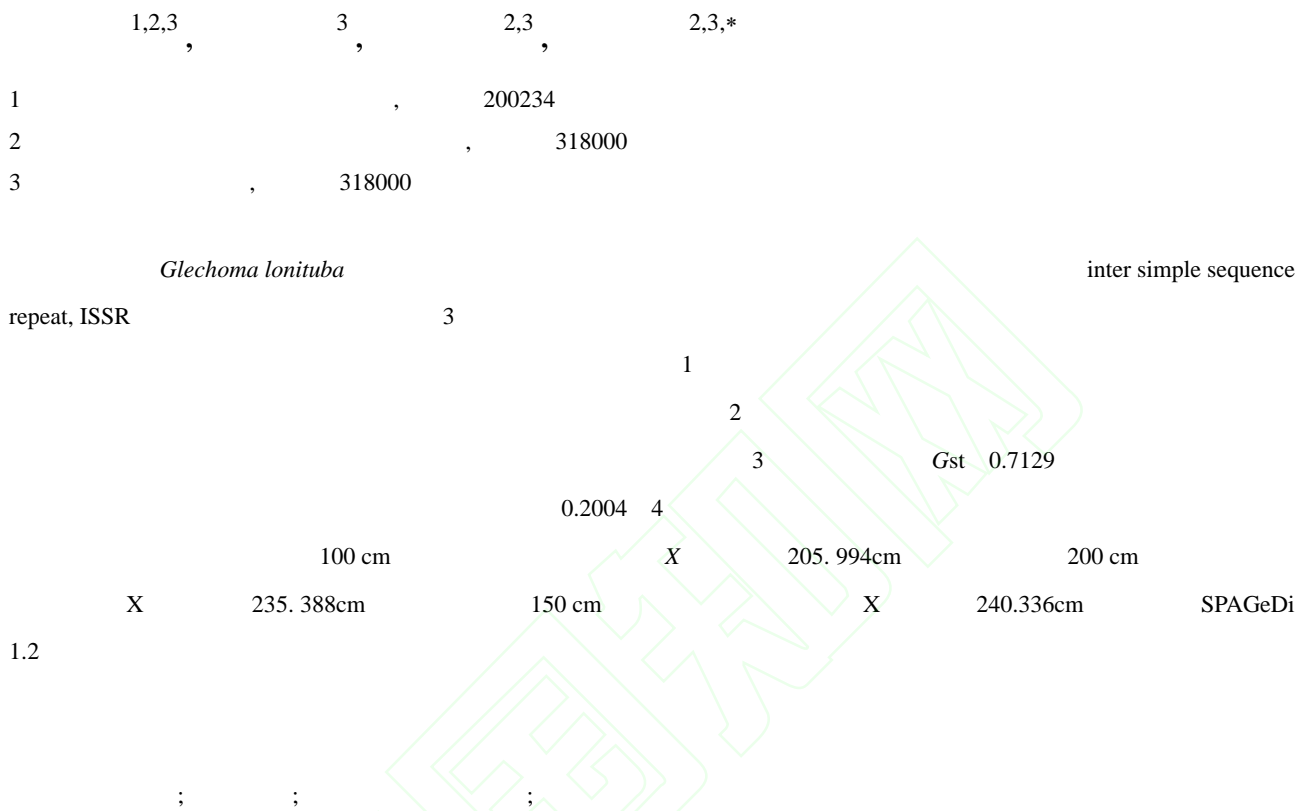


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Fine-scale spatial genetic structure of *Glechoma longituba*

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Abstract: Fine-scale spatial genetic structure, which indicates the nonrandom spatial distribution of genotypes or genetic diversity, has important consequences for population biology. The study of fine-scale spatial genetic structure can provide an understanding of the key processes and mechanisms involved in the maintenance of plant populations. *Glechoma longituba* is a perennial herbaceous clonal plant species that belongs to the Labiatae family. *Glechoma longituba* is a herb of medicinal importance that is widely distributed in China and its phenotypic characteristics are variable among different habitats. The genetic diversity, clonal diversity and fine-scale spatial genetic structure of *Glechoma longituba* plants collected from three different patches (Shuiqubian, Pinggecun, and Zhulinxia) with different habitats were analyzed using inter-simple sequence repeat (ISSR) molecular markers. In addition, the correlation with habitat he, propagule *omulgation*, and human disturbance were also ex ined in the study. The results the followi Genetic diversity of t the species level was relatively low (The percentage of

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polymorphic loci, $P = 31.15\%$; Shannon informative index, $I = 0.1601$; Nei's index, $h = 0.1096$). The genetic diversity of *Glechoma lonituba* was highest in the Shuiqubian patch ($P=21.31\%$, $I=0.0965$, $h=0.0627$), that in the Pinggecun patch was second highest ($P=8.20\%$, $I=0.0354$, $h=0.0226$), and that in the Zhulinxia patch was the lowest ($P=3.28\%$, $I=0.0120$, $h=0.0073$). 2) Clonal diversity of *Glechoma longituba* at the species level was relatively high (Number of genets, $G=73$; Ratio of genets to ramets, $G/N=0.2332$; Simpson's diversity index, $D=0.8843$; Genotypic evenness, $E =0.8192$). The clonal diversity of *Glechoma longituba* was highest in the Shuiqubian patch ($G=60$, $G/N=0.5660$, $D=0.9693$, $E =0.8747$), that in the Pinggecun patch was second highest ($G=10$, $G/N=0.1087$, $D=0.8430$, $E =0.9075$), and that in the Zhulinxia patch was the lowest ($G=3$, $G/N=0.0260$, $D=0.2642$, $E =0.3599$). 3) The genetic differentiation coefficient (G_{st}) was 0.7129, which indicated that most of the genetic variation existed among patches, whereas little genetic variation existed within patches. The estimated gene flow was as low as 0.2004. 4) Spatial autocorrelation analysis showed that the autocorrelation coefficient of *Glechoma longituba* in the Zhulinxia patch was significantly positive at a distance of 100 cm with an X -intercept of 205.994 cm but significantly negative at a distance of 350 cm. The autocorrelation coefficient in the Pinggecun patch was significantly positive at a distance of 200 cm with an X -intercept of 235.388 cm but significantly negative at a distance of 450 cm. The autocorrelation coefficient in the Shuiqubian patch was significantly positive at a distance of 150 cm with an X -intercept of 240.336 cm but significantly negative at a distance of 350 cm. Analysis with the SPAGeDi 1.2 software showed that the strength of spatial genetic structure in the Pinggecun patch was greater than those in the Shuiqubian and Zhulinxia patches. The Sp ratio (used to compare the extent of spatial genetic structure among populations) statistics for the Pinggecun, Shuiqubian, and Zhulinxia patches were 0.0944, 0.0558, and 0.0556, respectively. The genetic diversity, clonal diversity, and fine-scale spatial genetic structure of *Glechoma longituba* might be affected by propagule dispersal characteristics, human disturbance, and trade-off between investment in sexual reproduction and clonal propagation and might be a consequence of adaptation to habitat heterogeneity.

Key Words: *Glechoma longituba*; clonal plant; fine-scale spatial genetic structure; ISSR

[1]

[2]

[3]

[4]

(inter-

simple sequence repeat, ISSR) [5]

DNA (random amplified polymorphic DNA, RAPD) [6]

(amplified fragments length polymorphic, AFLP) [7]

(Simple sequence repeat, SSR)

[8]

ISSR

SSR ISSR

AFLP ISSR

[17]

RAPDs ISSR

[9]

ISSR

indica [4] *Potamogeton malaianus* [5] *Psammochloa villosa* [10] *Duchesnea*

3 [4] van Rossum Triest

Primula elatior [11] Bergf Hamrick (*Quercus*)

cerris) 5 m-10 m [12]

[11] Berg Hamrick

[12]

[4]

Glechoma longituba Lamiaceae *Glechoma*

[13] 50-2000 m

[14]

[15] ISSR 3

1 1.1 2008 4 50×50cm (x y) 1 [26,27]

1

1

Table 1 Conditions of the different patches of *Glechoma longituba*

Patch	Geographical location	Habitat	Slope	Altitude m	Size of patch
Shuiqubian	30°09.779N 119°13.176E		NW30°	97	600×400 cm
Zhulinxia	30°10.602N 119°11.794E		NW30°	144	600×400 cm
Pinggecun	30°10.038N 119°11.977E		SE30°	103	1020×200 cm

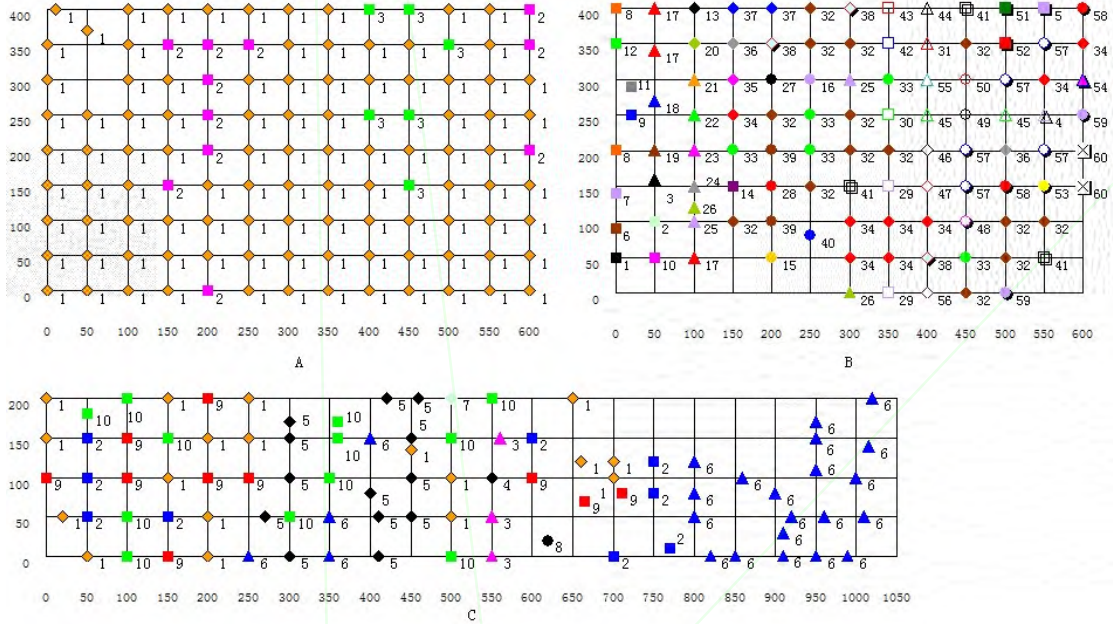


Fig.1 Distribution of the individuals and genotypes of *Glechoma lonituba* in different patches

A Zhulinxia patch B Shuiqubian patch C Pinggecun patch

1.2

1.2.1 DNA

DNA
Gel Doc XR

SDS [16] DNA DNA 0.8%
10 ng /μL -20

1.2.2 ISSR

ISSR

(University of British Columbia, Set No9, No.801-900)

PTC-220

DNA

dNTP

DNA

(bovine serum albumin,

BSA) ISSR

ISSR

10μL PCR

1μL Taq

10mmol/L Tris-HCL pH 9.0 50mmol/L KCl 0 1% TritonX-100 0.4 mmol/L MgCl₂

10 pmol

8 ng DNA 2mg/mL BSA dNTP 0.5mmol/L 1 U Taq

touch-down

PCR

94 5 m in 94 30s 57 1 m in 72 1.5min 0 5

10

94 30s 52 1 min 72 1.5min 25 72 5min DNA

2%

(0.5 μg/mL)

0.5 ×TBE Gel Doc XR

200 bp DNA

DNA

100 ISSR

PCR

ISSR

1.2.3

			"1"	"0"	ISSR
POPGEN 32	[17]		P Shannon	(I) Nei	(h)
POPGEN 32			(G_{st})		
4	[4]	(1)	()	$(G$	$B \text{ ù! } 7\frac{3}{4}p \text{ Aò } \frac{1}{4}j! \bullet _$

3

Table 3 Genetic diversity of *Glechoma lonituba* in different patches

Patch	Number of individuals	Number of polymorphic loci	Percentage of polymorphic loci <i>P</i> , %	Shannon informative index (<i>I</i>)	Shannon Nei index (<i>h</i>)	Nei's Nei's
Shuiqubian	106	13	21.31	0.0965	0.0627	
Zhulinxia	115	2	3.28	0.0120	0.0073	
Pinggecun	92	5	8.20	0.0354	0.0226	
Species level	313	19	31.15	0.1601	0.1096	

2.2

4

3

1 3

X

6 ISSR

3

15

98

24

SPAGeDi 1.2

X

[20]

200 cm

X

235.388cm

[44]

[21]

240.336 cm

[21]

150 cm

205.994 cm

[21]

[22]

[23]

[24]

[25]

[26]

SQB

PGC

[22]

References:

- [1] Alpert P. Nutrient sharing in natural clonal fragments of *Fragaria chiloensis*. *Journal of Ecology*, 1996, 84(3): 395-406
- [2] Jin Z X, Li J M, Liu L L. Fine-scale spatial genetic structure within age classes of the two fragmented populations of *Sinocalycanthus chinensis* Cheng et S.Y. Chang, an endangered plant species endemic to China. *Biochemical Systematics and Ecology*, 2012, 43: 117-124.
- [3] Stowe L G, Wade M J. The detection of small-scale patterns in vegetation. *Journal of Ecology*, 1979: 67(3):1047-1064.
- [4] Li J M, Jin Z X. Fine-scale clonal structure of the stoloniferous herb *Duchesnea indica*. *Acta Ecologica Sinica*, 2009, 29 (7): 3540 - 3548
- [5] Chen Y Y, Li Q, Wu W Y, Li W. Genetic diversity and clonal structure of *Potamogeton malaianus* populations. *Chinese Journal of Applied Ecology*, 2006, 17(11): 2034-2040.
- [6] Pluess A R, Stöcklin J. Population genetic diversity of the clonal plant *Geum reptans* (Rosaceae) in the Swiss Alps. *American Journal of Botany*, 2004, 91(12): 2013-2021.
- [7] Honnay O, Jacquemyn H, Roldán Ruiz I, Hermy M. Consequences of prolonged clonal growth on local and regional genetic structure and fruiting success of the forest perennial *Maianthemum bifolium*. *Oikos*, 2006, 112(1): 21-30.
- [8] Hämmerli A, Reusch T. Genetic neighbourhood of clone structures in eelgrass meadows quantified by spatial autocorrelation of microsatellite markers. *Heredity*, 2003, 91(5): 448-455.
- [9] Kojima T, Nagaoka T, Noda K, Ogihara Y. Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. *Theoretical and Applied Genetics*, 1998, 96(1): 37-45.
- [10] Li A, Ge S. Genetic variation and clonal diversity of *Psammodloa villosa* (Poaceae) detected by ISSR markers. *Annals of Botany*, 2001, 87(5): 585-590.
- [11] Van Rossum F, Triest L. Fine-scale genetic structure of the common *Primula elatior* (Primulaceae) at an early stage of population fragmentation. *American Journal of Botany*, 2006, 93(9): 1281-1288.
- [12] Berg E E , Hamrick J L. Fine-scale genetic structure of a turkey oak forest. *Evolution*, 1995,49(1): 110-120.
- [13] Zhang Q J , Yang X S, Zhu H Y, Hao X J. Chemical constituents and their pharmacological research progress in medicinal plants of *Glechoma Linn.* *Chinese Traditional and Herbal Drugs*, 2006, 37(6): 950-952.
- [14] Chen S, Li Z Z, Wang G. Advances in researches of growth form of the clonal plant. *Chinese Journal of Ecology* ,1997 ,16 (4) :59 - 63.
- [15] Tao J P, Zhong Z C. Morphological responses to different nutrient supply in the stoloniferous herb *Glechoma longituba*. *Acta Ecologica Sinica*, 2000, 20(2): 207-211.
- [16] Li JM eds. *Experiments in Molecular Biology*. Hangzhou: Press of Zhejiang University. 2010, 5.
- [17] Yeh F. Population genetic analysis of codominant and dominant markers and quantitative traits. *Belg. J. Bot*, 1997, 129: 157.
- [18] Vekemans X, Hardy OJ. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, 2004, 13: 921-935.

- [19] Zhang L L, Dong M, Li R Q, Wang Y H, Cui Q G, He W M. Soil-nutrient patch contrast modifies intensity and direction of clonal integration in *Glechoma longituba*. *Journal of Plant Ecology*, 2007, 31(4): 619-624.
- [20] Peakall R, Ruibal M, Lindenmayer DB. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution*, 2003, 57: 1182-1195.
- [21] Dong M. Clonal growth in plants in relation to resource heterogeneity: foraging behavior. *Acta Botanica Sinica*, 1996, 38(10): 828-835.
- [22] Liu Z G, Li Z Q. Perspectives on small-scale spatial structure of plant species in plant communities. *Acta Phytocologica Sinica*, 2005, 29(6): 1020-1028.
- [23] Widén B, Cronberg N, Widén M. Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. *Folia Geobotanica*, 1994, 29(2): 245-263.
- [24] Gillman M, Bullock J, Silvertown J, Hill B C. A density-dependent model of *Cirsium vulgare* population dynamics using field-estimated parameter values. *Oecologia*, 1993, 96(2): 282-289.
- [25] Nishitani S, Takada T, Kachi N. Optimal resource allocation to seeds and vegetative propagules under density-dependent regulation in *Syneilesis palmata* (Compositae). *Plant Ecology*, 1999, 141(1-2): 179-189.
- [26] Wang H Y, Wang Z W, Li L H, Chen Y J, Ren L Y. Reproductive tendency of clonal plants in various habitats. *Chinese Journal of Ecology*, 2005, 24(6): 670-676.

- [4] , . , 2009, 29(7): 3540-3548.
- [5] , , . , 2006, 17(11): 2034-2040.
- [13] , , . , 2006, 37(6): 950-952.
- [14] , , . , 1997, 16(4): 59-63.
- [15] , . (*Glechoma longituba*) , 2000, 20(2): 207-211.
- [16] . : . 2010, 5.
- [19] , , . , 2007, 31(4): 619-624.
- [21] . : . , 1996, 38(10): 828-835.
- [22] , . , 2005, 29(6): 1020-1028.
- [26] , , . , 2005, 24(6): 670-676.