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Fine-scale genetic diversity and genet dynamics of the fairy ring fungus *Floccularia luteovirens* on the Qinghai–Tibet plateau

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ABSTRACT

The fungus *Floccularia luteovirens* is mainly distributed in the alpine meadows of the Qinghai–Tibet plateau. Its fruit bodies tend to form fairy rings with a visible stimulating zone. Our previous studies have investigated the large-scale genetic structure among wild populations of F. luteovirens, but the mechanisms underlying the current genotype distribution pattern remain unknown. The balance between sexual and asexual reproduction affects the establishment and structure of populations. Measuring genet size and density is an effective approach to investigating the reproduction strategies of this species. In the current study, 234 fruit bodies and 79 soil samples were collected from three sampling sites over 3y, revealing that *F. luteovirens* exhibits relatively large genets. Very few new genets were detected over the 3y, illustrating that this species relies more on vegetative growth and can persist for long periods underground as mycelia. Moreover, the underground genet data showed a close relationship with the above-ground genet data. Our study found limited fine-scale gene flow, contrary to our previous large-scale genetic study of F. luteovirens, the present study found limited fine-scale gene flow of this species. The commercial trade of F. luteovirens fruit bodies by humans probably enhances the gene flow over QTP.

1. Introduction

Genetic structure analyses of fungal species are essential to better understand their genetic diversity and genotype distribution patterns (Rinaldi et al., 2008). However, most published studies concerning the genetic diversity and population structure of fungal species have only focused on the genotype distribution over a broad geological scale (Pringle et al., 2010; Xing et al., 2014; Duong et al., 2015; Rivera et al., 2015; Patrick and Jeffrey, 2016; Castillo et al., 2018). Few researchers have studied the mechanisms that result in the current genotype distributions of fungal species. The genotype distribution pattern of a specific fungal species depends on its ecological strategies (i.e., reproduction strategies and dispersibility). However, it is difficult to investigate the reproduction strategies of fungal species directly from wild populations (Wadud et al., 2014). The life cycle of fungi is composed of two components: sexual reproduction by spores and asexual reproduction by mycelia and asexual spores. The balance between sexual and asexual reproduction affects the establishment and structure of populations (Moore and Frazer, 2002). We can estimate the reproductive strategies of fungi by measuring the density and size of individual genets (genetically unique fungal individuals) (Douhan et al., 2011). Fungi that form large genets tend to spread vegetatively, while fungi that are genotypically diverse locally and produce small genets more often, rely on sexual reproduction. Because vegetative spread does not create new genotypes, it results in the enlargement of existing genets without forming new genets; in contrast, sexual reproduction involving meiosis and fusion of monokaryotic hyphae results in new genets (Bhatnagar et al., 2018).

Several studies have investigated the genet distribution pattern of fungi, demonstrating that many species have small genets (the diameter

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of genet is only a few meters) with high levels of genetic diversity (Fiore-Donno and Martin, 2001; Yu et al., 2004; Gryta et al., 2010). In contrast, other species tend to produce larger genets, sometimes measuring tens to hundreds of meters (Bonello et al., 1998; Dahlberg and Stenlid, 2010). Their large genets suggest that those species can expand vegetatively and persist for long periods of time below ground. Biotic and abiotic factors, including human activities, soil physical characteristics, and the age of host plants, may also influence the size and density of the genets of specific species (Dahlberg, 1997; Wang et al., 2015). Fine-scale genet distribution and diversity data have also been used to assess local gene flow. Most fungal spores fall from fruit bodies down to the nearby ground; thus, spatial clustering of similar genotypes is frequently observed in fungi (Bonello et al., 1998). The increase of genotypic similarity with decrease of the geographic distance between genets indicates a restricted gene flow. Furthermore, a lack of genotype clustering predicts unrestricted gene flow (Kretzer et al., 2005; Kathleen et al., 2017).

Floccularia luteovirens is associated with *Kobresia humilis* and *Carex* spp. in the alpine meadows of the Qinghai–Tibet plateau (QTP) (Wang et al., 2005; Wang and Xie, 2015). The fruit body is edible and famous in China for its unique flavor. This species can also stimulate mineral nutrient absorption of host plants and often forms fairy rings with a visible dark green stimulation zone (Cao et al., 2020). Our previous study found that the soil microbial community under the stimulated zone is significantly different from that of other zones; this is caused by the distinct soil properties changed by *F. luteovirens* (Xing et al., 2018). The phylogenetic analysis based on protein-coding genes showed that the ancient populations of *F. luteovirens* moved to the edge of the QTP during the Last Glacial Maximum. Additionally, the present distribution of the species on the QTP has resulted from recent population expansion; this is the historical factor influencing the current distribution pattern of *F. luteovirens* (Xing et al., 2017). A large-scale genetic diversity and population structure analysis (of 404 individuals from 23 wild populations around the QTP) based on microsatellite loci found low genetic differentiation and high gene flow among populations, although the distances between populations were 51.1–751.5 km (Xing et al., 2014).

The objective of the present study was to clarify the mechanism by which the current genotype distribution pattern of *F. luteovirens* was formed. This was undertaken through a fine-scale (i.e., single fairy ring) genetic structure analysis based on newly developed SSR markers, an investigation of local gene flow of *F. luteovirens*, and examination of the dynamics and persistence of the genets over 3y. Most recent studies of the fine-scale genetic diversity of fungi have only concerned the visible aboveground reproductive structures (i.e., fruit bodies) (Horton and Bruns, 2001). However, fungal fruit body formation is limited to a few days during the fruiting season, leading to inaccurate or biased results of fine-scale population genetics studies, overlooking the abundance of fungal biomass (i.e., fungal mycelia) below ground, which is undetected when only fruit bodies are sampled. Therefore, soil samples containing *F. luteovirens* mycelia were also included in the last sampling year (2017) of our study. The questions addressed in the present study were: (1) What is the fine-scale above-ground genet distribution pattern of *F. luteovirens*? (2) Is the method used in the study (extracting DNA from soil samples and testing by SSR) reliable for detecting underground genets? (3) Is there clustering of similar genotypes in *F. luteovirens* within a short distance? (4) Is there restricted gene flow of *F. luteovirens* on a fine distance scale? (5) What is the expansion rate of single fairy rings based on the distribution of fruit bodies? We hypothesized that the high gene flow and low genetic variation observed among populations throughout the QTP in our former studies were not caused by long-distance dispersal of *F. luteovirens* spores; rather, it is more likely caused by fruit body trade across the QTP.

2. Materials and methods

2.1. Study sites

The study species, *F. luteovirens,* is distributed across the alpine meadows of the QTP. The sampling sites were near Qilian Mountain, where the dominant plant species are *Kobresia humilis, Elymus nutans, Stipa aliena, Festuca ovina, Polygonum viviparum,* and *Leontopodium japonicum.* The 50-y means for temperature and rainfall in the area are −2.93 °C and 399.8 mm, respectively (Xing et al., 2018). Three sites (each measuring 700 square meters approximately) were included in the study. Site A (99°29′26.10″ N, 38°31′29.63″ E; 3373 m above sea level) contained three *F. luteovirens* fairy rings (F1, F2, and F3), while sites B (99°20′12.01″ N, E 38°37′06.89″ E; 3477 m above sea level) and C (99°32.43.40″ N, 38°27′48.80″ E; 3477 m above sea level) each contained only a single *F. luteovirens* fairy ring. The distances separating sites A from B, A from C, and B from C were 18.92 km, 6.197 km, and 24.37 km, respectively.

2.1.1. Fruit body and soil sampling

We collected every single *F. luteovirens* fruit body weekly at the three sampling sites (from a total of five fairy rings) during the fruiting season (July to October) from 2015 to 2017. All the *F. luteovirens* fruit bodies collected from each site were numbered and mapped according to their latitudinal and longitudinal coordinates and stored in a single sealed bag with color-changing silica gel. Over the 3y period, 234 *F. luteovirens* fruit bodies were collected from the five fairy rings at the three sites (not only from beneath the fruit bodies and stimulated zone, but also the areas inside and outside of the fairy ring). Soil samples (10 cm × 10 cm × 10 cm) were collected randomly in the last sampling year (2017) after fruit body collection to avoid affecting the generation of fruit bodies in that sampling year. After sampling, the surface (0–0.5 cm) soil was removed carefully to prevent the overestimation of the below-ground genets caused by contamination with material from the above-ground spores and mycelia. All soil samples were sieved through a 2-mm soil screen before DNA extraction.

2.2. DNA extraction and PCR

Genomic DNA of fruit bodies was extracted using the E.Z.N.A.® Fungal DNA Kit (Omega Bio-Tek, Norcross, GA, USA), and nine newly developed SSR markers (GSSR3L, GSSR7L, GSSR9L, GSSR11L, GSSR33L, GSSR36L, GSSR46L, GSSR47L, GSSR49L) were used in this study (Table S1) (Xing et al., 2019a,b). PCR was performed in 30 μL of solution containing 20 ng of genomic DNA, 400 mM 10 × PCR buffer, 3.0 mM of $MgCl_2$, 0.4 mM of dNTPs, 400 nM of each primer, and 1 U of Taq DNA polymerase (Takara, Dalian, China) (Xing et al., 2019a,b). The PCR program was as follows: initial denaturation for 5 min at 95 °C; 35 cycles of 30 s each at 95 °C, 30 s at the primer-specific annealing temperature (Table S1), and 40 s at 72 °C; and a final extension for 10 min at 72 °C. The PCR products were confirmed by electrophoresis in 1% agarose gels and purification of the obtained PCR product with a purification kit (Qiagen, Crawley, UK) followed by sequencing (Sangon Biotech Co., Ltd. Shanghai). All the sequences were submitted to GenBank under the accession numbers MW829813–MW830042 (fruit bodies) and MW924136–MW924168 (soil samples).

Genomic DNA extraction of soil samples was conducted with a FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, United States). The PCR method was the same as the one described above. However, most soil samples yielded poor PCR results owing to the low DNA concentration and complex DNA mixtures extracted from soil. Thus, we ran a second round of PCR reactions in which the first-round PCR products were used as the template. Then, the PCR products were sequenced (Sangon Biotech Co., Ltd. Shanghai) using an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). In our previous studies, we developed twelve primer pairs that were used in the

present study and found that the locus GSSR47L includes polymorphisms with SSR variants that cannot be detected by traditional methods (i.e., scoring of SSR fragment length) (Xing et al., 2019a,b). Thus, to obtain the most accurate results possible, we sequenced all PCR products instead of simply scoring the fragments by length.

2.3. Data analysis

The raw sequencing data were reviewed using Bioedit version 7.0 (Hall, 1999). Nine SSR fragments of each sample were concatenated into a single sequence for each sample. Multiple sequences were aligned using the program Clustal X v.2.0 (Thompson et al., 1997). The program DnaSP v.6.0 was used to identify the genotypes for all the fruit bodies and then calculate the gene diversity within the population (H_s), the coefficient of gene differentiation (G_{ST}), and the genetic differentiation coefficient (F_{ST}). The number of migrants per generation was estimated using the equation $N_m = 0.25(1 - F_{ST})/F_{ST}$. All fruit bodies with a unique genotype were considered to correspond to a single genet. The genet size was estimated by measuring the maximum distance between two fruit bodies with the same genotype. To estimate the below-ground genet size, within the same fairy ring, the distances between two soil samples with the same genotype were calculated.

We used the software package GenAlEx 6 (Peakall and Smouse, 2012) to identify significant genetic structure and spatial clustering of genotypes among genets in the three years. Genetic and geographic distance matrices were calculated for the analysis, and confidence intervals (95%) of r were generated based on resampling of 10,000 bootstrap replicates. The null distribution of r values was calculated from 999 permutations for each distance class. For the r values above or below the confidence range, significant positive autocorrelation (i.e., clustering of similar genotypes) was conformed. The Mantel test was used to test the relationship between the spatial distance matrix and the genetic distance matrix of SSR sequences using the mantel package in R (Dixon and VEGAN, 2010).

3. Results

All the observed fruit bodies were distributed only in the stimulating zone of the fairy ring. A total of 234 *F. luteovirens* fruit bodies were collected from the three sites over three years. In 2015, 2016, and 2017, the samples from all three sites included 74, 68, and 92 fruit bodies, respectively. We collected 172, 35, and 27 fruit bodies from sites A, B, and C, respectively (Table 1). A total of 230 combined SSR sequences were generated from 234 *F. luteovirens* fruit bodies, the lengths of the sequences were 144–154 bp, and a total of 13 genotypes were identified. The numbers of genets identified from each site were nine (site A), two (site B), and five (site C) (Fig. 1 and Fig. 2). The maximum fruit bodies per genet were 37 (site A, 2017), 12 (site B, 2015), and 15 (site C, 2017) (Table 2). The genet size of each fairy ring increased over time. For example, the genet sizes of fairy ring F1 from site A were 5.08 ± 0.76 m in 2015, 5.50 ± 0.54 m in 2016, and 5.82 ± 0.51 m in 2017 (Fig. 1), consistent with an expansion rate of 0.37 m/y (Table 2); however, the expansion rate of site B and C were −0.8 and −2.19 m/y (Table 2). We detected 80, 63, and 39 fruit bodies with genotypes H2, H1, and H5. In contrast, only one fruit body each was found with genotypes H10 and H13 (Table 3). There were only four genotypes detected across all three sampling years (genotypes H1 and H2 at site A, genotype H5 at site B, and genotype H12 at site C); most of the other genotypes (e.g., H3, H6, H7, and H9) were only found in two years in each site. Genotypes H6, H7, H8, H9, and H10 were unique to site A, and genotype H11 was only detected at site B in 2015 and 2016, but not in 2017. Genotype H12 was unique to site C across all the sampling years (Table 3). We collected 79 soil cores (40 soil cores from site A, 11 from site B, and 28 from site C) in the last sampling year (2017) (Table 1) and obtained 33 combined sequences successfully (Table 2). The five genotypes obtained from the soil samples matched the fruit bodies nearby (i.e., no new genets were found underground) (Table 3), but the underground genet number and underground genets per fairy ring were lower than the above-ground genets from fruit bodies.

For the above-ground genets, the overall mean F_{ST} among sites was between 0.32 and 0.75 across the three sampling years, and the N_m values were between 0.14 and 0.75 (Table 4). Moreover, the H_s values were between 0.33 and 0.63, and the G_{ST} values were between 0.17 and 0.46, with an average value of 0.30. For the below-ground genets, the mean values of H_s (0.49) and N_m (0.31) were lower than those for the above-ground genets (Table 4). The Mantel test showed a positive significant relationship between genetic distance and geographic distance in 2015 ($r = 0.67$ $p = 0.001$), 2016 ($r = 0.68$, $p = 0.001$), and 2017 ($r = 0.33$, $p = 0.001$), indicating limited fine-scale gene flow. Spatial autocorrelation analyses also revealed similar results over short distance classes (i.e., 0–5 m from 2015 to 2017), indicating spatial genetic structure and genet clustering at a fine scale (Fig. 3).

Table 1
The *Floccularia luteovirens* fruit body and soil samples collected in the three sampling years.

Sampling year	Sample Collected		
	site A	Site B	Site C
2015	56	13	5
2016	51	11	6
2017	65	11	16
2017 (soil mycelium)	40	11	28

4. Discussion

4.1. The genet size of F. luteovirens

Our present study successfully identified 13 genotypes from 230 fruit bodies, demonstrating that direct sequencing of SSR fragments is an effective method for fine-scale genetic structure analysis. Five genets (in 33 soil cores) from 79 soil cores taken randomly around fairy rings. Underground genets of *F. luteovirens* were strictly distributed within the range of fairy rings (stimulating zone). No new genets were identified from underground cores compared to the sampling of fruit bodies over 3y, indicating that the method for detecting underground gents used in the current study was reliable and that the underground genets were correlated with the dominant genets observed above-ground at all sites. Our results differ from those of Guidot et al. (2001) in that they revealed higher underground genet diversity (of ectomycorrhizas) in *Hebeloma cylindrosporum,* indicating that the number of the above-ground genets was not a good predictor of the underground genets. One possible explanation for this discrepancy is that the success rate for obtaining a complete combined sequence from our soil cores was only 41.8%; thus, more soil samples (regular sampling following a grid that covers the whole fairy ring) should be collected in future research investigating the full picture of underground genets.

Fungal genet size (as estimated from the greatest distance between two fruit bodies in the same genet) can reveal their reproduction strategies and growth conditions (Bhatnagar et al., 2018). Additionally, genet sizes can vary among taxa. Some fungi have a small genet size found in diverse populations. For example, in the genus *Russula,* many species have a small genet size (1–3m in diameter) (Liang et al., 2004; Bergemann et al., 2006; Riviere et al., 2007; Redecker et al., 2010). Other species such as *Rhizopogon vinicolor* (less than 2 m), *Amanita francheti* (1.5 m^2), and *Trichloma matsutake* (less than 2 m) also have a small genets (Kretzer et al., 2003; Lian et al., 2006). In contrast, some species are capable of forming relatively large genets. *Armillaria* spp. are considered to have an extensive range of genet sizes (over 4000 m^2) (Prospero et al., 2003). Additionally, *Suillus pungens* forms large genets (of more than 300 m^2). A species with a small genet is indicative of frequent sexual reproduction based on spores, through which new

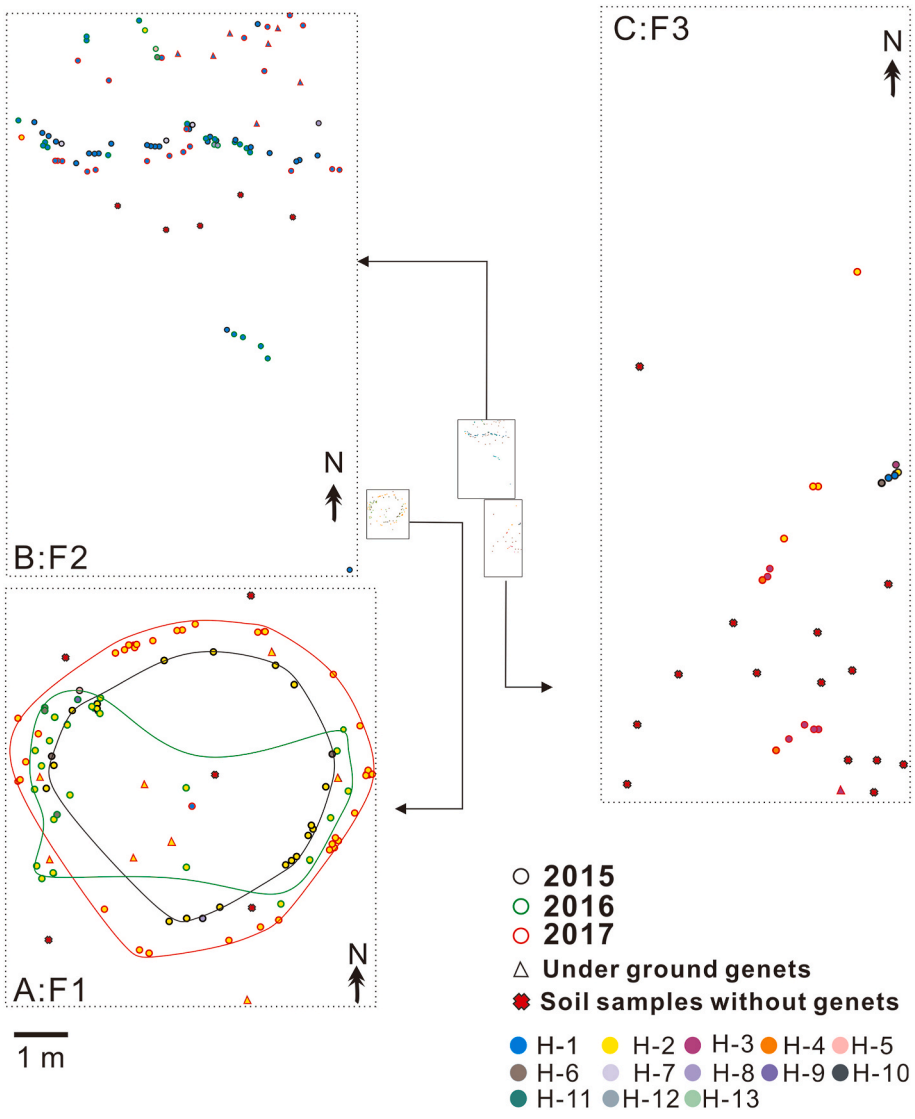

Fig. 1. Maps of fruit bodies and soil sampling locations in three fairy rings (F1(A), F2(B), and F3(C)) of site A (The shape of Fairy ring (F1) in each year are outlined).

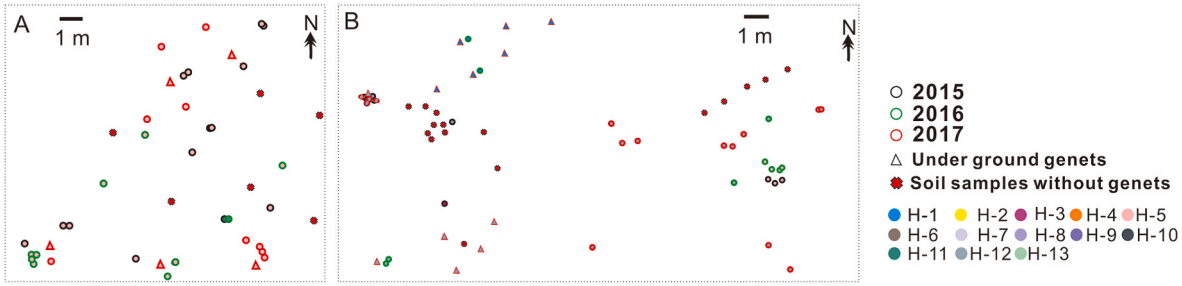

Fig. 2. Maps of fruit bodies and soil sampling locations in (A) site B and (B) site C.

genets can be created. In contrast, relatively large genets indicate that a specific species relies more on vegetative growth (asexual reproduction) and could persist for a long period of time underground as mycelia (Lian et al., 2006; Douhan et al., 2011). Our study found that *F. luteovirens* forms relatively large genets in the alpine meadows of the QTP; the longest distance between fruit bodies with the same genotype over the 3y at sites A (F1), B, and C were 5.82 m, 14.5 m, and 19.0 m, respectively. Only three genotypes (H4, H10, and H13) were identified as newly generated genotypes, suggesting a low frequency of sexual reproduction in this species. Additionally, the mean expansion rate of fairy ring F1 at site A was 0.37 m/y, indicating that this fairy ring formed at least 18 y ago at site A. Another piece of evidence indicating long survival times of *F. luteovirens* genets was the lack of a fruit body collected from fairy ring F3 at site A in 2016, though F3 fruit bodies reappeared in 2017. Additionally, a genotype H1 at site C was found above ground in 2016, but it was only found underground in 2017, indicating some of the genets of *F. luteovirens* can continue as mycelia through the harsh winters of the QTP.

Table 2
Genets of *Floccularia luteovirens* fruit body shown in detail.

Sampling year	Genets Identified				Genets Per FR (maximum)			Genet Size (m)			Expansion Rate of F1 in site A (m/y)	Fruit-body per genet (maximum)
	site A	Site B	Site C	Total	site A	Site B	Site C	site A	Site B	Site C		
2015	7	2	3	10	3	2	3	5.08 ± 0.76	14.5 ± 2.71	3.49 ± 0.20	−0.8	37
2016	6	2	4	11	2	2	4	5.50 ± 0.54	12.0 ± 0.10	16.94 ± 0.32	0.42[a]	12
2017	5	1	1	6	2	1	1	5.82 ± 0.51	12.6 ± 0.51	19.01 ± 0.73	0.32[a]	15
2017 (soil mycelium)	3	1	2		1	1	2	6.61 ± 0.34	11.9 ± 0.21	5.22 ± 0.11	−2.19	2
Total	10	2	4									

Average ± stand error of means.
Genet size: the longest distance between fruiting bodies within the same genotype in each fairy ring.
[a] Only the expansion rate of F1 in site A been shown due to its regular shape.

Table 3
Floccularia luteovirens genotype distribution over three sites and 3y.

Genotype	A			B			C		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
H1	21	10	30 (7)					2	(6)
H2	19	24	37 (8)						
H3	1		6 (1)				1		
H4			2						
H5			2	12	9	9 (5)	3	4	
H6	1	1							
H7	3	3							
H8	3								
H9	2	2							
H10		1							
H11				1	1				
H12							2	2	15 (6)
H13								1	

The number in the brackets represents the number of underground genets in each site.

Table 4
Population genetics parameters of *Floccularia luteovirens* at a fine scale.

Sampling Year	H_s	G_{st}	F_{st}	N_m
2015	0.63	0.17	0.32	0.53
2016	0.62	0.10	0.25	0.75
2017	0.33	0.46	0.75	0.14
Under ground (2017)	0.49	0.28	0.47	0.28

H_s, gene diversity within population; G_{st}, the coefficient of gene differentiation; F_{st}, genetic differentiation coefficient; N_m, the number of migrants per generation.

4.2. The priority effects on the genets of F. luteovirens

All the evidence from the current study shows that *F. luteovirens* more often relied on asexual reproduction to establish and expand its populations in the QTP and persist for long periods. Some biotic and abiotic factors may influence the size of genets (Wang et al., 2015). Previous research has shown that human activity (i.e., fruit body harvesting) may affect the fine-scale genetic structure within a short period. Guidot et al. (2003) found soil excavation and the collection of fruit bodies increased the formation of small and short-lived genets of *Hebeloma cylindrosporum*. In the present study, we collected all the fruit bodies in the fruiting season from three sites between 2015 and 2017. The genet density did not increase after fruit body collection (Table 4). In contrast, the number of genotypes in the three study sites decreased rapidly. Moreover, all the fairy rings were dominated by one or two genotypes in the last sampling year (Fig. 1).

The rhizosphere has a critical impact on ECM fungi. The host plants contribute their photosynthate (i.e., carbon resource) to ECM fungi, while ECM fungi provide mineral elements in exchange (Smith and Read, 2008). Thus, competition for roots between different fungal species and even between different genets of the same species is crucial for ECM fungal population establishment and expansion. The priority effects first described by Kennedy et al. (2009) indicate that the timing of colonization plays a vital role in the competition for root tips among ECM fungi. They found that the genets that arrive early can negatively affect the establishment of subsequent genets in *Rhizopogon* spp.. We also observed strong priority effects between different genets of *F. luteovirens* across all sites. The decrease in genet diversity over three years indicated fierce competition among genets of *F. luteovirens* on the alpine meadow of the QTP. Most studies conducted have shown that fairy rings initially come from one single spore and expand continuously via mycelial growth (Fox, 2006; Bonanomi et al., 2013). Thus, a particular fairy ring may be dominated by one unique genet in the majority of cases. However, in the present study, we observed the coexistence of at least two different genets in one fairy ring. The new genets in the fairy ring may be generated by sexual reproduction.

4.3. The fine-scale gene flow of F. luteovirens

Gene flow in fungi is mainly determined by the dispersibility of spores. Wind is the main factor that affects long-distance fungal spore dispersal. Moreover, the resistance of spores to drought (enhanced by hydrophobins in their walls) and ultraviolet radiation (as determined by the color of their walls) (Deacon, 2013) have also been determined to be

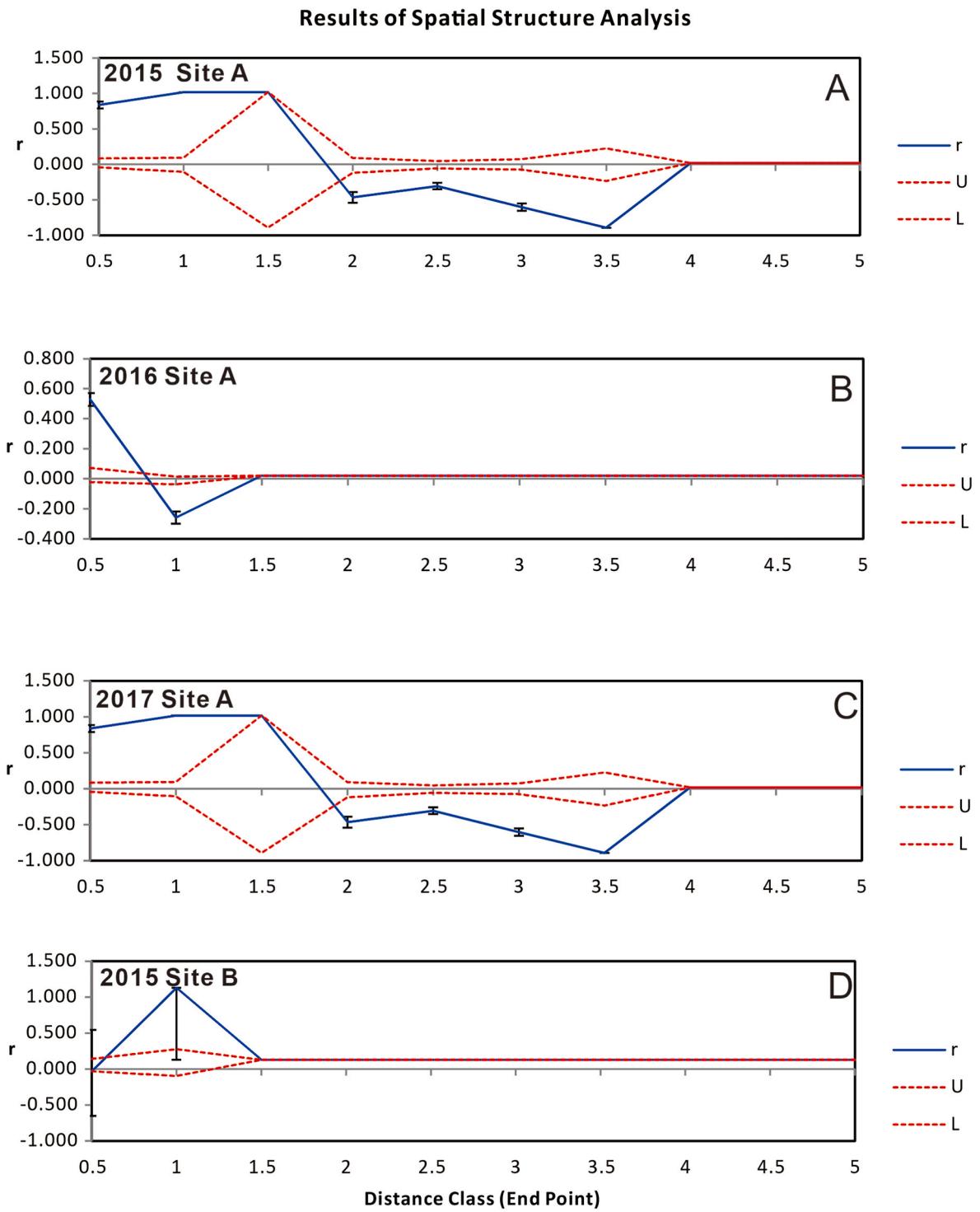

Fig. 3. Spatial autocorrelation of *Floccularia luteovirens* in (A) 2015, (B) 2016 and (C) 2017 in site A and (D) 2015 in site B. Solid lines show the observed correlation coefficients (r) and their 95% confidence intervals. Dashed lines represent the 95% upper and lower confidence intervals of correlation coefficients under the null hypothesis of random distribution.

critical factors associated with the survival of fungal spores that travel long distances. Other factors, such as rain, animal and insect dispersal, and human activities, also affect the long-distance dispersal of fungal spores (Gams, 1973). Our previous study on the large-scale genetic structure of *F. luteovirens* found low genetic variation and high gene flow among populations (the longest distance between populations was 751.5 km) (Xing et al., 2014). This previous work indicated unrestricted gene flow in this species (i.e., the spores of this species may travel long distances in the QTP). Indeed, most studies have suggested that the dispersal ability of fungal spores is quite low. For the fungal species that can form fruit bodies, spores typically fall directly under the fruit body (Savile and Ingold, 1971, Lacey, 1996, Dunham et al., 2006; Carriconde et al., 2008; Galante and Swaney, 2011). Similar conclusions can be obtained from the present study. Specifically, the results of the Mantel test (between genetic distance and geographic distance matrices), spatial clustering (positive spatial autocorrelation over a short distance),

and the relatively lower N_m value, all showed limited fine-scale *F. luteovirens* gene flow in the QTP. These results contradict our previous large-scale study of this species that low genetic variation and high gene flow among wild populations were being found (Xing et al., 2014). In the QTP, the influence of the South Asian monsoon, the East Asian monsoon, and the northern hemisphere westerly circulation are limited (Yeh, 1952; Wang et al., 2008). That is, it is unlikely that fungal spore dispersal via wind occurs in the QTP. Additionally, long-distance animal migration also does not occur on the QTP. In the QTP, only *Pantholops hodgsonii* has a range of migration beyond hundreds of kilometers (Zhou et al., 2005). On the other hand, because of the usage of fencing in grazing management, the migration of wild animals and livestock is also limited (You et al., 2013). The solar ultraviolet radiation in the QTP is also particularly serious owing to its high elevation; thus, ultraviolet radiation poses a great challenge to the survival of long-distance dispersed fungal spores. Previous studies have shown that the color of spores (i.e., the pigments in their walls) plays a key role in their resistance to ultraviolet radiation. For example, *Blumeria graminis* has colorless spores that can only survive for a short period during a sunny day. In contrast, the spores of *Puccinia graminis* and *Cladosporium* spp. can survive for weeks owing to their dark color (Deacon, 2013). The spores of *F. luteovirens* are white (Wang et al., 2005), indicating they are unlikely to survive under exposure to serious ultraviolet radiation. All the evidence above demonstrates that long-distance travel through the QTP of *F. luteovirens* spores does not occur via wind or wild animals, and it is also unlikely for its spores to remain viable for a long time. However, the results of our large-scale genetic structure study (covering most of its distribution area) showed very strong gene flow among its wild populations, though this may be enhanced by human activities. In recent years, the price of *F. luteovirens* fruit bodies has increased, which has promoted the trade of this species between local herdsmen and businessmen from nearby cities. This trade involves the transportation of fruit bodies from their origins to cities that are hundreds or even thousands of kilometers away, which could facilitate gene exchange between distant wild populations (Feng et al., 2006; Jiao et al., 2008).

Our investigation of the fine-scale genetic diversity structure of *F. luteovirens,* based on SSR markers, shows that *F. luteovirens* has formed relatively large genets in alpine meadows of the QTP. Only three genotypes were identified as newly generated genotypes across all sites, over the three sampling years, indicating a low frequency of sexual reproduction in this species. The observed underground genets of *F. luteovirens* were strictly distributed within the range of the visible fairy rings, and no genets were found underground that were not observed in the above-ground genets. The weak fine-scale gene flow between sites, positive spatial autocorrelation over short distances, and significant Mantel test results all indicate restricted gene flow in this species. We hypothesize that the high gene flow between wild populations of *F. luteovirens* over a large geographic distance is enhanced by the fruit body trade.

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