orinus* collected for this study

Population code	Species name	Locality	N	Latitude (N)	Longitude (E)	Altitude (m)	Voucher specimens
1	*O. kokonoricus*	Xiahe, Gansu	5	35°11′9.2″	102°30′59.7″	3,007	X. Su, 11,295
2		Gonghe, Qinghai	5	36°11′3.0″	101°59′16.9″	2,826	X. Su, 12,040
3		Xining, Qinghai	5	36°37′10.8″	101°44′1.7″	2,547	X. Su, 12,042
4		Haiyan, Qinghai	5	36°50′8.3″	100°50′6.1″	3,305	X. Su, 11,005
5		Gonghe, Qinghai	5	36°6′0.5″	100°24′16.0″	2,998	X. Su, 11,016
6		Gonghe, Qinghai	5	36°2′21.8″	100°18′55.6″	3,072	X. Su, 12,038
7		Yushu, Qinghai	5	32°58′55.6″	97°14′17.6″	3,493	X. Su, 13,095
8		Nangqian, Qinghai	5	32°32′50.6″	96°11′45.2″	4,119	X. Su, 11,075
9		Nangqian, Qinghai	5	32°29′24.4″	96°16′7.5″	3,728	X. Su, 11,080
10		Jiangda, Xizang	5	31°20′20.8″	98°8′2.2″	3,818	X. Su, 12,032
11		Changdu, Xizang	5	31°15′20.0″	97°9′42.4″	3,298	X. Su, 12,025
12		Changdu, Xizang	5	31°29′36.7″	97°12′21.1″	3,354	X. Su, 12,027
13		Dingqing, Xizang	5	31°15′57.4″	95°49′57.0″	3,603	X. Su, 11,152
14		Luolong, Xizang	5	30°46′1.2″	95°34′27.7″	3,762	X. Su, 13,081
15		Leiwuqi, Xizang	4	31°45′12.8″	96°19′51.2″	3,624	X. Su, 13,090
16		Dingqing, Xizang	5	31°36′18.9″	95°6′53.8″	3,786	X. Su, 13,087
17		Bianba, Xizang	5	30°49′19.1″	94°51′30.7″	3,999	X. Su, 13,082
18		Bianba, Xizang	5	30°58′40.3″	94°43′35.3″	3,597	X. Su, 13,083
19		Biru, Xizang	5	31°31′7.8″	93°31′59.7″	3,991	X. Su, 13,085
20	*O. intermedius*	Aba, Sichuan	5	32°45′26.7″	102°3′33.8″	3,319	X. Su, 12,003
21		Banma, Qinghai	4	33°1′28.9″	100°41′52.3″	3,852	X. Su, 13,032
22		Aba, Sichuan	4	32°54′45.0″	101°46′59.3″	3,379	X. Su, 11,285
23		Aba, Sichuan	5	32°54′28.2″	101°46′25.5″	3,358	X. Su, 12,001
24		Aba, Sichuan	4	31°46′16.2″	100°58′57.1″	3,478	X. Su, 12,007
25		Luhuo, Sichuan	5	31°38′35.0″	100°17′15.9″	3,534	X. Su, 13,058
26		Daofu, Sichuan	3	30°37′17.7″	101°24′15.5″	3,573	X. Su, 12,008
27		Mangkang, Xizang	5	29°32′28.8″	98°15′18.5″	3,522	X. Su, 13,075
28		Mangkang, Xizang	4	29°32′27.2″	98°15′3.3″	3,507	X. Su, 12,016
29	*O. thoroldii*	Zhanang, Xizang	4	29°15′23.9″	91°22′7.1″	3,586	X. Su, 11,195
30		Qushui, Xizang	5	29°29′46.0″	90°56′14.6″	3,617	X. Su, 11,010
31		Rikaze, Xizang	5	29°18′0.4″	89°46′7.3″	3,767	X. Su, 11,018
32		Kangma, Xizang	5	28°33′20.0″	89°41′2.0″	4,412	X. Su, 11,132
33		Lazi, Xizang	5	29°9′28.3″	88°10′16.9″	4,060	X. Su, 11,033
34		Dingjie, Xizang	5	28°21′51.0″	87°45′57.0″	4,324	X. Su, 11,120
35		Dingri, Xizang	5	28°39′34.2″	87°7′45.6″	3,852	X. Su, 11,123
36		Dingri, Xizang	5	28°39′34.2″	87°7′45.6″	3,852	X. Su, 11,119
37		Angren, Xizang	5	29°26′24.0″	86°39′52.6″	4,593	X. Su, 11,034
38		Jilong, Xizang	5	28°46′6.3″	85°32′14.3″	4,614	X. Su, 11,100
39		Shaga, Xizang	4	29°23′31.5″	85°30′57.4″	4,677	X. Su, 11,039
40		Shaga, Xizang	5	29°0′27.0″	85°26′48.8″	4,687	X. Su, 11,078
41		Shaga, Xizang	5	29°30′1.4″	84°33′39.6″	4,578	X. Su, 11,043
42		Zhongba, Xizang	5	29°41′7.9″	84°8′48.1″	4,563	X. Su, 11,044
43		Zhongba, Xizang	5	29°59′45.6″	83°31′43.1″	4,582	X. Su, 11,045
44		Pulan, Xizang	5	30°48′35.8″	81°34′22.5″	4,610	X. Su, 11,049
45		Pulan, Xizang	5	30°21′58.5″	81°9′8.3″	4,260	X. Su, 11,050
46		Pulan, Xizang	5	31°10′42.6″	80°45′26.8″	4,427	X. Su, 11,054
47		Ali, Xizang	5	32°34′17.9″	80°3′10.7″	4,451	X. Su, 11,056
48		Zhada, Xizang	5	31°28′46.0″	79°48′9.0″	4,434	X. Su, 11,070

Abbreviation: *N*, number of individuals sampled for amplified fragment length polymorphism experiments.

TABLE 2 Adapters and primer combination sequences used in this study

Primer	Name	Sequence
Adapters		
P-L	*Pst* I-adapter	5′-CTCGTAGACTGCGTACATGCA-3′
P-R	*Pst* I-adapter	5′-TGTACGCAGTCTAC-3′
M-L	*Mse* I-adapter	5′-GACGATGAGTCCTGAG-3′
M-R	*Mse* I-adapter	5′-TACTCAGGACTCAT-3′
Preamplification primer		
P01	*Pst* I	5′-GACTGCGTACATGCAG-3′
P02	*Mse* I	5′-GATGAGTCCTGAGTAAC-3′
Selective amplification primer		
A-1	*Pst* I-GAA	5′-GACTGCGTACATGCAGAA-3′
	Mse I-CAA	5′-GATGAGTCCTGAGTAACAA-3′
B-2	*Pst* I-GAC	5′-GACTGCGTACATGCAGAC-3′
	Mse I-CAC	5′-GATGAGTCCTGAGTAACAC-3′
B-3	*Pst* I-GAC	5′-GACTGCGTACATGCAGAC-3′
	Mse I-CAG	5′-GATGAGTCCTGAGTAACAG-3′
B-5	*Pst* I-GAC	5′-GACTGCGTACATGCAGAC-3′
	Mse I-CTA	5′-GATGAGTCCTGAGTAACTA-3′
C-1	*Pst* I-GAG	5′-GACTGCGTACATGCAGAG-3′
	Mse I-CAA	5′-GATGAGTCCTGAGTAACAA-3′
C-3	*Pst* I-GAG	5′-GACTGCGTACATGCAGAG-3′
	Mse I-CAG	5′-GATGAGTCCTGAGTAACAG-3′
C-7	*Pst* I-GAG	5′-GACTGCGTACATGCAGAG-3′
	Mse I-CTG	5′-GATGAGTCCTGAGTAACTG-3′
D-3	*Pst* I-GAT	5′-GACTGCGTACATGCAGAT-3′
	Mse I-CAG	5′-GATGAGTCCTGAGTAACAG-3′

marker would have a standard deviation of zero for the marker, and clonal individuals should have an average deviation of zero. However, clonal individuals may vary in AFLP analyses due to errors in obtaining or processing the data or due to somatic mutations. Thus, we regarded any population with less than 0.05 average deviation as being comprised exclusively of clones, and we sought to exclude these populations from downstream analyses.

We assessed genetic diversity in *Orinus*, including natural breaks potentially corresponding to species, by analyzing binary matrix of AFLP bands. We analyzed the matrix in POPGENE 1.32 (Yeh, Yang, & Boyle, 1999) to calculate the following summary statistics: percentage of polymorphic loci (*PPL*), observed number of alleles (N_a), effective number of alleles (*Ne*), expected heterozygosity (H_e; Kimura & Crow, 1964), and Shannon's information index (*I*; Lewontin, 1972). We also analyzed the binary matrix using the NTSYS-pc 2.l statistical package (Rohlf, 2000). Specifically, in NTSYS, we generated a pairwise similarity matrix with a simple matching coefficient according to the SIMQUAL algorithm. We also used SAHN in NTSYS package to construct a UPGMA tree based on Nei's genetic distance for assessment of relationships among individuals and populations of *Orinus*, and we estimated

support for the UPGMA tree using 2000 bootstrap replicates in Winboot software (Yap & Nelson, 1996; see also Liu et al., 2018). We calculated a genetic similarity matrix from the AFLP data according to the method of Nei and Li (1979) and visualized genetic variation among individuals with a principal coordinate analysis (PCoA) performed in GENALEX 6.5 (Peakall & Smouse, 2012). In addition, we constructed a similarity-based network using the Neighbor-Net algorithm based on Jaccard's distances within SplitsTree 4.13 (Huson & Bryant, 2006) to further depict relationships among individuals and populations and species based on the AFLP datasets.

We also sought to evaluate the genetic differentiation between and within populations of the three species of *Orinus* using average F_{ST}, analysis of molecular variance (AMOVA; Excoffier, Smouse, & Quattro, 1992), and a Mantel test. We calculated F_{ST} using Arlequin 3.11 (Excoffier, Laval, & Schneider, 2005) and determined significance of the pairwise F_{ST} comparisons via permutation tests (*n* = 1,000) with a sequential Bonferroni correction. For the AMOVA, we tested significance with nonparametric permutation using 9,999 replications. We performed Mantel tests on the distance matrix of Jaccard's coefficients calculated in GENALEX 6.5 (Peakall & Smouse, 2012) in order to detect the correlations between genetic distances generated from each of the AFLP primer pairs, and geographic distances of populations derived from geographic coordinates using AFLP datasets (Ehrich, 2006). For the Mantel tests, we computed correlation coefficients and assessed the significance with 1,000 permutations.

We conducted a Bayesian analysis of the population structure in *Orinus* using STRUCTURE 2.3 (Falush, Stephens, & Pritchard, 2007; Hubisz, Falush, Stephens, & Pritchard, 2009; Pritchard, Stephens, & Donnelly, 2000) to determine whether the structure was consistent with species boundaries and to infer the relative amounts of gene flow between each species. We performed the analyses using an admixture model with independent allele frequencies for 10 independent runs for the number of clusters (*K*) ranging from 1 to 10. We applied 1×10^6 Markov chain Monte Carlo repetitions with a burn-in rate of 25%. We summarized the outputs of all runs with the Web-based software Structure Harvester (Earl & von, 2012), and we calculated the average similarity coefficients among runs for each *K*. We determined the optimal *K* using two methods: the point of diminishing returns for adding additional *K* (i.e., elbow method) and the value representing the greatest change from the previous value (i.e., Δ*K*; Evanno, Regnaut, & Goudet, 2005; Pritchard et al., 2000).

2.3 | Testing AFLP data against explicit genetic models of hybridization

We tested the hybrid status of *O. intermedius* using the Bayesian implementation in NewHybrids (Anderson & Thompson, 2002) version 2.0+ Developmental (https://github.com/eriqande/newhybrids). Specifically, we tested the 231 sampled individuals for their compatibility with five genetic models: that each is genetically (a) *O. kokonoricus*, (b) *O. thoroldii*, (c) a true hybrid of *O. kokonoricus* and *O. thoroldii*, (d)
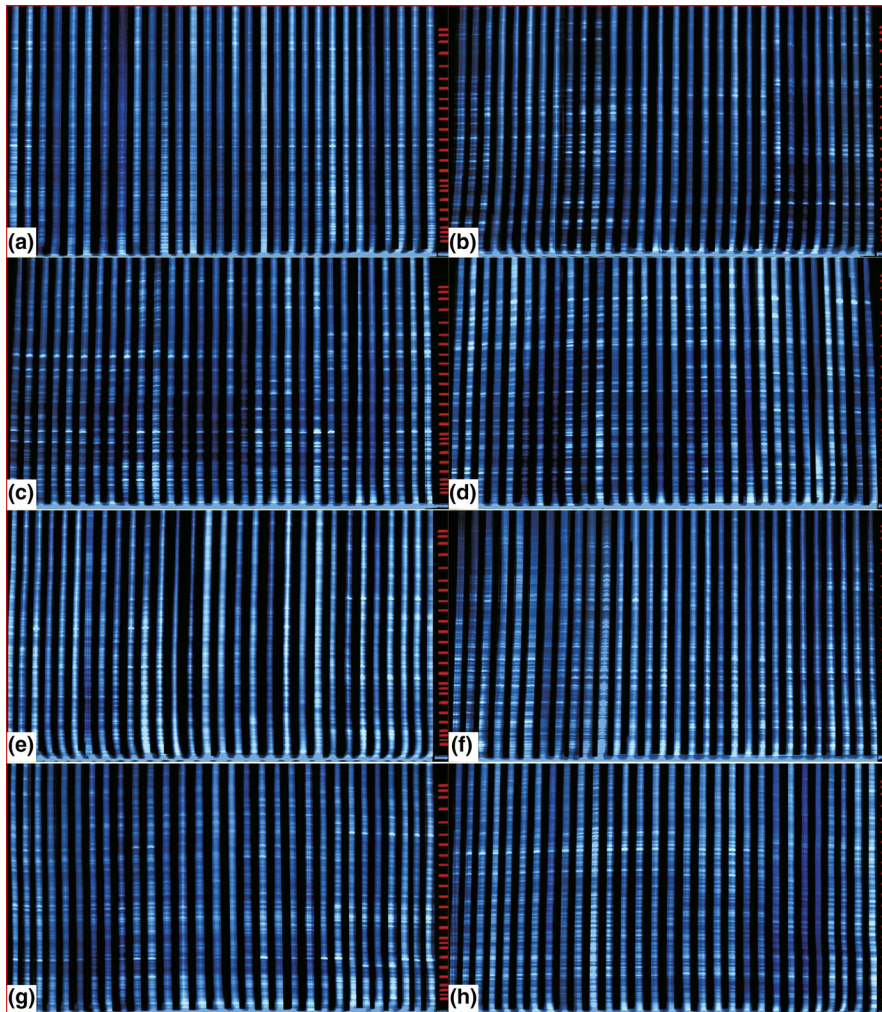
FIGURE 3 Fluorescently-labeled AFLPs generated using different primer combinations. (a) P-GAA/M-CAA, (b) P-GAC/M-CAC, (c) P-GAC/M-CTG, (d) P-GAG/M-CTA, (e) P-GAG/M-CAA, (f) P-GAG/M-CAG, (g) P-GAG/M-CTG, and (h) P-GAT/M-CAG

a hybrid of *O. kokonoricus* and *O. thoroldii* backcrossed with *O. kokonoricus*, and (e) a hybrid of *O. kokonoricus* and *O. thoroldii* backcrossed with *O. thoroldii*. These models cannot explicitly test the possibility that *O. intermedius* is an independent species not derived from hybrid origins. However, *O. intermedius* individuals should be resolved under model 1 or 2 if the species is not a hybrid but, instead, shared a common ancestor with either *O. kokonoricus* or *O. thoroldii* that does not include the other species. Additionally, models 4 and 5 cannot be differentiated from low levels of introgression that may occur among species that are differentiating in allopatry, but we interpret these results within the context of our other statistical analyses. For individuals within populations, we averaged the posterior probabilities of compatibility with each model. Thus, our results represent the average posterior probability for the best genetic model for each population. We also present the results for each individual in Appendix A.

3 | RESULTS

3.1 | Genetic diversity

Among 64 pairs of *Eco*RI/*Mse*I primer combinations, we successfully obtained eight pairs of selective AFLP primers that could amplify fragments with good coverage in the 231 individuals representing 48 populations of the three species of *Orinus* (Table 1, Figure 3). For the eight primer pairs, all summary and genetic statistics for the primer pairs are presented in Table 3. The eight pairs produced a total of 1,324 unambiguous and repetitious AFLP amplification bands across all the samples from *O. thoroldii* and *O. kokonoricus*, and 1,261 in *O. intermedius*. The total number of AFLP amplification bands for each primer pair ranged from 154 (P-GAC/M-CAC) to 185 (P-GAT/M-CAG) with an average of 166, 150 (P-GAA/M-CAA) to 179 (P-GAC/M-CAC) with an average of 166, and 143 (P-GAA/M-CAA) to 171 (P-GAG/M-CAA) with an average of 158. Among the AFLP amplification bands, 1,313 (99.17%) were polymorphic in *O. thoroldii*, 1,315 (99.32%) in *O. kokonoricus*, and 1,242 (98.49%) in *O. intermedius*. The total number of polymorphic bands for each primer pair varied from 152 (P-GAC/M-CAC) to 185 (P-GAT/M-CAG) with an average of 164, 150 (P-GAA/M-CAA) to 179 (P-GAC/M-CAC) with an average of 164, and 142 (P-GAA/M-CAA) to 171 (P-GAG/M-CAA) with an average of 155. Each primer pair yielded rich and clear patterns among the three species of *Orinus*. The allele size of *O. thoroldii* and *O. intermedius* ranged from 70 to 500 bp, while that of *O. kokonoricus* ranged from 60 to 500 bp. In addition, the percentage polymorphism of

TABLE 3 Summary Statistics for eight amplified fragment length polymorphism selective primer combinations in the present study

Species name	Selective nuclear	Polymorphism bands	Amplification bands	PPL (%)	Size range (bp)	N_a	N_e	H_e	I
O. thoroldii	P-GAA/M-CAA	161	164	98.17	70–500	1.98	1.33	0.20	0.32
	P-GAC/M-CAC	152	154	98.70	70–500	1.99	1.33	0.20	0.31
	P-GAC/M-CAG	167	170	98.24	70–500	1.98	1.27	0.17	0.27
	P-GAC/M-CTA	156	159	98.11	71–498	1.98	1.30	0.19	0.30
	P-GAG/M-CAA	174	174	100	70–499	2.00	1.30	0.19	0.31
	P-GAG/M-CAG	159	159	100	70–500	2.00	1.34	0.21	0.33
	P-GAG/M-CTG	159	159	100	70–494	2.00	1.33	0.21	0.33
	P-GAT/M-CAG	185	185	100	71–498	2.00	1.33	0.21	0.34
	Total	1,313	1,324	99.17	560–3,988	–	–	–	–
	Mean	164	166	99.15	70–499	1.99	1.32	0.20	0.31
O. kokonoricus	P-GAA/M-CAA	150	150	100	70–500	2.00	1.37	0.23	0.35
	P-GAC/M-CAC	179	179	100	70–498	2.00	1.29	0.19	0.31
	P-GAC/M-CAG	159	166	95.78	71–498	1.96	1.29	0.18	0.29
	P-GAC/M-CTA	161	163	98.77	70–499	1.99	1.32	0.20	0.31
	P-GAG/M-CAA	170	170	100	70–499	2.00	1.33	0.20	0.33
	P-GAG/M-CAG	164	164	100	60–497	2.00	1.32	0.20	0.32
	P-GAG/M-CTG	174	174	100	70–493	2.00	1.31	0.20	0.37
	P-GAT/M-CAG	158	158	100	71–500	2.00	1.34	0.21	0.33
	Total	1,315	1,324	99.32	551–3,984	–	–	–	–
	Average	164	166	99.32	69–498	1.99	1.32	0.20	0.32
O. intermedius	P-GAA/M-CAA	142	143	99.30	70–499	1.99	1.38	0.23	0.37
	P-GAC/M-CAC	163	163	100	71–498	2.00	1.33	0.21	0.34
	P-GAC/M-CAG	155	161	96.27	70–499	1.96	1.29	0.19	0.30
	P-GAC/M-CTA	152	154	98.70	70–500	1.99	1.36	0.22	0.35
	P-GAG/M-CAA	171	171	98.28	70–489	1.98	1.31	0.20	0.32
	P-GAG/M-CAG	142	146	97.26	70–490	1.97	1.34	0.21	0.33
	P-GAG/M-CTG	152	155	98.06	70–498	1.98	1.31	0.20	0.33
	P-GAT/M-CAG	165	168	98.21	71–497	1.98	1.32	0.21	0.33
	Total	1,242	1,261	98.49	562–3,969	–	–	–	–
	Average	155	158	98.26	70–496	1.98	1.33	0.21	0.33

Note. PPL, percentage of polymorphic loci; N_a, observed number of alleles; N_e, effective number of alleles; H_e, expected heterozygosity; I, Shannon's information index.

each species of *Orinus* varied from 98.11% to 100% with an average of 99.15% in *O. thoroldii*, 95.78% to 100% with an average of 99.32% in *O. kokonoricus*, and 96.27% to 100% with an average of 98.26% in *O. intermedius* among the primer pairs.

The mean N_a of *O. thoroldii* was 1.99, which varied from 1.98 to 2.00, while the mean N_e and H_e varied from 1.27 to 1.34 with a mean value of 1.32 and from 0.17 to 0.21 with the mean value of 0.20, respectively. The mean value of I was 0.31 and ranged from 0.27 to 0.34. For *Orinus kokonoricus*, the N_a, N_e, H_e, and I ranged, respectively, from 1.96 to 2.00, 1.29 to 1.37, 0.18 to 0.23, and 0.29 to 0.35. The mean values were 1.99, 1.32, 0.20, and 0.32, also respectively. Similarly, the mean values of N_a, N_e, H_e, and I for *O. intermedius* were 1.98, 1.33, 0.21, and 0.33, and the variation of these ranged from 1.96 to 2.00, 1.29 to 1.38, 0.19 to 0.23, and 0.30 to

0.37, all respectively. All measures revealed high levels of genetic diversity among the three species of *Orinus*. In particular, *O. intermedius* showed the highest level of genetic diversity among the three species according to Shannon's information index (I = 0.33).

3.2 | Population genetic structure

Analysis of molecular variance (AMOVA) based on AFLP datasets and inbreeding coefficients (F_{ST}; Table 4) indicated significant interspecific genetic differentiation across the natural distribution of the three species of *Orinus* (F_{ST} = 0.19, p < 0.01). Variation within populations represented 56.65% of the total genetic variation, while variation among species comprised 30.04%, and variation among populations within each species was 13.31%. Among

TABLE 4 Results of analyses of molecular variance (AMOVAs) based on amplified fragment length polymorphism markers for the three species of *Orinus*

Grouping regions	Source of variation	df	SS	VC	Percent variation (%)	Fixation index
O. thoroldii	Among populations	19	4,692.26	25.25	16.86	$F_{ST} = 0.17^*$
	Within populations	77	9,589.10	124.53	83.14	
O. kokonoricus	Among populations	18	4,905.54	29.99	19.45	$F_{ST} = 0.20^*$
	Within populations	75	9,313.95	124.19	80.55	
O. intermedius	Among populations	8	2,340.80	38.79	23.68	$F_{ST} = 0.24^*$
	Within populations	30	3,749.92	124.80	76.32	
O. thoroldii and *O. kokonoricus*	Among species	1	7,664.98	77.17	33.67	$F_{ST} = 0.46^*$
	Among populations within species	37	9,609.03	27.46	11.98	$F_{SC} = 0.18^*$
	Within populations	153	19,053.35	124.53	54.34	$F_{CT} = 0.34^*$
O. thoroldii and *O. intermedius*	Among species	1	4,147.07	69.73	31.21	$F_{ST} = 0.44^*$
	Among populations within species	27	7,044.29	28.77	12.88	$F_{SC} = 0.19^*$
	Within populations	108	13,489.32	124.90	55.91	$F_{CT} = 0.31^*$
O. kokonoricus and *O. intermedius*	Among species	1	2,171.07	34.42	18.00	$F_{ST} = 0.35^*$
	Among populations within species	26	7,246.34	32.45	16.97	$F_{SC} = 0.21^*$
	Within populations	105	13,063.87	124.42	65.04	$F_{CT} = 0.18^*$
Total	Among species	2	10,080.42	66.08	30.04	$F_{ST} = 0.19^*$
	Among populations within species	45	11,949.83	29.27	13.31	$F_{SC} = 0.43^*$
	Within populations	183	22,803.27	124.61	56.65	$F_{CT} = 0.30^*$

Note. df, degrees of freedom; SS, sum of squares; VC, variance components; F_{ST}, variance among populations; F_{SC}, variance among populations within species; F_{CT}, variance among groups relative to total variance. Significant level:
$^*p < 0.01$.

the three species of *Orinus*, the genetic variation between *O. thoroldii* and *O. kokonoricus* was the highest ($F_{ST} = 0.46$, $p < 0.01$), with 33.67% of the variation between species and 54.34% within populations. For *O. thoroldii* and *O. intermedius*, the genetic variation was also high ($F_{ST} = 0.44$, $p < 0.01$), and 31.21% of the total variation was interspecific while 55.91% was within populations. Genetic variation between *O. kokonoricus* and *O. intermedius* was lower, at 18.00% with a corresponding average F_{ST} value of 0.35, while 65.04% of the variation was within populations. In all species, intrapopulational genetic variation was much higher than interpopulational.

The UPGMA tree indicated that the 231 individuals from 48 populations of *Orinus* comprised three clades (Figure 4) corresponding to three geographically clustered groups of populations within the QTP and consistent with species identities. Thus, the UPGMA tree revealed geographic structure within the genus *Orinus* with three independent clades consisting of *O. thoroldii*, *O. kokonoricus*, and *O. intermedius*, which is sister to *O. kokonoricus* (Figure 4). Similarly, the Mantel tests agreed that geography is positively correlated with genetic divergence ($r = 0.05$, $p < 0.05$). Discrete cluster corresponding to species and geography was supported by the principal coordinate analysis (PCoA; Figure 5). The first two axes in the PCoA plot explained 23.31% and 18.20% of variation, respectively (data not shown). The PCoA axis 1 separated *O. thoroldii* from the other two species, while axis 2 yielded greater separation for *O. intermedius*. Additionally, the split

network revealed three splits, corresponding to the three species of *Orinus* (Figure 6). According to STRUCTURE (Figure 7), the optimum *K* value was *K* = 3 and the highest peak of Δ*K* values also appeared at *K* = 3. The three clusters predicted by the STRUCTURE analysis corresponded to the three recognized species of *Orinus* (Figure 7d). In a separate analysis with *K* = 2, *O. kokonoricus* and *O. intermedius* were clustered together (Figure 7d).

3.3 | Results of genetic models of hybridization

The results of NewHybrids show that models of a single genetic origin are best suited to most populations (Figure 8; Appendix A). All populations of *O. kokonoricus* correspond to *O. kokonoricus* origins with posterior probability (pp) of 1.0 except populations 14 and 17 (Figure 2), in which one individual each (Appendix A) showed trivial pp (<0.01) support for hybrid backcrossing into *O. kokonoricus*. Most populations of *O. thoroldii* had 1.0 pp of having a genetic origin of solely *O. thoroldii* stock. For population 33 of *O. thoroldii*, one individual showed a trivial pp of representing a hybrid with backcrossing into *O. thoroldii*. In contrast, populations 29 and 30 had nontrivial pp for representing hybrid backcrosses to *O. thoroldii* (0.48 and 0.21, respectively). Nevertheless, these support values were lower than for an origin from exclusively *O. thoroldii* genetic stock. Among populations of *O. intermedius*, four populations (20, 21, 25, and 26) showed 1.0 pp for exclusive origins from *O. kokonoricus* stock, while three populations (22, 23,
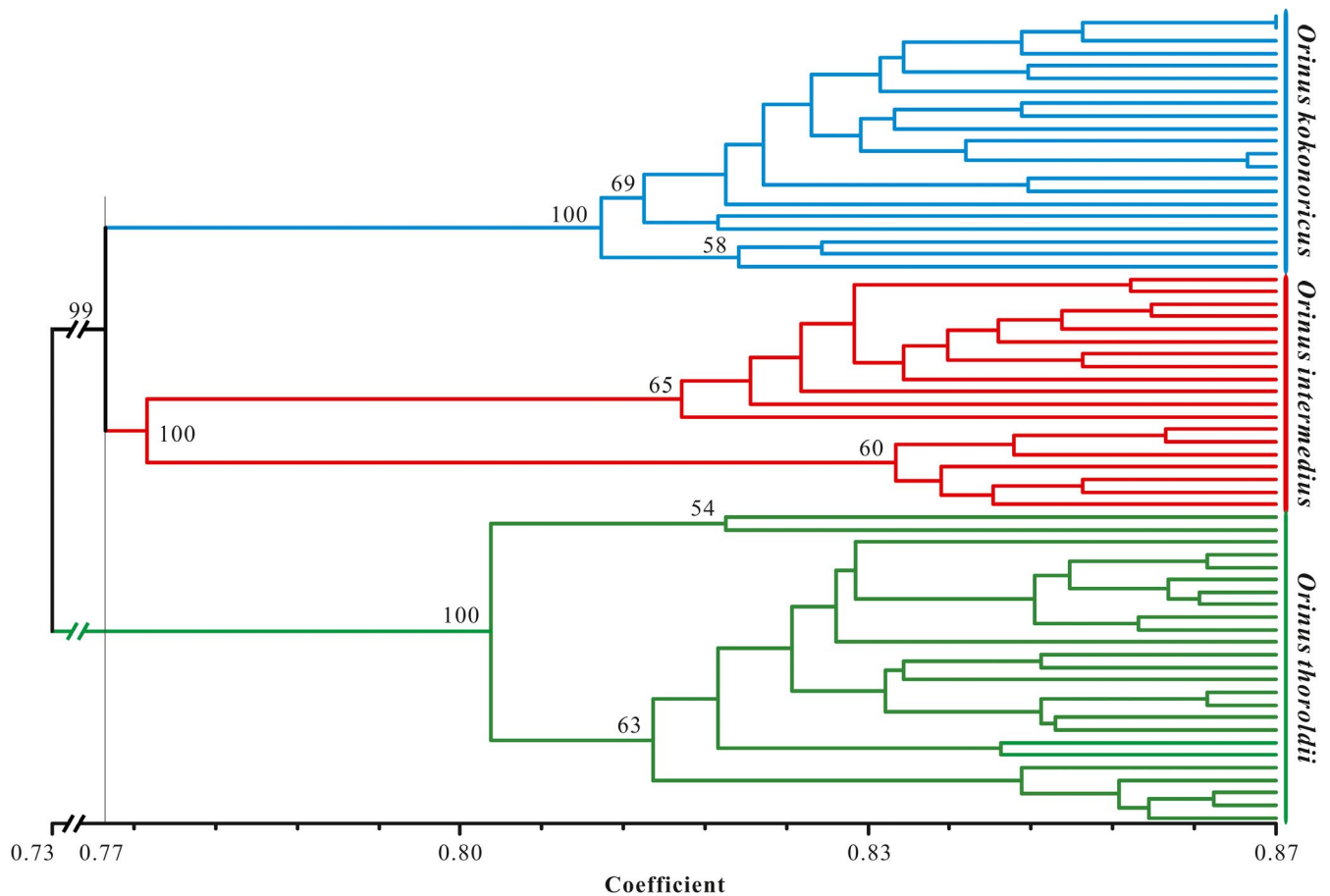
FIGURE 4 Dendrogram of the three species of *Orinus* generated by unweighted pair group method analysis (UPGMA) cluster analysis from the similarity matrix obtained using amplified fragment length polymorphism genetic distance
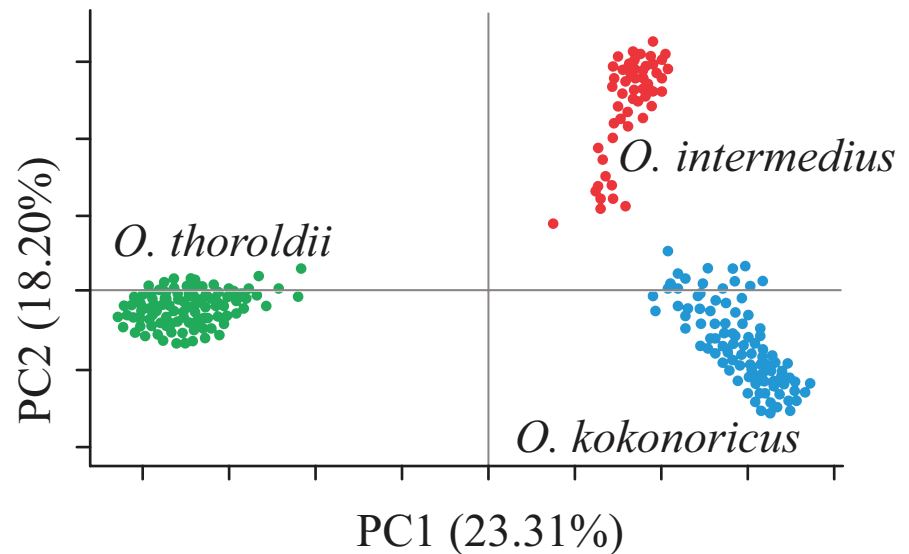
FIGURE 5 A two-dimensional plot of the principal coordinate analysis (PCoA)-based variation of amplified fragment length polymorphism markers within the three species of *Orinus*. Tick marks on axes are in increments of 1.0, and 0.0 on each axis is indicated by a gray line

and 24) showed high pp for *O. kokonoricus* origins and trivial pp for representing hybrid backcrosses with *O. kokonoricus*. Notably, two populations of *O. intermedius*, 27 and 28, had 1.0 pp and 0.77 pp, respectively, for representing hybrid backcrosses with *O. kokonoricus*.

4 | DISCUSSION

4.1 | The hybrid origin of *Orinus intermedius*

In our prior work, we have hypothesized that *Orinus intermedius* (Su et al., 2017) may be either a hybrid of *O. kokonoricus* and *O. thoroldii* or an

FIGURE 6 Neighbor-Net split network of the three species of *Orinus* based on amplified fragment length polymorphism data using Jaccard's distances. Lines of green, blue and red represent *O. thoroldii*, *O. kokonoricus*, and *O. intermedius*, respectively

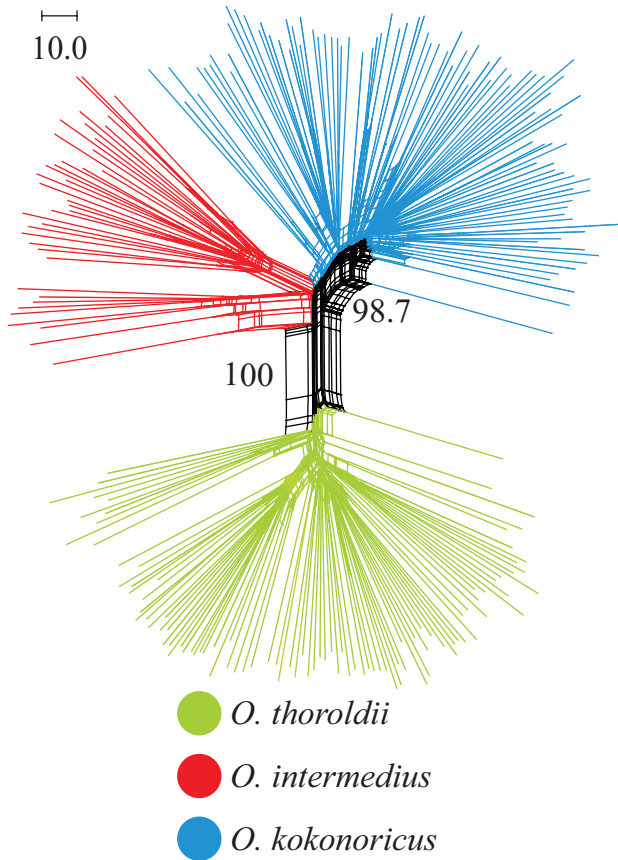incompletely diverged sister of *O. kokonoricus*. Here, our AFLP data are consistent with most populations of *O. kokonoricus* and *O. intermedius* sharing a common ancestor, and, thus, a common genetic stock, that is not shared with *O. thoroldii*. Therefore, our data do not support a hybrid origin for *O. intermedius*. However, we observed that two populations of *O. intermedius* are consistent with a backcrossing model. However, this likely represents a level of introgression occurring contemporaneously with speciation processes, rather than backcrossing, as we did not detect any true hybrid individuals or populations.

4.2 | Species limits in *Orinus* and introgression

Previously, we suggested that *Orinus* represents three species and noted that genetic isolation among all species of *Orinus* is nearly, but not entirely, complete (Liu et al., 2018; Su et al., 2017, 2015). The present study is congruent with our prior work in showing that the

three species of *Orinus* are largely distinct, especially according to the UPGMA (Figure 4), STRUCTURE (Figure 7), AMOVA (Table 4), and SplitsTree (Figure 6). However, some gene flow does continue to occur among all species based on the results of these same analyses and may also explain the nonzero probabilities of backcrosses within some populations of *O. kokonoricus* and *O. thoroldii* according to NewHybrids (Figure 8; Appendix A).
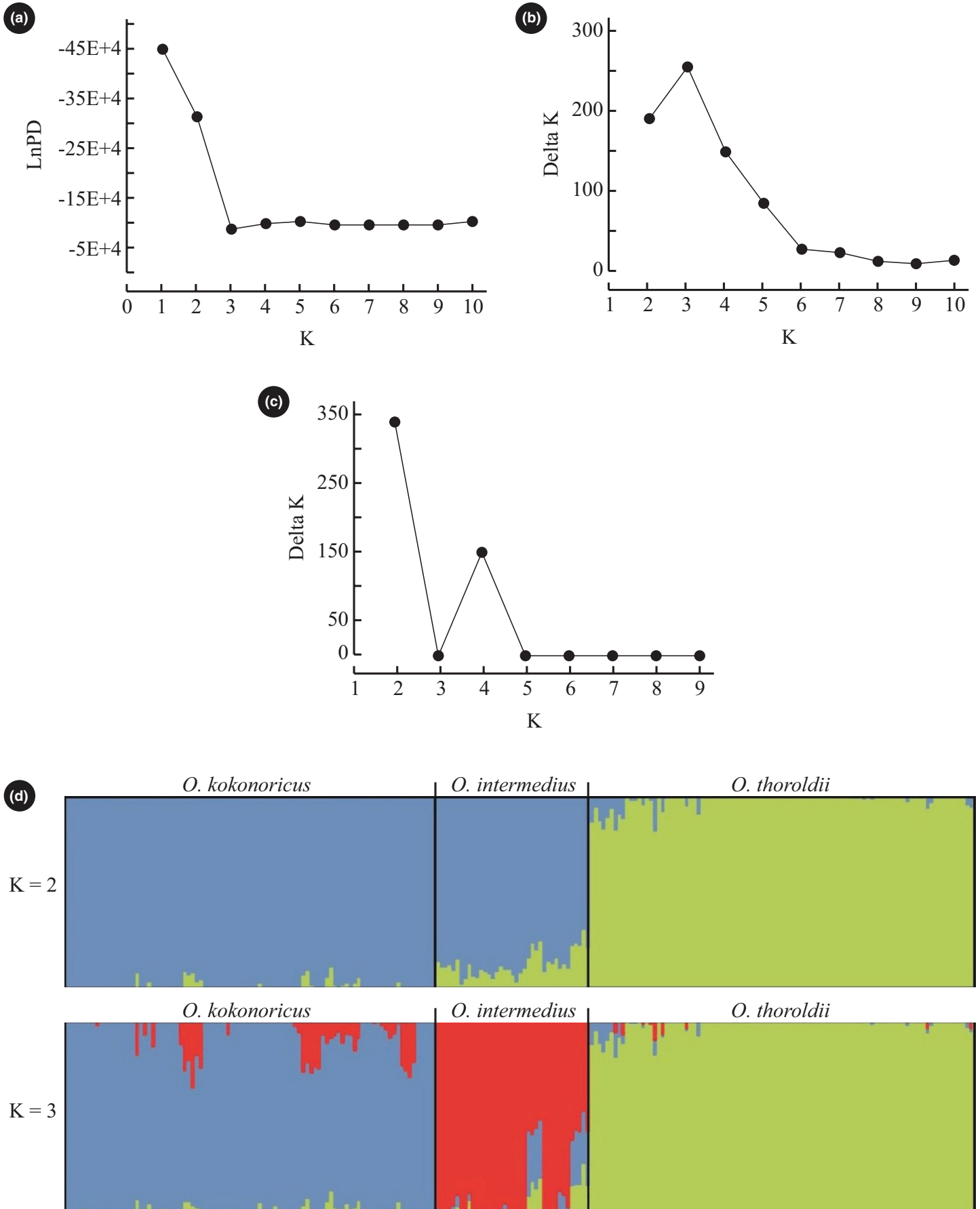
Gene flow between *O. intermedius* and *O. kokonoricus* may enable them to maintain the higher levels of genetic diversity that we detected, compared with *O. thoroldii*, which is more genetically isolated. In contrast to our results, it is relatively common that more widely spread species, such as *O. thoroldii*, maintain greater genetic diversity than more geographically restricted species (Hamrick & Godt, 1989; Karron, 1987; Xue, Wang, Korpelainen, & Li, 2005), such as *O. intermedius* and *O. kokonoricus*. High diversity within *O. intermedius* and *O. kokonoricus* is likely due to their ongoing speciation (Liu et al., 2018), in which barriers to gene flow remain incomplete. Relatedly, due to earlier divergence time of *O. thoroldii*, it may have had more time in isolation to undergo some degree of genetic drift. *Orinus thoroldii* is not only more genetically distant from its congeners (Figure 5), but also more geographically distant. Thus, genetic differentiation in *Orinus* may be mediated by reduced gene flow over greater geographic distances as is consistent with an allopatric mode of speciation as has been observed in other plant species of the QTP (Ge, Zhang, Yuan, Hao, & Chiang, 2005; Hu et al., 2016; Liu, Wang, Geng, et al., 2006; Zhang, Chiang, George, Liu, & Abbott, 2005).

4.3 | Population and species diversification history in *Orinus* compared to other clonal species

Many clonal species exhibit a common pattern of diversity, which is low or intermediate within populations and very high among them (Ellstrand & Roose, 1987; Li & Ge, 2001). This pattern has been documented in other clonal grasses, such as *Psammochloa villosa* Hitchc. (Poaceae; Li & Ge, 2001; Yu, Dong, & Krüsi, 2004). Overall, for clonal species, this pattern suggests that interpopulation movement of propagules is rare and that diversity within populations may be largely explained by the founder genotypes and, in some cases, outcrossing among genotypes (e.g., *Carex curvula*, *Dryas octopetala* L., *Salix herbacea* L., and *Vaccinium uliginosum* L.; de Witte, Armbruster, Gielly, Taberlet, & Stöcklin, 2012).

Orinus differs from other clonal plants by showing the highest diversity within populations and limited diversity among them, including populations within and among species. This is an uncommon pattern of diversity for clonal species, but one which has been previously observed (Pluess & Stöcklin, 2004). In particular, *Geum reptans* L. (Rosaceae), a clonal alpine species of the Swiss Alps, exhibits

FIGURE 7 Results of the Bayesian clustering analysis in STRUCTURE of the 231 individuals representing three species of *Orinus*. (a) The probability of the data Ln *P*(*D*) (±*SD*) against the number of *K* cluster, and increase of Ln *P*(*D*) given *K*, calculated as (Ln *P*(*D*)*k*−Ln *P*(*D*)*k*−1). (b) △*K* values from the mean log-likelihood probabilities from STRUCTURE runs where inferred cluster (*K*) ranged from one to ten. (c) Bayesian inference of the number of clusters (*K*) for the three species of *Orinus*. (d) Estimated genetic clustering for *K* = 2 and 3, where unique colors correspond to assignment to different clusters
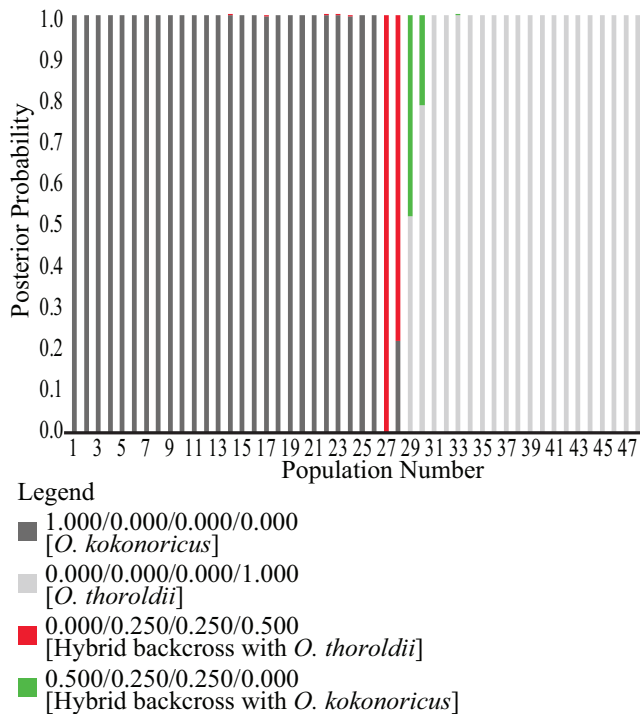
(a)

(b)

(c)

(d)

K = 2

O. kokonoricus | *O. intermedius* | *O. thoroldii*

K = 3

O. kokonoricus | *O. intermedius* | *O. thoroldii*

FIGURE 8 Results of testing explicit genetic models in NewHybrids. Posterior probability of models shown on the *y*-axis, averaged for populations shown on the *x*-axis. We tested five models, but the true hybrid model is not shown, because it received 0.0 pp for all individuals and, thus, all populations. Bars represent populations 1-48 consecutively

this pattern of diversity probably due to ongoing gene flow, despite geographic isolation of populations on sky islands (Pluess & Stöcklin, 2004; see also sky islands in Hughes & Atchison, 2015; Körner, 2004). In *Orinus*, gene flow is unlikely to account for this pattern of diversity, especially among species, because the species are, overall, distinct, and because the species probably experience limited gene flow by rare dispersals of rhizome sections and occasional pollen movement by wind, water, and animal visitors. Within *Orinus*, there is no obvious mechanism for seed dispersal. Therefore, alternatively to ongoing, regular gene flow, recency of isolation of species and populations of *Orinus* within the QTP may explain the limited genetic diversity at the interspecific and interpopulational levels, respectively (e.g., as in Cruickshank & Hahn, 2014). Indeed, *Orinus* may have begun diversifying within the QTP during the latter part of the Pliocene (2.85 million years ago; Liu et al., 2018), which represents the end of a global period of evolution of modern alpine species (Hughes & Atchison, 2015). This alternative also requires that the original populations possessed high genetic diversity that has been preserved, at least partially, to present times. High diversity within ancestral populations often results from isolation by vicariance, rather than dispersal, events (Mayr, 1942; see also Harris, Ickert-Bond, & Rodríguez, 2018; Kropf, Comes, & Kadereit, 2006). Vicariance within the QTP is often invoked to explain commonly observed patterns in the diversification of plant populations or species (e.g., Yang, Li, Ding, & Wang, 2008), especially the divergence of western lineages, such as *O. thoroldii*, from eastern ones, such as *O.*

kokonoricus and *O. intermedius*. Moreover, vicariance in the region may be attributed to either the topographic or climatic effects of recent geomorphism (Jia, Liu, Wang, Zhou, & Liu, 2011; Liu et al., 2013; Wen et al., 2014; Yang et al., 2008), and topology may be a better explanation for divergence in the case of *Orinus*, because the ecological niches of species are similar (Su et al., 2015). Overall, the pattern of genetic diversity within *Orinus* could eventually come to resemble patterns overserved for other clonal species (Ellstrand & Roose, 1987; Li & Ge, 2001) given sufficient evolutionary time. However, as a caveat of the present study, we also cannot rule out that our limited sampling within populations accounts for some parts of the patterns in diversity that we observed, and expanded sampling is needed in the future.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

XS conceived and designed the study. XS, YL, and QG performed the laboratory work. YL, AJ-H, QG, XS, and ZR contributed to performing data analyses, interpreting results, and writing the manuscript. All authors approved the manuscript as written.

DATA ACCESSIBILITY

All data are provided within the text, tables, appendix, and figures, except for the binary scoring of AFLP bands, which we have submitted to the Dryad Digital Repository (https://doi.org/10.5061/dryad.403j5s4).

ORCID

AJ Harris https://orcid.org/0000-0003-3215-1201

REFERENCES

Anderson, E. C., & Thompson, E. A. (2002). A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*, 160(3), 1217–1229.

Beger, M., McGowan, J., Treml, E., Green, A., White, A., Wolff, N., … Possingham, H. (2015). Integrating regional conservation priorities for multiple objectives into national policy. *Nature Communications*, 6, 8208. https://doi.org/10.1038/ncomms9208

Bowman, W. D. (2000). Biotic controls over ecosystem response to environmental change in alpine tundra of the Rocky Mountains. *Aliso*, 29, 396–400. https://doi.org/10.1579/0044-7447-29.7.396

Cai, L. B. (2004). Two new recorded species of *Orinus* from Qinghai Province. *Bulletin of Botanical Research*, 24, 394–395.

Cortés, A. J., Waeber, S., Lexer, C., Sedlacek, J., Wheeler, J. A., van Kleunen, M., … Karrenberg, S. (2014). Small-scale patterns in snowmelt timing affect gene flow and the distribution of genetic diversity in the alpine dwarf shrub *Salix herbacea*. *Heredity*, 113, 233–239. https://doi.org/10.1038/hdy.2014.19

Cruickshank, T. E., & Hahn, M. W. (2014). Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. *Molecular Ecology*, 23, 3133–3157. https://doi.org/10.1111/mec.12796

Crutzen, P. J., & Stoermer, E. (2000). The "Anthropocene". *Global Change Newsletter*, 41, 12–14.

Davies, T. J., Savolainen, V., Chase, M. W., Moat, J., & Barraclough, T. G. (2004). Environmental energy and evolutionary rates in flowering plants. *Proceedings of the Royal Society of London B Biological Sciences*, 271, 2195–2200. https://doi.org/10.1098/rspb.2004.2849

de Witte, L. C., Armbruster, G. F. J., Gielly, L., Taberlet, P., & Stöcklin, J. (2012). AFLP markers reveal high clonal diversity and extreme longevity in four key arctic-alpine species. *Molecular Ecology*, 21, 1081–1097. https://doi.org/10.1111/j.1365-294X.2011.05326.x

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochemical Bulletin*, 19, 11–15.

Earl, D. A., & von, H. B. M. (2012). Structure Harvester: A website and program for visualizing Structure output and implementing the Evanno method. *Conservation Genetic Resources*, 4, 359–361. https://doi.org/10.1007/s12686-011-9548-7

Ehrich, D. (2006). AFLPDAT: A collection of R functions for convenient handling of AFLP data. *Molecular Ecology Notes*, 6, 603–604. https://doi.org/10.1111/j.1471-8286.2006.01380.x

Ellstrand, N. C., & Roose, M. L. (1987). Patterns of genotypic diversity in clonal plant species. *American Journal of Botany*, 74, 123–131. https://doi.org/10.1002/j.1537-2197.1987.tb08586.x

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, 14, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1, 47–50. https://doi.org/10.1177/117693430500100003

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, 131, 479–491.

Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molecular Ecology Notes*, 7, 574–578. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Feng, C., Tong, C., Zhang, R., Li, G., Wanghe, K., Tang, Y., … Zhao, K. (2017). Biodiversity and distribution patterns of *Triplophysa* species in the northeastern margin of the Tibetan Plateau. *Biodiversity Science*, 25, 53–61. https://doi.org/10.17520/biods.2016259

Garnhart, N. (2001). *Binthere V1.0, a program to bin AFLP data*. Durham, NH: University of New Hampshire.

Ge, X. J., Zhang, L. B., Yuan, Y. M., Hao, G., & Chiang, T. Y. (2005). Strong genetic differentiation of the East Himalayan *Megacodon stylophorus* (Gentianaceae) detected by inter-simple sequence repeats (ISSR). *Biodiversity Conservation*, 14, 849–861. https://doi.org/10.1007/s10531-004-0655-6

Gordon, S. P., Sloop, C. M., Davis, H. G., & Cushman, J. H. (2012). Population genetic diversity and structure of two rare vernal pool grasses in central California. *Conservation Genetics*, 13, 117–130. https://doi.org/10.1007/s10592-011-0269-y

Guo, Z. T., Ruddiman, W. F., Hao, Q. Z., Wu, H. B., Qiao, Y. S., Zhu, R. X., … Liu, T. S. (2002). Onset of Asian desertification by 22 Myr ago inferred from loess deposits in China. *Nature*, 416, 159–163.

Hamrick, J., & Godt, M. (1989). Allozyme diversity in plant species. In A. Brown, M. Clegg, A. Kahler, & B. Weir (Eds.), *Plant population genetics, breeding and genetic resources* (pp. 43–63). Sunderland, MA: Sinauer Associates Publishers.

Han, M., Brierley, G. J., Cullum, C., & Li, X. (2016). Climate, vegetation and human land-use interactions on the Qinghai–Tibet Plateau through the Holocene. In G. J. Brierley, X. Li, C. Cullum, & J. Gao (Eds.), *Landscape and ecosystem diversity, dynamics and management in the Yellow River Source Zone* (pp. 253–274). Berlin, Germany: Springer.

Han, W. X., Fang, X. M., & Berger, A. (2012). Tibet forcing of mid-Pleistocene synchronous enhancement of East Asian winter and summer monsoons revealed by Chinese loess record. *Quaternary Research*, 78, 174–184. https://doi.org/10.1016/j.yqres.2012.05.001

Harris, AJ, Ickert-Bond, S., & Rodríguez, A. (2018). Long distance dispersal in the assembly of floras: A review of progress and prospects in North America. *Journal of Systematics and Evolution*, 56, 430–448.

Hedrick, P. (2001). Conservation genetics: Where are we now? *Trends in Ecology and Evolution*, 16, 629–636. https://doi.org/10.1016/s0169-5347(01)02282-0

Hu, Q. J., Peng, H. C., Bi, H., Lu, Z. Q., Wan, D. S., Wang, Q., & Mao, K. S. (2016). Genetic homogenization of the nuclear ITS loci across two morphologically distinct gentians in their overlapping distributions in the Qinghai-Tibet Plateau. *Scientific Reports*, 6, 34244. https://doi.org/10.1038/srep34244

Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, 9, 1322–1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x

Hughes, C. E., & Atchison, G. W. (2015). The ubiquity of alpine plant radiations: From the Andes to the Hengduan Mountains. *New Phytologist*, 207, 275–282. https://doi.org/10.1111/nph.13230

Huson, D. H., & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, 23, 254–267. https://doi.org/10.1093/molbev/msj030

Jia, D. R., Liu, T. L., Wang, L. Y., Zhou, D. W., & Liu, J. Q. (2011). Evolutionary history of an alpine shrub *Hippophae tibetana* (Elaeagnaceae): Allopatric divergence and regional expansion. *Biological Journal of the Linnean Society*, 102, 37–50. https://doi.org/10.1111/j.1095-8312.2010.01553.x

Jump, A. S., Marchant, R., & Peñuelas, J. (2009). Environmental change and the option value of genetic diversity. *Trends Plant Science*, 14, 51–58. https://doi.org/10.1016/j.tplants.2008.10.002

Kaljund, K., & Jaaska, V. (2010). No loss of genetic diversity in small and isolated populations of *Medicago sativa* subsp. *falcata*. *Biochemical Systematics and Ecology*, 38, 510–520. https://doi.org/10.1016/j.bse.2010.05.007

Karron, J. (1987). A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. *Evolutionary Ecology*, 1, 47–58. https://doi.org/10.1007/BF02067268

Kimura, M., & Crow, J. F. (1964). The number of alleles that can be maintained in a finite population. *Genetics*, 49, 725–738.

Körner, C. (2004). Mountain biodiversity, its causes and function. *Ambio*, 13, 11–17.

Kropf, M., Comes, H. P., & Kadereit, J. W. (2006). Long-distance dispersal vs vicariance: The origin and genetic diversity of alpine plants in the Spanish Sierra Nevada. _New Phytologist_, _172_, 169–184. https://doi.org/10.1111/j.1469-8137.2006.01795.x

Lewontin, R. C. (1972). Testing the theory of natural selection. _Nature_, _236_, 181–182. https://doi.org/10.1038/236181a0

Li, A., & Ge, S. (2001). Genetic variation and clonal diversity of _Psammochloa villosa_ (Poaceae) detected by ISSR markers. _Annals of Botany_, _87_, 585–590. https://doi.org/10.1006/anbo.2001.1390

Li, Y., Wen, L., Dong, S., Liu, S., Wang, X., & Wu, Y. (2014). The interaction between poisonous plants and soil quality in response to grassland degradation in the alpine region of the Qinghai-Tibetan Plateau. _Plant Biology_, _215_, 809–819. https://doi.org/10.1007/s11258-014-0333-z

Liu, J. Q. (2004). Uniformity of karyotypes in _Ligularia_ (Asteraceae: Senecioneae), a highly diversified genus of the eastern Qinghai–Tibet Plateau highlands and adjacent areas. _Botanical Journal of the Linnean Society_, _144_, 329–342. https://doi.org/10.1111/j.1095-8339.2003.00225.x

Liu, J. Q., Gao, T. G., Chen, Z. D., & Lu, A. M. (2002). Molecular phylogeny and biogeography of the Qinghai-Tibet Plateau endemic _Nannoglottis_ (Asteraceae). _Molecular Phylogenetics and Evolution_, _23_, 307–325. https://doi.org/10.1016/S1055-7903(02)00039-8

Liu, J., Luo, Y. H., Li, D. Z., & Gao, L. M. (2017). Evolution and maintenance mechanisms of plant diversity in the Qinghai-Tibet Plateau and adjacent regions: Retrospect and prospect. _Biodiversity Science_, _25_, 163–174. https://doi.org/10.17520/biods.2016293

Liu, J., Möller, M., Provan, J., Gao, L. M., Poudel, R. C., & Li, D. Z. (2013). Geological and ecological factors drive cryptic speciation of yews in a biodiversity hotspot. _New Phytologist_, _199_, 1093–1108. https://doi.org/10.1111/nph.12336

Liu, J. M., Wang, L., Geng, Y. P., Wang, Q. B., Luo, L. J., & Zhong, Y. (2006). Genetic diversity and population structure of _Lamiophlomis rotata_ (Lamiaceae), an endemic species of Qinghai–Tibet Plateau. _Genetica_, _128_, 385–394. https://doi.org/10.1007/s10709-006-7517-y

Liu, J. Q., Wang, Y. J., Wang, A. L., Hideaki, O., & Abbott, R. J. (2006). Radiation and diversification within the _Ligularia-Cremanthodium-Parasenecio_ complex (Asteraceae) triggered by uplift of the Qinghai-Tibetan Plateau. _Molecular Phylogenetics and Evolution_, _38_, 31–49. https://doi.org/10.1016/j.ympev.2005.09.010

Liu, Y. P., Ren, Z. M., Harris, AJ, Peterson, P. M., Wen, J., & Su, X. (2018). Phylogeography of _Orinus_ (Poaceae), a dominant grass genus on the Qinghai-Tibet Plateau. _Botanical Journal of the Linnean Society_, _186_, 202–223. https://doi.org/10.1093/botlinnean/box091

Liu, Y. P., Su, X., He, Y. H., Han, L. M., Huang, Y. Y., & Wang, Z. Z. (2015). Evolutionary history of _Orinus thoroldii_ (Poaceae), endemic to the western Qinghai-Tibetan Plateau in China. _Biochemical Systematics and Ecology_, _59_, 159–167. https://doi.org/10.1016/j.bse.2015.01.014

Ma, Z., Liu, H., Mi, Z., Zhang, Z., Wang, Y., Xu, W., … He, J.-S. (2017). Climate warming reduces the temporal stability of plant community biomass production. _Nature Communications_, _8_, 15378. https://doi.org/10.1038/ncomms15378

Madriñán, S., Cortés, A. J., & Richardson, J. E. (2013). Páramo is the world's fastest evolving and coolest biodiversity hotspot. _Frontiers in Genetics_, _4_, 192. https://doi.org/10.3389/fgene.2013.00192

Maréchaux, I., Rodrigues, A. S. L., & Charpentier, A. (2016). The value of coarse species range maps to inform local biodiversity conservation in a global context. _Ecography_, _40_, 1166–1176. https://doi.org/10.1111/ecog.02598

Mayr, E. (1942). _Systematics and the origin of species, from the viewpoint of a zoologist_. New York, NY: Columbia University Press.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. _Nature_, _403_(6772), 853–858.

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetical variation in terms of restriction endonucleases. _Proceedings of the National Academy of Science of the United States of America_, _76_, 5269–5273.

Peakall, R., & Smouse, P. (2012). GenAlEx 6.5: Genetic analysis in Excel: Population genetic software for teaching and research – An update. _Bioinformatics_, _28_, 2537–2539.

Peterson, P. M., Romaschenko, K., & Arrieta, Y. H. (2016). A molecular phylogeny and classification of the Cynodonteae (Poaceae: Chloridoideae) with four new genera: _Orthacanthus_, _Triplasiella_, _Tripogonella_, and _Zaqiqah_; three new subtribes: Dactylocteniinae, Orininae, and Zaqiqahinae; and a subgeneric classification of _Distichlis_. _Taxon_, _65_, 1263–1287.

Pluess, A. R., & Stöcklin, J. (2004). Population genetic diversity of the clonal plant _Geum reptans_ (Rosaceae) in the Swiss Alps. _American Journal of Botany_, _91_, 2013–2021. https://doi.org/10.3732/ajb.91.12.2013

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. _Genetics_, _155_, 945–959.

Rahimmalek, M., Tabatabaei, B. E. S., Arzani, A., & Etemadi, N. (2009). Assessment of genetic diversity among and within _Achillea_ species using amplified fragment length polymorphism (AFLP). _Biochemical Systematics and Ecology_, _37_, 354–361. https://doi.org/10.1016/j.bse.2009.06.002

Ren, G. P., Conti, E., & Salamin, N. (2015). Phylogeny and biogeography of _Primula_ sect. _Armerina_: Implications for plant evolution under climate change and the uplift of the Qinghai-Tibet Plateau. _BMC Evolutionary Biology_, _15_, 161. https://doi.org/10.1186/s12862-015-0445-7

Risser, P. (1987). Landscape ecology: State of the art. In M. Turner (Ed.), _Landscape heterogeneity and disturbance_ (pp. 3–14). New York, NY: Springer.

Rohlf, F. (2000). _NTSYS-pc version 2.1: Numerical Taxonomy and Multivariance Analysis System_.

Sedlacek, J., Cortés, A. J., Wheeler, J., Bossdorf, O., Hoch, G., Klápště, J., … van Kleunen, M. (2016). Evolutionary potential in the Alpine: Trait heritabilities and performance variation of the dwarf willow _Salix herbacea_ from different elevations and microhabitats. _Ecology and Evolution_, _6_, 3940–3952.

Sedlacek, J., Wheeler, J. A., Cortés, A. J., Bossdorf, O., Hoch, G., Lexer, C., … Rixen, C. (2015). The response of the alpine dwarf shrub _Salix herbacea_ to altered snowmelt timing: Lessons from a multi-site transplant experiment. _PLoS ONE_, _10_(4), e0122395. https://doi.org/10.1371/journal.pone.0122395

Shi, Y. F. (2002). Characteristics of late Quaternary monsoonal glaciation on the Tibetan Plateau and in East Asia. _Quaternary International_, _97_, 79–91. https://doi.org/10.1016/S1040-6182(02)00053-8

Shi, Y., Li, J., & Li, B. (1998). _Uplift and environmental changes of Qinghai-Tibetan Plateau in the late Cenozoic_. Guangzhou, China: Guangdong Science and Technology Press.

Soreng, R. J., Peterson, P. M., Romaschenko, K., Davidse, G., Zuloaga, F. O., Judziewicz, E. J., … Morrone, O. (2017). A worldwide phylogenetic classification of the Poaceae (Gramineae) II: An update and a comparison of two 2015 classifications. _Journal of Systematics and Evolution_, _55_, 259–290. https://doi.org/10.1111/jse.12262

Su, X., Liu, Y. P., Wu, G. L., Luo, W. C., & Liu, J. Q. (2017). A taxonomic revision of _Orinus_ (Poaceae) with a new species, _O. intermedius_, from the Qinghai-Tibet Plateau. _Novon_, _25_, 206–213.

Su, X., Wu, G. L., Li, L. L., & Liu, J. Q. (2015). Species delimitation in plants using the Qinghai-Tibetan Plateau endemic _Orinus_ (Poaceae: Tridentinae) as an example. _Annals of Botany_, _116_, 35–48.

Su, X., Yue, W., & Liu, J. Q. (2013). Germplasm collection and preservation of _Orinus_ (Poaceae) in the Qinghai-Tibet Plateau. _Plant Diversity Research_, _35_, 343–347.

Wang, W., Chen, L., Yang, P., Hou, L., He, C., Gu, Z., & Liu, Z. (2007). Assessing genetic diversity of populations of topmouth culter (_Culter alburnus_) in China using AFLP markers. _Biochemical Systematics and Ecology_, _35_, 662–669. https://doi.org/10.1016/j.bse.2007.04.008

Wen, J., Zhang, J. Q., Nie, Z. L., Zhong, Y., & Sun, H. (2014). Evolutionary diversifications of plants on the Qinghai-Tibetan Plateau. *Frontiers in Genetics*, 5, 4. https://doi.org/10.3389/fgene.2014.00004

Wilcox, B. P., Sorice, M. G., & Young, M. H. (2011). Dryland ecohydrology in the Anthropocene: Taking stock of human–ecological interactions. *Geography Compass*, 5(3), 112–127. https://doi.org/10.1111/j.1749-8198.2011.00413.x

Willis, K. J., Bennett, K. D., & Birks, H. J. B. (2009). Variability in thermal and UV-B energy fluxes through time and their influence on plant diversity and speciation. *Journal of Biogeography*, 36, 1630–1644. https://doi.org/10.1111/j.1365-2699.2009.02102.x

Wu, S. G., Yang, Y. P., & Fei, Y. (1995). On the flora of the alpine region in the Qinghai-Xizang (Tibet) plateau. *Acta Botanica Yunnanica*, 17, 233–250.

Wu, Y. (2008). *The vascular plants and their eco-geographical distribution of the Qinghai-Tibetan Plateau*. Beijing, China: Science Press.

Xue, X., Wang, Y., Korpelainen, H., & Li, C. (2005). Assessment of AFLP-based genetic variation in the populations of *Picea asperata*. *Silvae Genetica*, 54, 24–30. https://doi.org/10.1515/sg-2005-0004

Yang, F. S., Li, Y. F., Ding, X., & Wang, X. Q. (2008). Extensive population expansion of *Pedicularis longiflora* (Orobanchaceae) on the Qinghai-Tibetan Plateau and its correlation with the Quaternary climate change. *Molecular Ecology*, 17, 5135–5145.

Yang, M. X., Wang, S. L., Yao, T. D., Gou, X. H., Lu, A. X., & Guo, X. J. (2004). Desertification and its relationship with permafrost degradation in Qinghai-Xizang (Tibet) Plateau. *Cold Region Science and Technology*, 39, 47–53. https://doi.org/10.1016/j.coldregions.2004.01.002

Yap, I., & Nelson, R. (1996). Winboot: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. *International Rice Research Institute Discussion Paper Series*, 14, 1–22.

Yeh, F., Yang, R., & Boyle, T. (1999). *POPGENE: Microsoft Windows-based freeware for population genetic analysis. Version 1.31*. Edmonton, Canada: University of Alberta.

Yi, S. H., Zhou, Z. Y., Ren, S. L., Xu, M., Qin, Y., Chen, S. Y., & Ye, B. S. (2011). Effects of permafrost degradation on alpine grassland in a semi-arid basin on the Qinghai-Tibetan Plateau. *Environmental Research Letters*, 6, 45403–45409. https://doi.org/10.1088/1748-9326/6/4/045403

Yu, F., Dong, M., & Krüsi, B. (2004). Clonal integration helps *Psammochloa villosa* survive sand burial in an inland dune. *New Phytologist*, 162(3), 697–704. https://doi.org/10.1111/j.1469-8137.2004.01073.x

Zhang, Q., Chiang, T. Y., George, M., Liu, J. Q., & Abbott, R. J. (2005). Phylogeography of the Qinghai-Tibetan Plateau endemic *Juniperus przewalskii* (Cupressaceae) inferred from chloroplast DNA sequence variation. *Molecular Evolution*, 14, 3513–3524. https://doi.org/10.1111/j.1365-294X.2005.02677.x

Zheng, B., & Nat, R. (1998). On the problem of Quaternary glaciations, and the extent and patterns of Pleistocene ice cover in the Qinghai-Xizang (Tibet) Plateau. *Quaternary International*, 45, 109–122.

Zhou, S. L., & Qian, P. (2003). Matrix Generator (MG): A program for creating 0/1 matrix from DNA fragments. *Acta Botanica Sinica*, 45, 766–769.

Zuo, Y. J., Wen, J., Ma, J. S., & Zhou, S. L. (2015). Evolutionary radiation of the *Panax bipinnatifidus* species complex (Araliaceae) in the Sino-Himalayan region of eastern Asia as inferred from AFLP analysis. *Journal of Systematics and Evolution*, 53, 210–220.

APPENDIX A

TABLE A1 Posterior probabilities for five genetic models tested in NewHybrids for individual samples of species of *Orinus*

Putative Species	Population Number (Figure 2)	1.000/0.000/0.000/0.000/0.000 (O. thoroldii)	0.000/0.000/0.000/0.000/1.000 (O. kokonoricus)	0.000/0.500/0.500/0.000 (true hybrid)	0.500/0.250/0.250/0.000 (backcross into O. thoroldii)	0.000/0.250/0.250/0.500 (backcross into O. kokonoricus)
O. kokonoricus	4	0	1	0	0	0
O. kokonoricus	4	0	1	0	0	0
O. kokonoricus	4	0	1	0	0	0
O. kokonoricus	4	0	1	0	0	0
O. kokonoricus	4	0	1	0	0	0
O. kokonoricus	5	0	1	0	0	0
O. kokonoricus	5	0	1	0	0	0
O. kokonoricus	5	0	1	0	0	0
O. kokonoricus	5	0	1	0	0	0
O. kokonoricus	5	0	1	0	0	0
O. kokonoricus	8	0	1	0	0	0
O. kokonoricus	8	0	1	0	0	0
O. kokonoricus	8	0	1	0	0	0
O. kokonoricus	8	0	1	0	0	0
O. kokonoricus	8	0	1	0	0	0
O. kokonoricus	9	0	1	0	0	0
O. kokonoricus	9	0	1	0	0	0
O. kokonoricus	9	0	1	0	0	0
O. kokonoricus	9	0	1	0	0	0
O. kokonoricus	9	0	1	0	0	0
O. kokonoricus	13	0	1	0	0	0
O. kokonoricus	13	0	1	0	0	0
O. kokonoricus	13	0	1	0	0	0
O. kokonoricus	13	0	1	0	0	0
O. kokonoricus	13	0	1	0	0	0
O. kokonoricus	1	0	1	0	0	0
O. kokonoricus	1	0	1	0	0	0
O. kokonoricus	1	0	1	0	0	0
O. kokonoricus	1	0	1	0	0	0
O. kokonoricus	1	0	1	0	0	0
O. kokonoricus	12	0	1	0	0	0

(Continues)

TABLE A1 (Continued)

Putative Species	Population Number (Figure 2)	1.000/0.000/0.000/0.000 (O. thoroldii)	0.000/0.000/0.000/1.000 (O. kokonoricus)	0.000/0.500/0.500/0.000 (true hybrid)	0.500/0.250/0.250/0.000 (backcross into O. thoroldii)	0.000/0.250/0.250/0.500 (backcross into O. kokonoricus)
O. kokonoricus	12	0	1	0	0	0
O. kokonoricus	12	0	1	0	0	0
O. kokonoricus	12	0	1	0	0	0
O. kokonoricus	12	0	1	0	0	0
O. kokonoricus	11	0	1	0	0	0
O. kokonoricus	11	0	1	0	0	0
O. kokonoricus	11	0	1	0	0	0
O. kokonoricus	11	0	1	0	0	0
O. kokonoricus	11	0	1	0	0	0
O. kokonoricus	10	0	1	0	0	0
O. kokonoricus	10	0	1	0	0	0
O. kokonoricus	10	0	1	0	0	0
O. kokonoricus	10	0	1	0	0	0
O. kokonoricus	10	0	1	0	0	0
O. kokonoricus	6	0	1	0	0	0
O. kokonoricus	6	0	1	0	0	0
O. kokonoricus	6	0	1	0	0	0
O. kokonoricus	6	0	1	0	0	0
O. kokonoricus	6	0	1	0	0	0
O. kokonoricus	2	0	1	0	0	0
O. kokonoricus	2	0	1	0	0	0
O. kokonoricus	2	0	1	0	0	0
O. kokonoricus	2	0	1	0	0	0
O. kokonoricus	2	0	1	0	0	0
O. kokonoricus	3	0	1	0	0	0
O. kokonoricus	3	0	1	0	0	0
O. kokonoricus	3	0	1	0	0	0
O. kokonoricus	3	0	1	0	0	0
O. kokonoricus	3	0	1	0	0	0
O. kokonoricus	14	0	1	0	0	0

(Continues)

TABLE A1 (Continued)

Putative Species	Population Number (Figure 2)	1.000/0.000/0.000/0.000 (O. thoroldii)	0.000/0.000/0.000/1.000 (O. kokonoricus)	0.000/0.500/0.500/0.000 (true hybrid)	0.500/0.250/0.250/0.000 (backcross into O. thoroldii)	0.000/0.250/0.250/0.500 (backcross into O. kokonoricus)
O. kokonoricus	14	0	0.99999	0	0	0.00001
O. kokonoricus	14	0	1	0	0	0
O. kokonoricus	14	0	1	0	0	0
O. kokonoricus	14	0	1	0	0	0
O. kokonoricus	17	0	1	0	0	0
O. kokonoricus	17	0	1	0	0	0
O. kokonoricus	17	0	0.99735	0	0	0.00265
O. kokonoricus	17	0	1	0	0	0
O. kokonoricus	17	0	1	0	0	0
O. kokonoricus	18	0	1	0	0	0
O. kokonoricus	18	0	1	0	0	0
O. kokonoricus	18	0	1	0	0	0
O. kokonoricus	18	0	1	0	0	0
O. kokonoricus	19	0	1	0	0	0
O. kokonoricus	19	0	1	0	0	0
O. kokonoricus	19	0	1	0	0	0
O. kokonoricus	19	0	1	0	0	0
O. kokonoricus	19	0	1	0	0	0
O. kokonoricus	16	0	1	0	0	0
O. kokonoricus	16	0	1	0	0	0
O. kokonoricus	16	0	1	0	0	0
O. kokonoricus	16	0	1	0	0	0
O. kokonoricus	15	0	1	0	0	0
O. kokonoricus	15	0	1	0	0	0
O. kokonoricus	15	0	1	0	0	0
O. kokonoricus	15	0	1	0	0	0
O. kokonoricus	7	0	1	0	0	0
O. kokonoricus	7	0	1	0	0	0
O. kokonoricus	7	0	1	0	0	0

(Continues)

TABLE A1 (Continued)

Putative Species	Population Number (Figure 2)	1.000/0.000/0.000/0.000 (O. thoroldii)	0.000/0.000/0.000/1.000 (O. kokonoricus)	0.000/0.500/0.500/0.000 (true hybrid)	0.500/0.250/0.250/0.000 (backccross into O. thoroldii)	0.000/0.250/0.250/0.500 (backccross into O. kokonoricus)
O. kokonoricus	7	0	1	0	0	0
O. kokonoricus	7	0	1	0	0	0
O. intermedius	22	0	0.99999	0	0	0.00001
O. intermedius	22	0	1	0	0	0
O. intermedius	22	0	1	0	0	0
O. intermedius	22	0	1	0	0	0
O. intermedius	23	0	1	0	0	0
O. intermedius	23	0	0.99918	0	0	0.00082
O. intermedius	23	0	1	0	0	0
O. intermedius	23	0	1	0	0	0
O. intermedius	23	0	0.99999	0	0	0.00001
O. intermedius	20	0	1	0	0	0
O. intermedius	20	0	1	0	0	0
O. intermedius	20	0	1	0	0	0
O. intermedius	20	0	1	0	0	0
O. intermedius	25	0	1	0	0	0
O. intermedius	25	0	1	0	0	0
O. intermedius	25	0	1	0	0	0
O. intermedius	25	0	1	0	0	0
O. intermedius	25	0	1	0	0	0
O. intermedius	26	0	1	0	0	0
O. intermedius	26	0	1	0	0	0
O. intermedius	26	0	1	0	0	0
O. intermedius	26	0	1	0	0	0
O. intermedius	28	0	0.88592	0	0	0.11408
O. intermedius	28	0	0	0	0	1
O. intermedius	28	0	0.00012	0	0	0.99988
O. intermedius	28	0	0	0	0	1
O. intermedius	21	0	1	0	0	0

(Continues)

TABLE A1 (Continued)

Putative Species	Population Number (Figure 2)	1.000/0.000/0.000/0.000 (O. thoroldii)	0.000/0.000/0.000/1.000 (O. kokonoricus)	0.000/0.500/0.500/0.000 (true hybrid)	0.500/0.250/0.250/0.000 (backcross into O. thoroldii)	0.000/0.250/0.250/0.500 (backcross into O. kokonoricus)
O. intermedius	21	0	1	0	0	0
O. intermedius	21	0	1	0	0	0
O. intermedius	24	0	0.99743	0	0	0.00257
O. intermedius	24	0	1	0	0	0
O. intermedius	24	0	1	0	0	0
O. intermedius	24	0	1	0	0	0
O. intermedius	27	0	0	0	0	1
O. intermedius	27	0	0	0	0	1
O. intermedius	27	0	0	0	0	1
O. intermedius	27	0	0	0	0	1
O. intermedius	27	0	0	0	0	1
O. thoroldii	29	0.11855	0	0	0.88145	0
O. thoroldii	29	1	0	0	0	0
O. thoroldii	29	0.00002	0	0	0.99998	0
O. thoroldii	29	0.95236	0	0	0.04764	0
O. thoroldii	30	0.98688	0	0	0.01312	0
O. thoroldii	30	1	0	0	0	0
O. thoroldii	30	0.00092	0	0	0.99908	0
O. thoroldii	30	0.99997	0	0	0.00003	0
O. thoroldii	30	0.94228	0	0	0.05772	0
O. thoroldii	31	1	0	0	0	0
O. thoroldii	31	1	0	0	0	0
O. thoroldii	31	1	0	0	0	0
O. thoroldii	31	1	0	0	0	0
O. thoroldii	31	1	0	0	0	0
O. thoroldii	33	1	0	0	0	0
O. thoroldii	33	1	0	0	0	0
O. thoroldii	33	0.99999	0	0	0.00001	0
O. thoroldii	33	1	0	0	0	0
O. thoroldii	33	1	0	0	0	0

TABLE A1 (Continued)

Putative Species	Population Number (Figure 2)	1.000/0.000/0.000/0.000 (O. thoroldii)	0.000/0.000/0.000/1.000 (O. kokonoricus)	0.000/0.500/0.500/0.000 (true hybrid)	0.500/0.250/0.250/0.000 (backcross into O. thoroldii)	0.000/0.250/0.250/0.500 (backcross into O. kokonoricus)
O. thoroldii	37	1	0	0	0	0
O. thoroldii	37	1	0	0	0	0
O. thoroldii	37	1	0	0	0	0
O. thoroldii	37	1	0	0	0	0
O. thoroldii	37	1	0	0	0	0
O. thoroldii	39	1	0	0	0	0
O. thoroldii	39	1	0	0	0	0
O. thoroldii	39	1	0	0	0	0
O. thoroldii	39	1	0	0	0	0
O. thoroldii	41	1	0	0	0	0
O. thoroldii	41	1	0	0	0	0
O. thoroldii	41	1	0	0	0	0
O. thoroldii	41	1	0	0	0	0
O. thoroldii	41	1	0	0	0	0
O. thoroldii	42	1	0	0	0	0
O. thoroldii	42	1	0	0	0	0
O. thoroldii	42	1	0	0	0	0
O. thoroldii	42	1	0	0	0	0
O. thoroldii	42	1	0	0	0	0
O. thoroldii	43	1	0	0	0	0
O. thoroldii	43	1	0	0	0	0
O. thoroldii	43	1	0	0	0	0
O. thoroldii	43	1	0	0	0	0
O. thoroldii	43	1	0	0	0	0
O. thoroldii	44	1	0	0	0	0
O. thoroldii	44	1	0	0	0	0
O. thoroldii	44	1	0	0	0	0
O. thoroldii	44	1	0	0	0	0
O. thoroldii	44	1	0	0	0	0
O. thoroldii	45	1	0	0	0	0
O. thoroldii	45	1	0	0	0	0

(Continues)

TABLE A1 (Continued)

Putative Species	Population Number (Figure 2)	1.000/0.000/0.000/0.000 (O. thoroldii)	0.000/0.000/0.000/1.000 (O. kokonoricus)	0.000/0.500/0.500/0.000 (true hybrid)	0.500/0.250/0.250/0.000 (backcross into O. thoroldii)	0.000/0.250/0.250/0.500 (backcross into O. kokonoricus)
O. thoroldii	45	1	0	0	0	0
O. thoroldii	45	1	0	0	0	0
O. thoroldii	45	1	0	0	0	0
O. thoroldii	46	1	0	0	0	0
O. thoroldii	46	1	0	0	0	0
O. thoroldii	46	1	0	0	0	0
O. thoroldii	46	1	0	0	0	0
O. thoroldii	47	1	0	0	0	0
O. thoroldii	47	1	0	0	0	0
O. thoroldii	47	1	0	0	0	0
O. thoroldii	47	1	0	0	0	0
O. thoroldii	48	1	0	0	0	0
O. thoroldii	48	1	0	0	0	0
O. thoroldii	48	1	0	0	0	0
O. thoroldii	48	1	0	0	0	0
O. thoroldii	40	1	0	0	0	0
O. thoroldii	40	1	0	0	0	0
O. thoroldii	40	1	0	0	0	0
O. thoroldii	40	1	0	0	0	0
O. thoroldii	38	1	0	0	0	0
O. thoroldii	38	1	0	0	0	0
O. thoroldii	38	1	0	0	0	0
O. thoroldii	38	1	0	0	0	0
O. thoroldii	38	1	0	0	0	0
O. thoroldii	36	1	0	0	0	0
O. thoroldii	36	1	0	0	0	0

(Continues)

TABLE A1 (Continued)

Putative Species	Population Number (Figure 2)	1.000/0.000/0.000/0.000 (O. thoroldii)	0.000/0.000/0.000/1.000 (O. kokonoricus)	0.000/0.500/0.500/0.000 (true hybrid)	0.500/0.250/0.250/0.000 (backcross into O. thoroldii)	0.000/0.250/0.250/0.500 (backcross into O. kokonoricus)
O. thoroldii	36	1	0	0	0	0
O. thoroldii	36	1	0	0	0	0
O. thoroldii	36	1	0	0	0	0
O. thoroldii	34	1	0	0	0	0
O. thoroldii	34	1	0	0	0	0
O. thoroldii	34	1	0	0	0	0
O. thoroldii	34	1	0	0	0	0
O. thoroldii	34	1	0	0	0	0
O. thoroldii	35	1	0	0	0	0
O. thoroldii	35	1	0	0	0	0
O. thoroldii	35	1	0	0	0	0
O. thoroldii	35	1	0	0	0	0
O. thoroldii	35	1	0	0	0	0
O. thoroldii	32	1	0	0	0	0
O. thoroldii	32	1	0	0	0	0
O. thoroldii	32	1	0	0	0	0
O. thoroldii	32	1	0	0	0	0
O. thoroldii	32	1	0	0	0	0