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Genetic Diversity and Population Differentiation of Main Species of *Dendrolimus* (Lepidoptera) in China and Influence of Environmental Factors on Them

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1. Introduction

As the major forestry pest insects in China, *Dendrolimus* included *Dendrolimus punctatus* Walker, *D. punctatus tabulaeformis* Tsai *et* liu, *D. punctatus spectabilis* Butler, *D. superans* Butler, *D. houi* Lajonquiere and *D. kikuchii* Matsumura. During sequential outbreaks, economical damage can be so extensive for forest appears to be burned and unable to withstand such a long period of defoliation. Gene diversity and genetic structure among the main species of *Dendrolimus* were assessed using morphological diversities, allozyme, Random Amplified Polymorphism DNA（RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSR), mitochondrial DNA and Simple Sequence Repeat (SSR), which provide the powerful tool for investigation of genetic variation. The influence of ecological factors on the genetic diversity is also discussed by the correlation analysis.

2. Genetic diversities among the main species and geographical populations of *Dendrolimus*

2.1 Morphological diversities among 4 species of *Dendrolimus*

For investigating the morphological diversity, many pupae of *D. punctatus* Walker, *D. superans* Butler, *D. houi* Lajonquiere and *D. kikuchii* Matsumura were collected in 2006(table1).

Species	Location	Code	Latitude and Longitude
D.punctatus Walker:	Yujiang , Jiangxi	YJM	28° 11′N 116°54′E
D.superans Butler:	Zhangjiakou, Hebei	ZJKL	40° 50′N 114°53′E
D.houi Lajonquiere:	Guangyuan, Sichuan	GYYN	31° 53′N 105°16′E
D.kikuchii Matsumura:	Liangping, Chongqing	LPS	30° 25′N 107°24′E

Table 1. Origin of 4 different species of the tested *Dendrolimus* materials

Morphological diversities among 4 species of *Dendrolimus* by analyzing characters such as pupae weight, pupae length, female weight, female wing length, female length, male weight, male wing length and male length. Analysis of variance for all characteristics was significantly different among species and among individuals within populations. Coefficient of variation of morphological traits showed that pupae weight is significantly higher than other traits. Pupae length, female weight and female wing length were the main traits account for the phenotypic variations according to the PCA and discriminate analysis. Based on the UPGMA cluster, four species of *Dendrolimus* may be divided into two groups: the first branch in the dendrogram included *D. punctatus* Walker and *D. superans* Butler, and *D. houi* Lajonquiere and *D. kikuchii* Matsumura were grouped together into second branch(table2-3,figure1)

Code	(PW)	(PL)	(FW)	(ML)	(FL)	(MW)	(MWL)	(FWL)	Mean
YJM	54.15	14.13	20.15	4.38	6.01	27.30	4.53	7.87	17.32
GYYN	46.62	12.89	12.85	5.20	3.72	23.09	3.53	2.59	13.81
LPS	58.14	5.21	26.56	8.29	6.00	32.13	4.65	7.58	18.57
ZJKL	49.04	9.68	33.52	4.03	8.57	50.87	9.15	9.21	21.76
Total	51.99	10.48	23.27	5.48	6.08	33.35	5.47	6.81	17.87

Note:PW:pupae weight, PL: pupae length, FW: female weight, FWL: female wing length, FL: female length, MW: male weight, MWL: male wing length, ML: male length.

Table 2. Coefficient of variation of morphological traits of 4 different species of the tested *Dendrolimu* materials

code	Eigenvalue	Contribution rate %	Cumulative contribution rate %
PW	6.0824	76.03	76.03
PL	1.0501	13.13	89.16
FW	0.3807	4.76	93.92
FWL	0.2334	2.92	96.83
FL	0.0886	1.11	97.94
MW	0.0734	0.92	98.86
MWL	0.0515	0.64	99.50
ML	0.0400	0.50	100.00

Note: PW: pupae weight, PL: pupae length, FW: female weight, FWL: female wing length, FL: female length, MW: male weight, MWL: male wing length, ML: male length.

Table 3. Eigenvalue and principle proportion of 8 character of 4 different species of the tested *Dendrolimus* materials

2.2 Genetic diversities among 4 species of *Dendrolimus* by allozyme

Gene diversity and genetic structure among 4 species of *Dendrolimus* were assessed by allozyme, which provides a powerful tool for investigation of genetic variation (table4,figure2).

A total of 12 presumed loci and 22 alleles were scored in the sampled populations by allozyme method, which 6 loci were polymorphic. At population level, the mean number of alleles per locus (*A*) was 1.2708, the percentage age of polymorphic loci (*P*) was 25.00%, and the mean expected heterozygosity per locus (*He*) was 0.0828. At species level, *A*=1.8333,

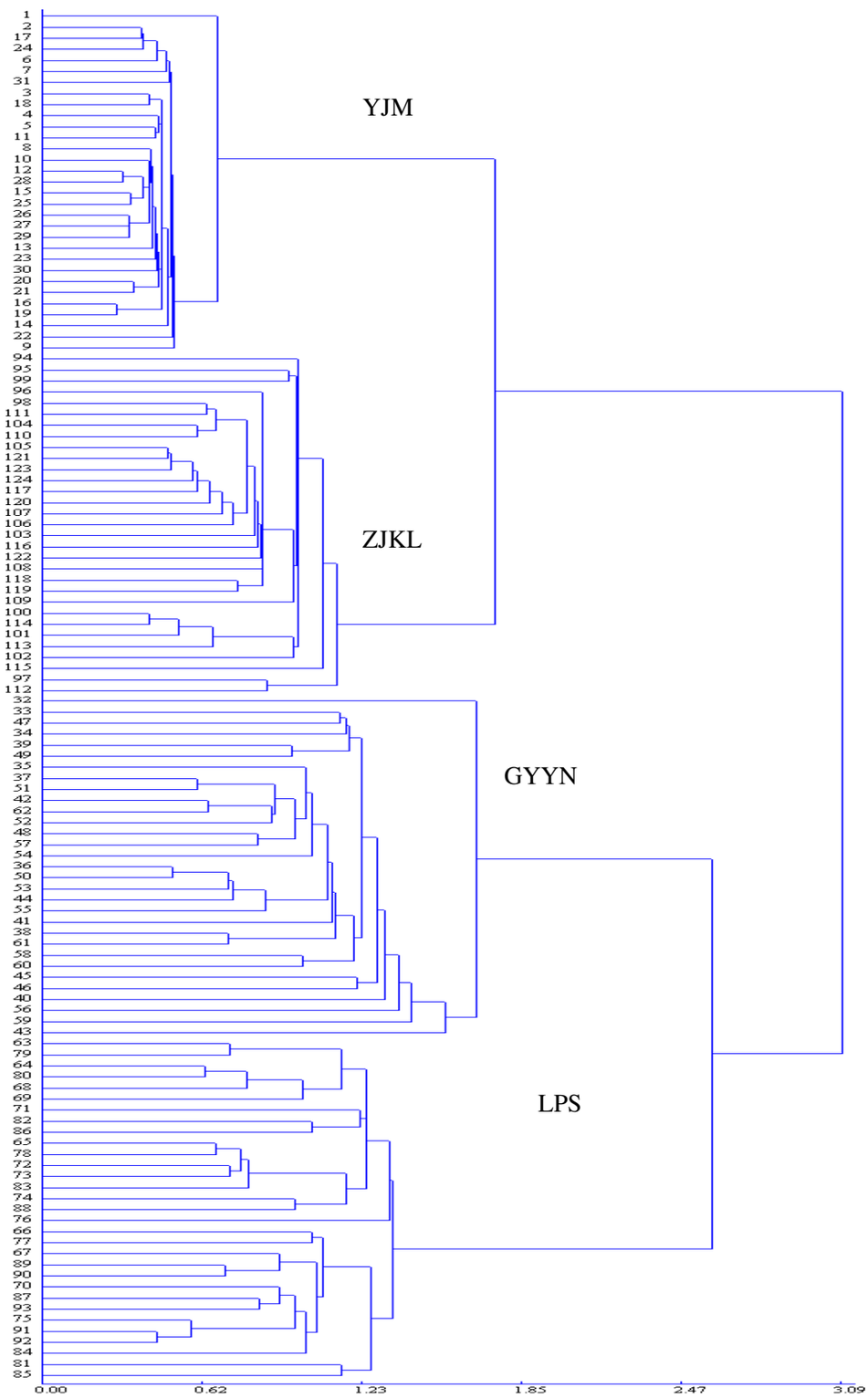


Fig. 1. UPGMA cluster based on morphological traits of 4 different species of the tested *Dendrolimus* materials

Enzyme	Abbreviation	E.C. code
Lactate dehydrogenase	LDH	E.C. 1. 1. 1. 27
Malate dehydrogenase	MDH	E.C. 1. 1. 1. 37
Malic enzyme	ME	E.C. 1. 1. 1. 40
Alcohol dehydrogenase	ADH	E.C. 1. 1. 1. 1
Formate dehydrogenase	FDH	E.C. 1.2. 1. 2
Glutamate dehydrogenas	GDH	E.C. 1.4. 1.2
Catalase	CAT	E.C. 1. 11. 1. 6
Peroxidase	POD	E.C. 1. 1. 1. 1

Table 4. The kinds of allozymes be choosed

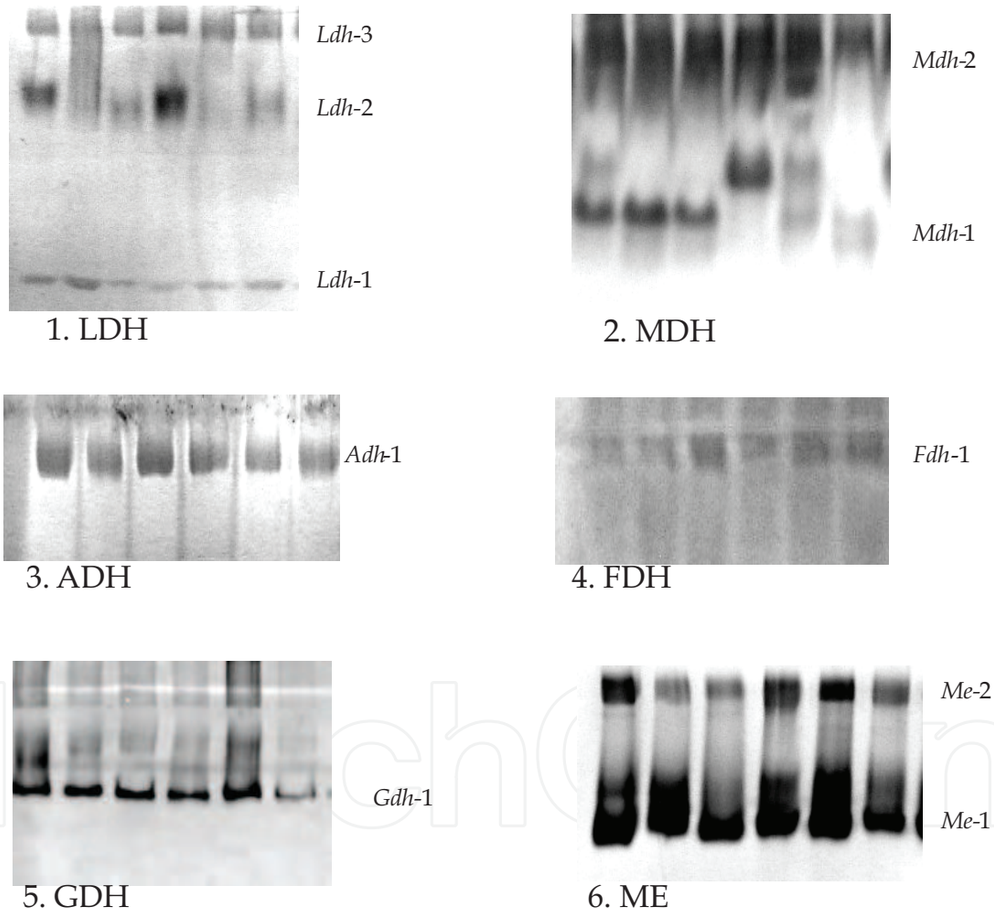


Fig. 2. Electrophoretic forms of LDH, ADH, FDH, GDH, MDH, ME
From left to right: SYC, QDC, JZC, HTM, TDM, YJM

P= 50.00%, *He* = 0.2323. So, the genetic diversity at species level was rather high than that at population level (table5,6). A UPGMA dendrogram was generated based on *Nei's* genetic distance showed 4 species of *Dendrolimus* were clustered into two groups: the first branch in the dendrogram included *D. punctatus* Walker and *D. superans* Butler, and the second branch included *D. houi* Lajonquiere and *D. kikuchii* Matsumura (figure3).

population	*A*	*Ae*	I	*P(%)*	*Ho*	*He*
YJM	1.3333	1.1462	0.1403	33.33	0.0944	0.0921
ZJKL	1.1667	1.1569	0.1129	16.67	0.1458	0.0828
GYYN	1.3333	1.1325	0.1207	25.00	0.0877	0.0771
LPS	1.2500	1.1424	0.1258	25.00	0.0972	0.0856
Mean	1.2708	1.1445	0.1249	25.00	0.1063	0.0828
Overall loci	1.8333	1.5564	0.3795	50.00	0.1076	0.2323

Note: A: Number of alleles per locus; Ae: Effective number of alleles per locus; I: The Shannon information index;
Ho: Observed heterozygosity; He: Expected heterozygosity; P: Percentage of polyporphic loci

Table 5. Genetic diversity parameters of 4 different species of the tested *Dendrolimus* materials

population	YJM	ZJKL	GYYN	LPS
YJM	****	0.9142	0.8319	0.7157
ZJKL	0.0897	****	0.7845	0.6747
GYYN	0.1840	0.2427	****	0.7967
LPS	0.3345	0.3935	0.2272	****

Note: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 6. Nei's genetic identity and genetic distance of 4 different species of the tested *Dendrolimus* materials

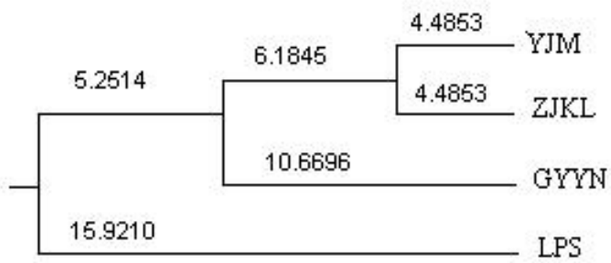
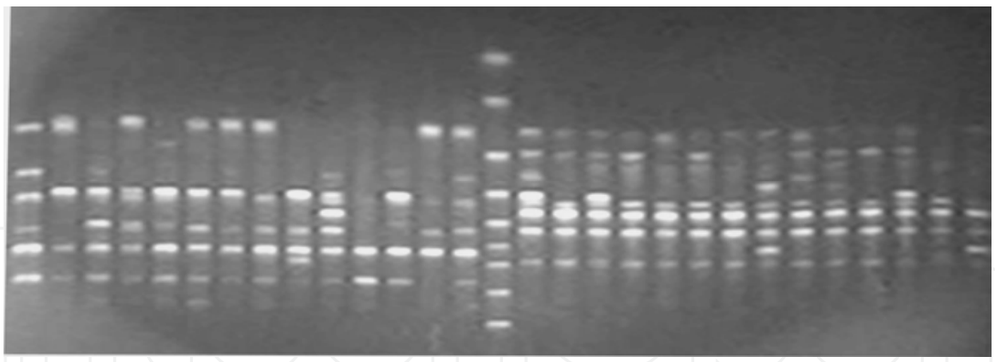


Fig. 3. UPGMA dendrograms based on Nei's genetic distance of 4 different species of the tested *Dendrolimus* materials

2.3 Genetic diversities among 3 species of *Dendrolimus* by ISSR

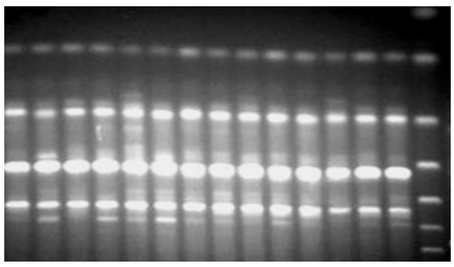
A set of optimized response system of ISSR has been developed, which provides a powerful tool for investigation of genetic variation of 9 natural populations(*D. punctatus spectabilis*; *D. punctatus tabulaeformis*; *D. superans*) (figure4-8,table7). Shannon index shows highest genetic diversity in Weichang population(*D. superans*) and lowest genetic diversity in Shenyang population(*D. spectabilis*), which is identical with Nei's index(table8-10).

Different geographical groups in the same populations has a genetic divergence in some extent. Genetic similarities and cluster analysis shows that inter-species heredity difference is obviously bigger than the homogeneous between populations in the heredity difference, which reflects the heredity difference degree is consistent from the DNA level with the phenotype level. *D. punctatus spectabilis* has nearer genetic distance with *D. punctatus tabulaeformis* than *D. superans*, which is identical with that researched in sexual information of pine caterpillars(figure9).



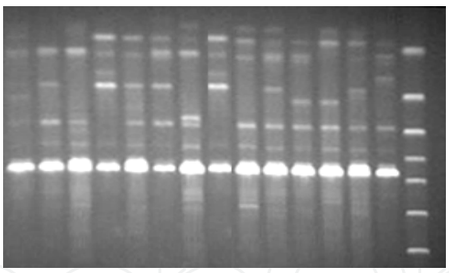
Sequence (from left to right): WL1~WL14,Marker, SC1~SC14

Fig. 4. ISSR-PCR fingerprints of 14 samples of *Dendrolimus superans* from WL and *Dendrolimus punctatus spectabilis* from SC populations using primer 106



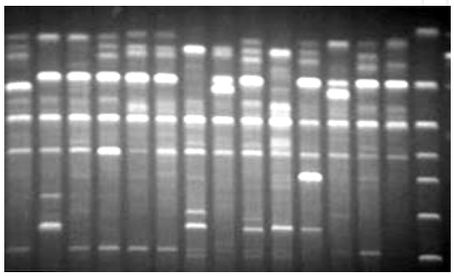
1~14: Qingdao population of D. punctatus Spectabilis M: molecular marker(DL3000bp DNA marker)

Fig. 5. ISSR profile amplified by primer 30



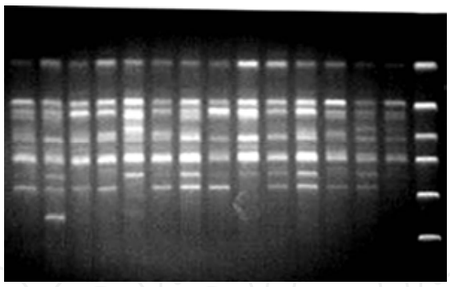
1 ~ 14: Qingdao population of D. punctatus spectabilis M: molecular marker(DL3000bp DNA marker)

Fig. 6. ISSR profile amplified by primer 32



1~14: Weichang population of D.superans M:molecular marker(DL3000bp DNA marker)

Fig. 7. ISSR profile amplified by primer 33



1~14: Shenyang population of D. Punctatus spectabilis M: molecular marker(DL3000bp DNA marker)

Fig. 8. ISSR profile amplified by primer 35

Primer order	Sequence ofprimer(5′-3′)	Total bands	Polymorphic bands	Percentage of Polymorphic bands
30	AGAGAGAGAGAGAGAGG	11	9	81.82
32	GAGAGAGAGAGAGAGAC	17	16	94.12
33	ACCACCACCACCACCACC	14	13	92.86
35	AGCAGCAGCAGCAGCAGC	17	16	94.12
102	CACACACACACACACAG	11	11	100.00
104	ATGATGATGATGATGATG	16	14	87.50
105	GGATGGATGGATGGAT	14	13	92.86
106	VDVCTCTCTCTCTCTCT	14	14	100.00
108	CACACACACACAAC	16	15	93.75
112	AGAGAGAGAGAGAGAGTA	15	15	100.00
118	GTCGTCGTCGTCGTCGTC	17	16	94.12
120	GAGGAGGAGGAGGC	18	18	100.00
121	GTGCGTGCGTGCGTGC	15	14	93.33
	all locus	195	184	94.36%

Note: V= (G/A/C)

Table 7. Result of ISSR on adults of 3 species of *Dendrolimus* with 13 primers

population	*Na*	*Ne*	*h*	*I*	*PPB*
TC	1.1949±0.3971	1.0983±0.2359	0.0605±0.1371	0.0927±0.2035	19.49
JC	1.2256±0.4191	1.1383±0.2989	0.0794±0.1624	0.1182±0.2345	22.56
SC	1.2051±0.4048	1.0913±0.2293	0.0565±0.1310	0.0883±0.1944	20.51
QC	1.2103±0.4085	1.1158±0.2647	0.0691±0.1484	0.1047±0.2176	21.03
ZL	1.3846±0.4878	1.2055±0.3245	0.1230±0.1791	0.1873±0.2608	38.46
WL	1.4308±0.4965	1.2605±0.3588	0.1518±0.1959	0.2264±0.2824	43.08
CY	1.2256±0.4191	1.1287±0.2805	0.0759±0.1557	0.1143±0.2271	22.56
PY	1.1846±0.3890	1.1159±0.2805	0.0662±0.1517	0.0984±0.2190	18.46
SY	1.2205±0.4157	1.1561±0.3244	0.0869±0.1736	0.1268±0.2482	22.05
Average	1.2535±0.4264	1.1456±0.2886	0.0855±0.1594	0.1286±0.2319	25.35
All locus	1.9436±0.2313	1.4926±0.3278	0.2955±0.1610	0.4495±0.2127	94.36

Note: CY: *D. tabulaeformis* (Chengde population) ; PY:*D.tabulaeformis* (Pingquan population) ; SY: *D.tabulaeformis* (Shenyang population) QC:*D.spectabilis* (Qingdao population) ; TC :*D.spectabilis* (Taian population) ; SC: *D.spectabilis* (Shenyang population) ; JC: *D.spectabilis* (Jinzhou population) ; ZL: *D.superans* (Zhangjiakou population) ; WL: *D. superans* (Weichang population)

Table 8. The genetic variation among 9 populations of *Dendrolimus*

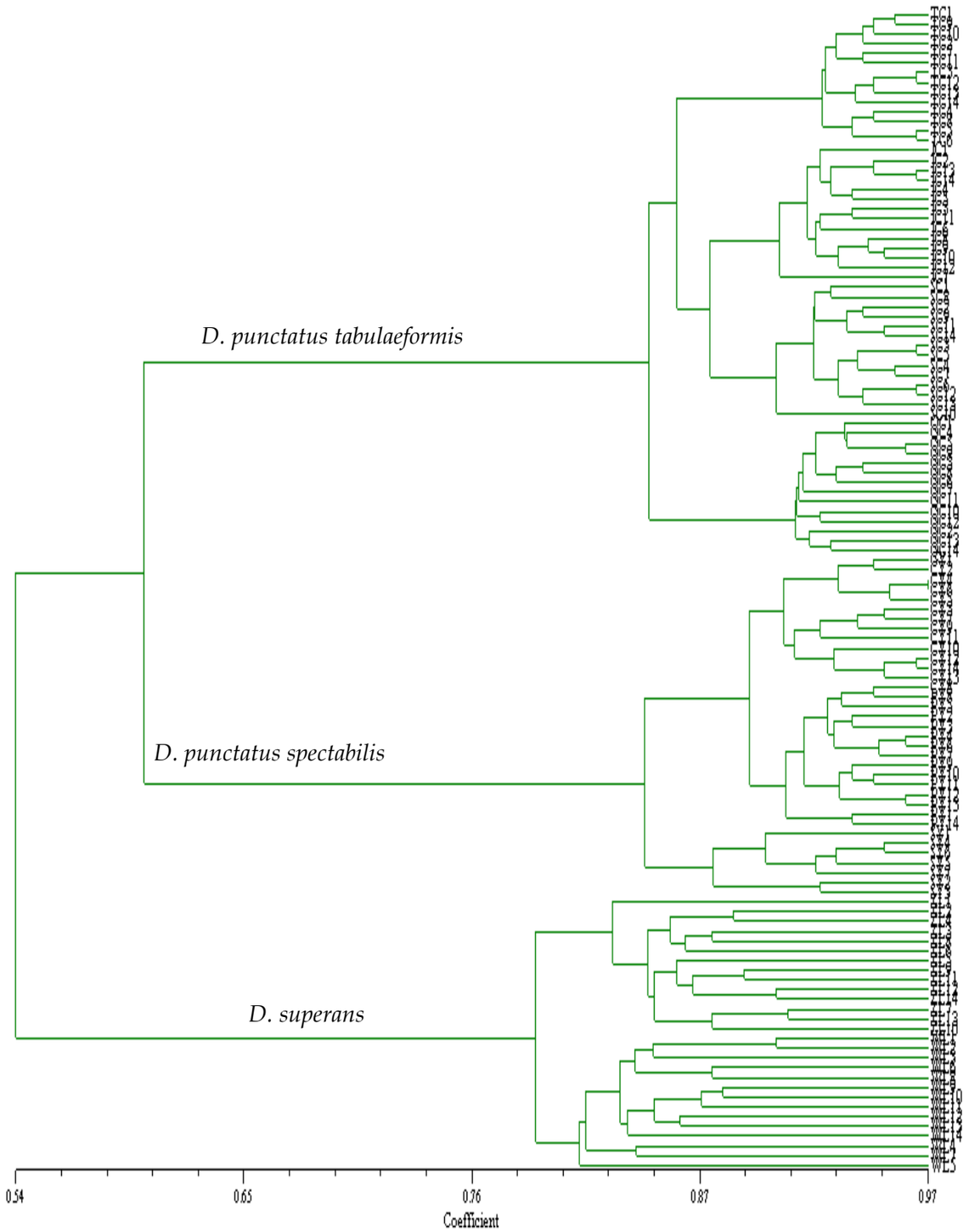


Fig. 9. Dendrogram of all D. punctatus spectabilis, D. punctatus tabulaeformis and D.superans clustered by NTSYS

species	Total gene diversity, *Ht*	Within population gene diversity, *Hs*	Genetic differentiation among populations, *Gst*	*Nm*
D.punctatus spectabilis	0.1143	0.0664	0.4192	0.6927
D.punctatus tabulaeformis	0.1132	0.0763	0.3257	1.0351
D.superans	0.1626	0.1374	0.1550	2.7265

Table 9. Genetic differentiations among 3 species of *Dendrolimus*

populations	TC	JC	SC	QC	ZL	WL	CY	PY	SY
TC	****	0.9492	0.9293	0.9305	0.6429	0.6434	0.6729	0.6735	0.6501
JC	0.0521	****	0.9512	0.9219	0.6104	0.6239	0.6833	0.6839	0.6654
SC	0.0733	0.0500	****	0.9224	0.6446	0.6535	0.7072	0.7071	0.6967
QC	0.0720	0.0813	0.0808	****	0.6154	0.6209	0.6648	0.6605	0.6510
ZL	0.4418	0.4936	0.4392	0.4855	****	0.9473	0.6970	0.6872	0.6784
WL	0.4410	0.4717	0.4254	0.4766	0.0541	****	0.6969	0.6954	0.7076
CY	0.3961	0.3808	0.3464	0.4083	0.3609	0.3612	****	0.9772	0.9259
PY	0.3952	0.3799	0.3466	0.4147	0.3751	0.3632	0.0230	****	0.9292
SY	0.4306	0.4074	0.3614	0.4292	0.3880	0.3459	0.0769	0.0735	****

Note: Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Table 10. Genetic identity and genetic distance among 9 populations of *Dendrolimus*

2.4 Genetic diversities among 3 species of *Dendrolimus* by AFLP

The genetic structure and diversity among the 3 natural populations of the *D. punctatus tabulaeformis* Tsai *et* Liu, *D. punctatus spectabilis* Butler and *D. punctatus* Walker were tested with AFLP technique(table11). At population level, gene diversity with in populations (Hs) was 0.0895; coefficient of population differentiation (Gst) was 0.7623. Genetic variation among populations accounted for the genetic diversity of the total population of 76.23%, few portions of variation exist in the population (23.77%). Gene flow between populations Nm (0.1559) on the exchange of genes between populations is not strong, can not be effectively offset by genetic drift caused by population differentiation(table12,13). Clustering results show that *D. punctatus tabulaeformis* Tsai and *D. punctatus* Walker together with a category, with the *D. punctatus spectabilis* Butler together (figure10).

Primer combination	Total bands	Polymorphic bands	Polymorphism(%)
P1-T4	46	42	91.30
P2-T3	39	33	84.62
P2-T4	62	59	95.16
P2-T5	56	52	92.86
P7-T9	33	33	100.00
P8-T1	40	39	97.50
P8-T3	40	38	95.00
P8-T4	34	31	91.18
P8-T5	34	31	91.18
P8-T6	34	33	97.06

Table 11. List of AFLP primer labels,primer sequences and amplification results of 3 different species of the tested *Dendrolimus*

population	*Na*	*Ne*	*h*	*I*	*PPB*
PYC	1.6746	1.5184	0.2870	0.4140	67.46%
SC	1.6555	1.4951	0.2739	0.3961	65.55%
HM	1.7010	1.5452	0.3003	0.4326	70.10%
Average	1.6770	1.5196	0.2871	0.4142	67.70%
All locus	1.9354	1.6707	0.3765	0.5487	93.54%

Note: PYC: *D. punctatus tabulaeformis* (Pingquan population), SC: *D.punctatus spectabilis* (Shenyang population), HM: *D.punctatus* (Walker) (Hunan population)

Table 12. The genetic diversity of 3 different species of the tested *Dendrolimus*

Pop	PYC	SC	HM
PYC	****	0.8097	0.8271
SC	0.2111	****	0.8190
HM	0.1898	0.1997	****

Note: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 13. Nei's genetic identity and genetic distance of 3 different species of the tested *Dendrolimus* materials

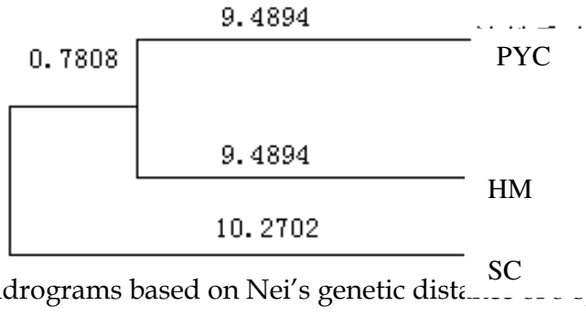


Fig. 10. UPGMA dendrograms based on Nei's genetic distance of 3 species of the tested *Dendrolimus* materials

2.5 The genetic polymorphism and genetic differentiation in mitochondrial DNA of 3 species of *Dendrolimus*

In order to provide the scientific basis for prevention and treatment of *Dendrolimus*, the genetic structure of 3 species of *Dendrolimus* were probed by mitochondrial DNA. Cyt b exhibits an A/T (73.6%) bias across all sites which was the most prominent at the third position of codon with the highest content of 86.5%. A/T bias was no significant difference among the populations. Nucleotide substitution occurred mostly at the third position (table14,15). Transitions were greater than transversions, while substitutions of intraspecific populations were higher than interspecific populations.

condon site	*T*	*C*	*A*	*G*	*A+T*	*Ts*	*Tv*	*Ts/Tv*
First	*23*	*10.0*	*46.9*	*20.0*	*69.9*	*0*	*1*	*0*
Second	*31*	*17.5*	*34.0*	*17.8*	*65*	*1*	*1*	*1.33*
Third	*47*	*1.1*	*39.5*	*12.8*	*86.5*	*6*	*2*	*3.04*
total	*33.5*	*9.5*	*40.1*	*16.9*	*73.6*	*6*	*4*	*1.45*

Table 14. Nucleotide Frequency and Substitution of Coden in Vary Site

condon site		populations					
		GYM	SYC	HBY	HBY		
					HTLZYC	PQYC	HTLZYH
Avg.	T	33.2	33.4	33.5	33.5	33.5	33.5
	C	9.6	9.1	9.6	9.7	9.6	9.5
	A	39.8	40.4	40.1	40.1	40.1	40.2
	G	17.5	17.1	16.7	16.7	16.7	16.8
	A+T	73	73.8	7.6	73.6	73.6	73.7
First	T	23	24	23	23	23	23
	C	10.0	9.6	10.0	10.0	10.0	10.0
	A	46.9	46.5	46.9	46.9	46.9	46.9
	G	20.0	20.0	20.0	20.0	20.0	20.0
	A+T	69.9	70.5	69.9	69.9	69.9	69.9
Second	T	31	30	31	31	31	31
	C	17.7	16.5	17.6	17.7	17.7	17.5
	A	33.3	35.0	33.9	33.8	33.8	34.2
	G	18.5	18.1	17.7	17.7	17.7	17.7
	A+T	64.3	65.0	64.9	64.8	64.8	64.8
Third	T	46	46	47	47	47	47
	C	1.0	1.2	1.1	1.2	1.2	0.9
	A	39.0	39.5	39.5	39.5	39.5	39.5
	G	14.0	13.2	12.5	12.4	12.4	12.7
	A+T	85.0	85.5	86.5	86.5	86.5	86.5

Table 15. Nucleotide Frequencey of Cyt b gene of different populations of *Dendrolimus*

Thirty nine nucleotide sites showed mutation in this sequence fragment and the sequence variability was 10.1%, the measurement sequence encoding 129 amino acids, of which 11 mutations, accounting for 8.5% (table16,17). Nucleotide sequences and amino acid sequences in genetic distance was 0.000-0.100 and 0. 000-0.086, indicating a low genetic variation(table18).

Average nucleotide differences of interspecies and intraspecific are 8.968 and 3.934, genetic difference mainly exist in interspecies(table19).

Defined eight different haplotypes based on the mtDNA Cyt b of *Dendrolimus* populations with one shared. Four haplotypes were identified in three populations of *D. punctatus tabulaeformis* Tsai *et* Liu, accounting for 50%, and with two shared. Analysis of the haplotype and its distribution based on the mtDNA Cyt b, in interspecies has more exclusive haplotypes but the exclusive haplotypes equal the shared haplotypes. *Fst* value and gene flow showed that genetic differentiation mostly existed in interspecies, and among the intraspecific not only the gene flow occurred, but also the genetic differences did(table20,21).

codon site	ii	si	sv	R	TT	TC	TA	TG	CC	CA	CG	AA	AG	GG
Avg.	380	6	4	1.45	139	1	3	0	37	1	0	152	5	63
First	130	0	1	0	30	0	1	0	13	0	0	61	0	26
Second	129	1	1	1.33	39	0	0	0	23	0	0	44	0	13
Third	122	6	2	3.04	59	1	2	0	1	0	0	48	5	14

Table 16. The base frequency and substitution of nucleotide codes each sites

code	site	ii	si	sv	R	TT	TC	TA	TG	CC	CA	CG	AA	AG	GG
GYM	1st	130	0	0	-	30	0	0	0	13	0	0	61	0	26
	2nd	129	0	1	0	39	0	1	0	23	0	0	43	0	24
	3rd	128	1	1	1	59	1	1	0	1	0	0	50	0	18
	Avg.	387	1	1	0.5	128	1	1	0	37	0	0	154	0	68
SYC	1st	127	0	3	0	30	0	2	0	12	1	0	59	0	26
	2nd	126	2	2	1	39	0	0	1	21	1	0	44	2	22
	3rd	126	1	2	0.5	59	0	1	0	1	0	1	50	1	16
	Avg.	379	3	7	0.43	128	0	3	1	34	2	1	153	3	64
HBY	1st	130	0	0	-	30	0	0	0	13	0	0	61	0	26
	2nd	130	0	0	0.54	40	0	0	0	23	0	0	44	0	23
	3rd	126	3	0	5.3	60	1	0	0	1	0	0	50	2	15
	Avg.	385	3	1	3.78	130	1	1	0	37	0	0	155	2	64
HTLZYC	1st	130	0	0	-	30	0	0	0	13	0	0	61	0	26
	2nd	130	0	0	-	40	0	0	0	23	0	0	44	0	23
	3rd	128	1	0	-	60	1	0	0	1	0	0	51	0	16
	Avg.	388	1	0	-	130	1	0	0	37	0	0	156	0	65
HBY — PQYC	1st	130	1	0	-	30	0	0	0	13	0	0	61	0	26
	2nd	130	0	0	-	40	0	0	0	23	0	0	44	0	23
	3rd	128	1	0	-	60	1	0	0	1	0	0	51	0	16
	Avg.	388	1	0	-	130	1	0	0	37	0	0	156	0	65
HBY — HTLZYH	1st	130	0	0	-	30	0	0	0	13	0	0	61	0	26
	2nd	129	0	1	0.67	39	0	1	0	23	0	0	44	0	23
	3rd	120	7	2	4.5	59	0	1	0	1	0	0	47	7	13
	Avg.	379	8	2	3.45	129	1	2	0	37	0	0	152	7	62

Note: GYM: *D. punctatus punctatus* Walker(Guiyang population); SYC:*D. punctatus spectabilis* Butler (Shenyang population); HTLZYC, PQYC, HTLZYH: *D.punctatus tabulaeformis* (Pingquan population)

Table 17. The base substitution of nucleotide of different populations of *Dendrolimus*

Code	1	2	3	4	5
GYM	***	0.086	0.016	0.016	0.016
SYC	0.100	***	0.070	0.070	0.070
HTLZYC	0.013	0.094	***	0.000	0.000
PQYC	0.013	0.094	0.000	***	0.000
HTLZYH	0.010	0.091	0.003	0.003	***

Note: nucleotides, lower triangle; amino acid, up triangle

Table 18. Pairwise distance of Cyt b gene sequence of different populations of *Dendrolimus*

Pop		*n*	*S*	*H*	*Hd*	*Pi*	*K*
GYM		3	3	3	1.000	0.00514	2.000
SYC		2	10	2	1.000	0.02571	10.000
HBY		15	24	4	0.670	0.01011	3.934
	HTLZYC	9	1	2	0.556	0.00143	0.556
HBY	PQYC	2	1	2	1.000	0.00257	1.000
	HTLZYH	4	23	4	0.833	0.02999	11.667
total		20	39	8	0.805	0.02306	8.968

Note:n-number of individuals; *H*-Haploid; *Hd*-Haploid diversity index; *S*-sites of diversity; *K*—Average nucleotide differences; *Pi*—Polymorphic index

Table 19. Genetic diversity between different populations of *Dendrolimus*

pop	GYM	SYC	HBY
GYM	***	0.12	0.36
SYC	0.808	***	0.17
HBY	0.583	0.744	***

Note: Fst, lower triangle; Nm, up triangle

Table 20. Genetic differentiation and gene flow between any specie of *Dendrolimus*

pop	HTLAYC	PQYC	HTLZYH
HTLZYC	***	19.00	6.88
PQYC	-0.555	***	-1.40
HTLZYH	0.025	0.067	***

Note:Fst, lower triangle; Nm, up triangle

Table 21. Genetic differentiation and gene flow between any population of *D. punctatus tabulaeformis*

Cluster analysis showed that the genetic distance between *D. punctatus* Walker and sub-species of *D. punctatus tabulaeformis* is relatively close, genetic differentiation exists between *D. punctatus* Walker and sub-species of *D. punctatus spectabilis* Butler and the population genetic differentiation was related to ecological environment(figure11).

Fig. 11. NJ dendrogram based on the Cyt b gene sequence of *Dendrolimus*

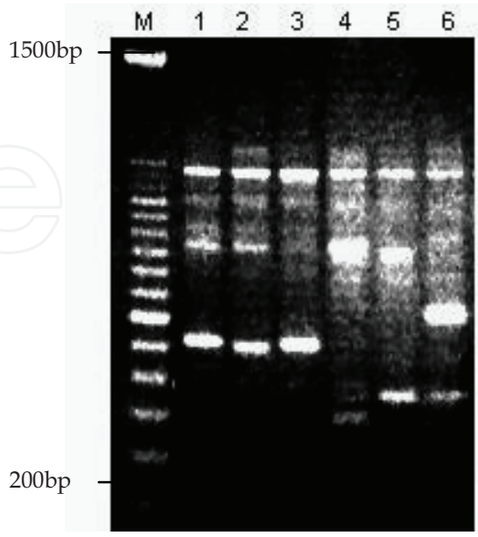
2.6 The genetic diversity analysis of 6 populations of *D. punctatus* Walker by RAPD

Rrandom amplified polymorphic DNA (RAPD) were used to evaluate genetic diversity of 6 populations of the masson moth *D. punctatus* Walker. Three populations of *D. punctatus* Walker and three populations of geogrophic subspecies of *D. punctatus* Walker - *D. punctatus spectabilis* Bulter were examined and compared(table22).

Populations	Location	Code	Latitude and Longitude
D. punctatus punctatus Walker:	Huitong , Hunan	HTM	26° 40′N 109°26′E
	Tongdao, Hunan	TDM	26° 07′N 109°46′E
	Yujiang , Jiangxi	YJM	28° 11′N 116°54′E
D. punctatus spectabilis Butler:	Shenyang , Liaoning	SYC	41° 11′ N 119°23′ E
	Jinzhou, Liaoning	JZC	40° 27′ N 119°51′ E
	Qingdao, Shandong	QDC	41° 07′N 121°05′ E

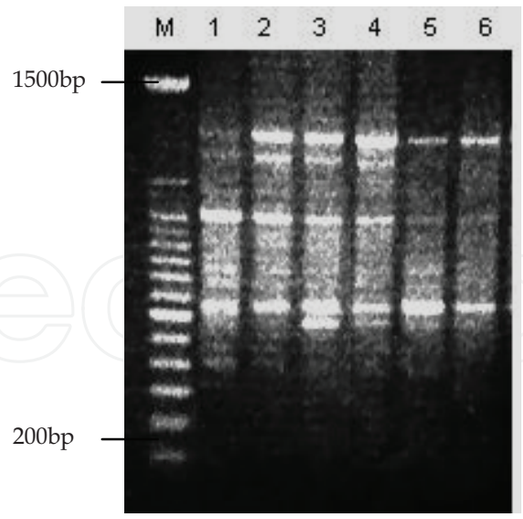
Table 22. Origin of the tested *D. punctatus* Walker materials

In total, 88 bands whose size ranged between 350 to 1500 bp were produced using 8 primers in RAPD analysis method (figure12-13,table23-24). Out of 88 loci, 73 bands were polymorphic at the species level. The percentages of polymorphic loci (*P*) was 25.19 %, the mean number of alleles per locus (*A*) was 1.252, the mean *Nei's* gene diversity (*h*) was 0.052, the mean Shannon's information index (*I*) was 0.090. For the species level, *P*= 82.95 %, *A*= 1.830, *h*= 0.234(table25). The coefficient of genetic differentiation between populations based on Shannon information index was 0.7490, which revealed a very high level of genetic differentiation among populations, the number of migrants per generation among populations (*Nm*) was 0.168, which revealed a very low gene flow among populations. The coefficient of genetic differentiation between populations based on *Nei's* genetic diversity was 0.7780 which revealed a very high level of genetic differentiation among populations, the number of migrants per generation among populations (*Nm*) was 0.143(table26,27).



1~6: SYC, JZC, QDC, HTM, TDM, YJM. M: molecular marker (100bp DNA ladder)

Fig. 12. RAPD profile amplified by primer OP03



1~6: SYC, JZC, QDC, HTM, TDM, YJM. M: molecular marker (100bp DNA ladder)

Fig. 13. RAPD profile amplified by primer OP05

Primer	Sequences (5′-3′)	Primer	Sequences (5′-3′)
OP03	CTGAGACGGA	OP12	ACGACCGACA
OP05	CTGACGTCAC	OP13	GTCAGGGCAA
OP06	AGGGCCGTCT	SB01	TTCGAGCCAG
OP07	TGCCCGTCGT	SB09	TGTCATCCCC
OP08	CTCTCCGCCA	P01	GGTCCCTGAC

Table 23. RAPD Primers of the tested *D. punctatus* Walker materials

Primer order	Sequence of primer (5′-3′)	Total bands	Polymorphic bands	Percentage of polymorphic bands
OP03	CTGAGACGGA	18	12	66.67
OP05	CTGACGTCAC	20	14	70.00
OP06	AGGGCCGTCT	25	19	76.00
OP07	TGCCCGTCGT	22	22	100.00
OP08	CTCTCCGCCA	30	30	100.00
OP12	ACGACCGACA	29	13	44.83
OP13	GTCAGGGCAA	28	28	100.00
SB01	TTCGAGCCAG	29	23	79.31
SB09	TGTCATCCCC	29	23	79.31
P01	GGTCCCTGAC	28	22	78.57
Total		88	73	82.95

Table 24. Result of RAPD on adults of 6 populations of *D. punctatus* Walker materials with 10 primers

population	*A*	*Ae*	*I*	*h*	*P*
SYC	1.239	1.058	0.081	0.046	23.86
JZC	1.193	1.056	0.071	0.042	19.32
QDC	1.273	1.059	0.087	0.048	27.27
HTM	1.273	1.076	0.099	0.058	27.27
TDM	1.250	1.070	0.089	0.052	25.00
YJM	1.284	1.096	0.114	0.069	28.41
At population level	1.252	1.069	0.090	0.052	25.19
At species level	1.830	1.398	0.359	0.234	82.95

Note: *A:* Number of alleles per locus; *Ae:* Effective number of alleles per locus;
I: The Shannon information index; *h*: Nei's genetic diversity;
P: Percentage of polymorphic loci

Table 25. The genetic variation statistic among populations of *D. punctatus* Walker materials

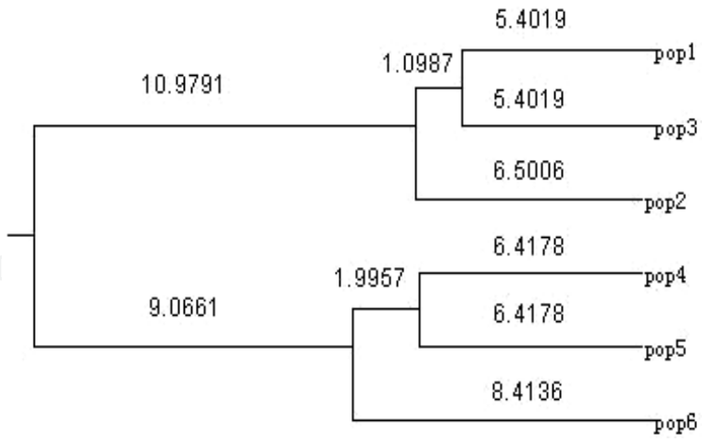
species	*D. punctatus* Walker	
Shannon's information index	*Hsp*	0.090
	Hpop	0.359
	(*Hpop-Hsp*) /*Hpop*	0.749
	N m	0.168
Nei's genetic index	*Hs*	0.052
	Ht	0.234
	Gst	0.778
	Nm	0.143

Table 26. Genetic differentiations among populations of *D. punctatus* Walker materials

population	SYC	JZC	QDC	HTM	TDM	YJM
SYC	****	0.870	0.724	0.725	0.691	0.610
JZC	0.121	****	0.747	0.732	0.687	0.623
QDC	0.108	0.139	****	0.880	0.871	0.711
HTM	0.351	0.323	0.292	****	0.820	0.637
TDM	0.351	0.322	0.313	0.128	****	0.677
YJM	0.448	0.370	0.376	0.138	0.199	****

Table 27. RAPD estimates of Nei's unbiased genetic distance among 6 populations of *D. punctatus* Walker

Three populations of *D. punctatus* Walker and three populations of *D. punctatus spectabilis* were clustered into one branch independently: pop1 and pop3 grouped firstly in the first branch, and the pop 4 and pop 5 were first grouped together in the second branch (figure14).



From pop1 to pop6: SYC, JZC, QDC, HTM, TDM, YJM, See table 22 for abbreviations of population codes.

Fig. 14. UPGMA dendrograms based on Nei's genetic distance of RAPD markers

2.7 Genetic diversities among 5 sub-populations of *D. punctatus tabulaeformis* Tsai *et* liu

The pupae of 5 sub-populations of *D. punctatus tabulaeformis* were collected in Pingquan, Hebei Province(table28). And the gene diversity and genetic structure of them were assessed by SSR (table29).

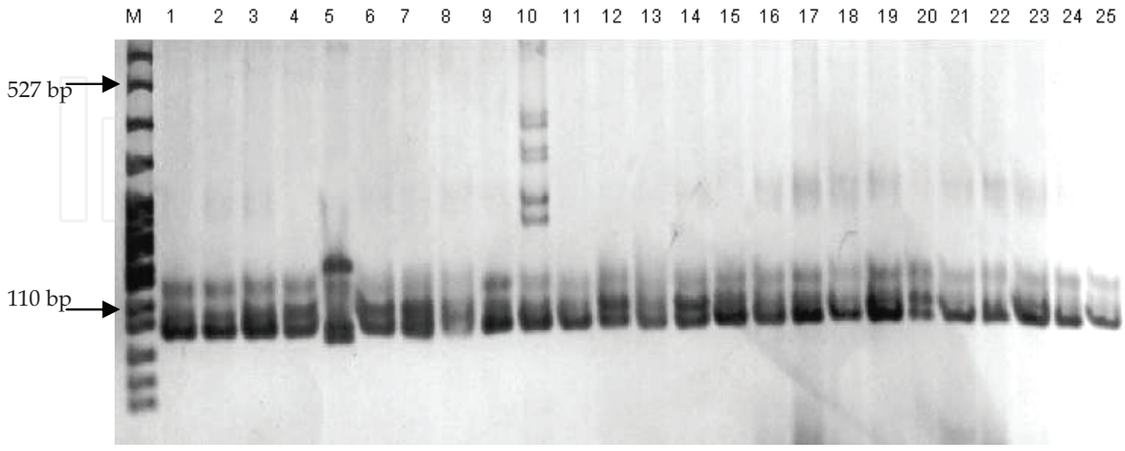
Sub-Population Name	Forest type	Mixed tree	Direction	Vegetation under the forest	Insect prevention
pop1	Artificial pure *Pinus tabulaeformis* forest	none	North	Few vegetation, pine needle leaf cover	Not control
pop2	Artificial mixed *P. tabulaeformis* forest	*Larix gmelinii* (Ruprecht) Kuzeneva	West	Few herb, pine needle leaf cover	
pop3	Artificial mixed *P. tabulaeformis* forest	*Quercus mongolicus* Fisch.	Northeast	Ferns and bryophyta, pine needle, broad-leaf leaf cover.	Not control
pop4	Natural pure *P. tabulaeformis* forest	None	South	Few herb, pine needle leaf cover	Matrine aircraft control district
pop5	Artificial mixed *P. tabulaeformis* forest	*Pinus armandii* Franch	Southeast	Herb, pine needle leaf cover	Matrine aircraft control district

Table 28. Origin of the tested *D. punctatus tabulaeformis* materials in Chengde City, Pingquan county

Primer	Sequences (5′—3′)	Number of allele	Size range (bp)	Tm(°C)
SSR4	TCATCCCGAGTCCCACTCA ATTGCTCTTCCTATCTGGCTA	3	78~188	52
SSR5	GTTCTCGGTCGTGGTTTTAG AACCGCTTCCGCCGATTAC	1	161	50
SSR6	CTGGCACCCCGCCGATTAC AACAAAACAATTATAAACTCTTAC	2	155~160	50
SSR7	ACCAACTTCGACACCTTCT CACTGCCCCGAACCTATAC	3	217~333	50
SSR8	ACTTCTACTGCGTGTGAACT GTCCCTTTGTCCGATAATATG	4	193~207	51
SSR9	GGAGCACCAATGAAGAATGT GTTTCTACCTCATGGGATCTTTTAGCTC	3	234~430	52
SSR10	ACGTAAAACTAATCAA CTGTCCAAAGCAAACTATC	3	187~220	52
SSR11	CTGCTAGAGCTTTCTGTGTT AAGAATTTCAATTTAAGACTGAC	6	158~241	50

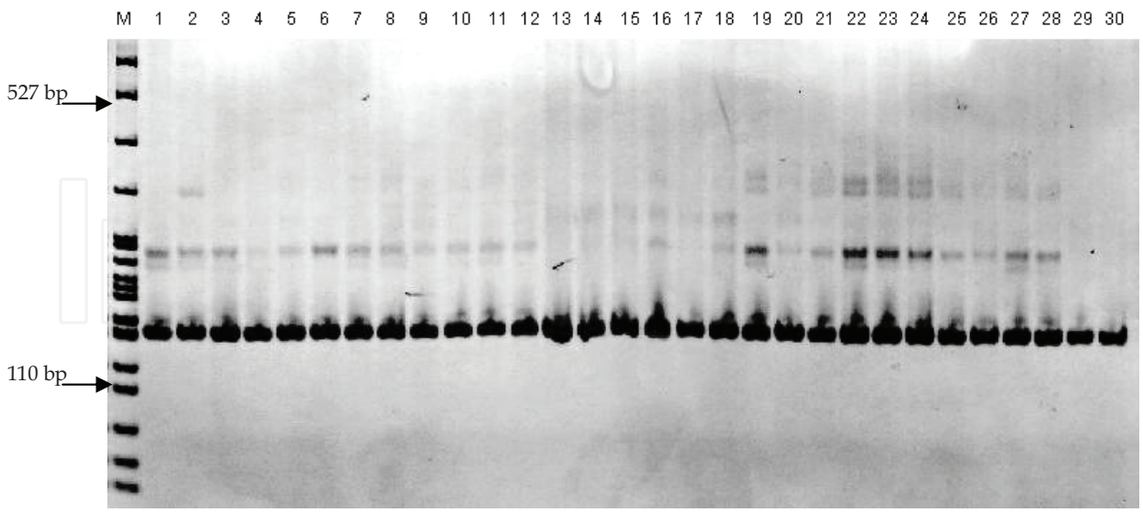
Table 29. SSR primers of the tested *D. punctatus tabulaeformis* materials in this study

For genetic diversities, among 5 sub-populations of *D. punctatus tabulaeformis*, in total, polymorphic bands were produced using 8 primers in SSR analysis method (table29, figure15-17). The percentage of polymorphic loci (*P*) was 80.00%, the mean number of alleles per locus (*A*) was 2.6250, and the mean expected heterozygosity per locus (*He*) was 0.3765. For the species level, *P*=87.50%, *A*=3.1250, *He*=0.4747(table30). From the summary of F-statistics at polymorphic loci of 6 populations, we could found the *Fst* was 0.2159, which mean there show high genetic diversity among populations.The number of migrants per generation among populations (*Nm*) was 0.9081, suggesting the occurrence of rather low



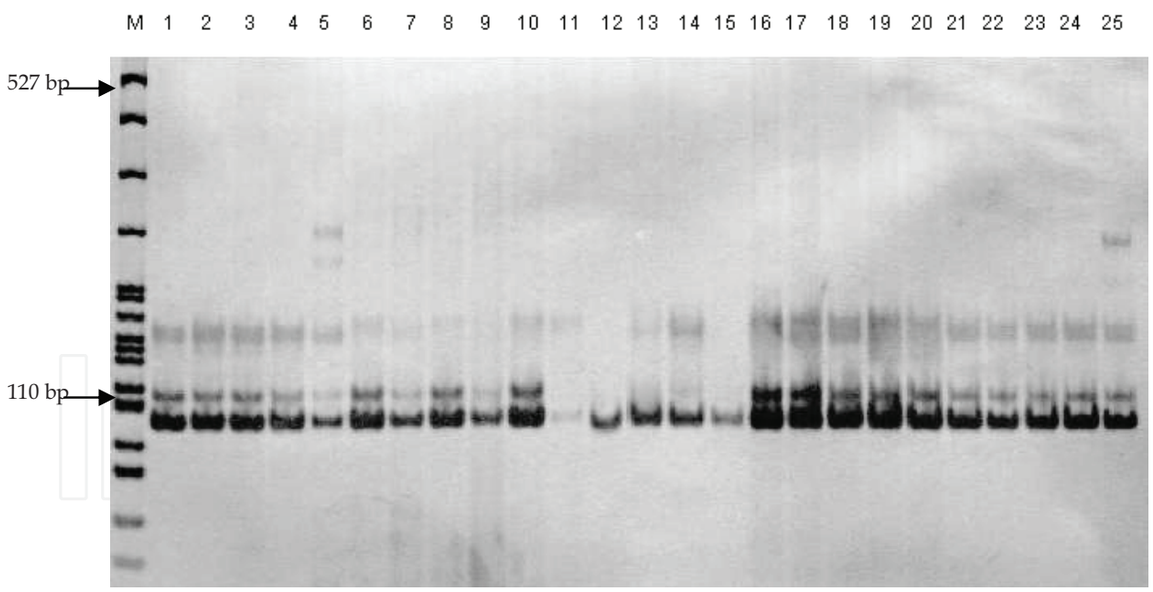
1~5,6~10,11~15,16~20,21~25 are pop1, pop2, pop3, pop4, pop5 of *D. punctatus tabulaeformis*;
M: molecular marker (PBR322/Msp I marker)

Fig. 15. SSR profile amplified by primer SSR4



1~6, 7~12, 13~18, 19~24, 25~30 are pop1, pop2, pop3, pop4, pop5 of *D. punctatus tabulaeformis*;
M: molecular marker(PBR322 / Msp I marker)

Fig. 16. SSR profile amplified by primer SSR5



1~5,6~10,11~15,16~20,21~25 are pop1, pop2, pop3, pop4, pop5 of *D. punctatus tabulaeformis*;
M: molecular marker(PBR322 / Msp I marker)

Fig. 17. SSR profile amplified by primer SSR6

gene flow among sub-populations. A UPGMA dendrogram based on *Nei's* genetic distance showed 5 sub-populations of *D. punctatus tabulaeformis* Tsai *et* liu were clustered into two groups: the first branch in the dendrogram included pop1 and pop2, and the remaining three populations were grouped together into second branch (table31,figure18).

population	*A*	*Ae*	*I*	*Ho*	*He*	*h*	*P*
pop1	2.5000	1.8045	0.6501	0.4903	0.4109	0.4063	87.50
pop2	2.5000	1.4960	0.4981	0.3587	0.2811	0.2781	75.00
pop3	2.6500	1.7922	0.6245	0.4417	0.3690	0.3649	75.00
pop4	3.0000	2.2521	0.8020	0.4054	0.4543	0.4481	87.50
pop5	2.5000	1.8122	0.6180	0.4472	0.3674	0.3634	75.00
Mean	2.6250	1.8314	0.6385	0.4287	0.3765	0.3722	80.00
Overall	3.1250	2.1201	0.8091	0.4302	0.4747	0.4736	87.50

Note: *A:* Number of alleles per locus; *Ae:* Effective number of alleles per locus; *I:* The Shannon information index;
Ho: Observed heterozygosity; *He:* Expected heterozygosity; *P:* Percentage of polyporphic loci

Table 30. Genetic diversity parameters of 5 sub-populations in *D. punctatus tabulaeformis*

POP	pop1	pop2	pop3	pop4	pop5
pop1	****	0.9322	0.7555	0.8826	0.7714
pop2	0.0703	****	0.6573	0.8262	0.6953
pop3	0.2804	0.4197	****	0.8568	0.7905
pop4	0.1249	0.1909	0.1546	****	0.8775
pop5	0.2596	0.3635	0.2350	0.1306	****

Note: See table 3-1 for abbreviations of population codes.

Table 31. Nei's genetic identity and genetic distance of 5 sub-populations in *D. punctatus tabulaeformis*

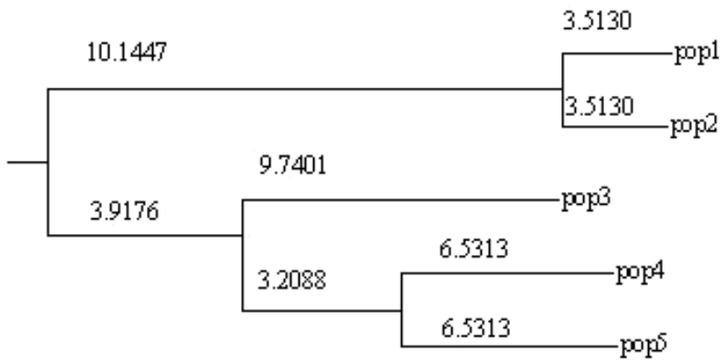


Fig. 18. Phylogenetic relationship of 5 sub-populations of *D. punctatus tabulaeformis* based on Nei's distance of SSR markers and clustered using UPGMA

3. The effect of environmental conditions on the genetic diversity of *Dendrolimus*

3.1 The effect of environmental conditions on the genetic diversity of *D. punctatus tabulaeformis* populations

The genetic diversity among 3 natural populations of the Chinese pine caterpillar (*Dendrolimus punctatus tabulaeformis*) were tested by AFLP method in one *Pinus tabulaeformis-Dahurian larch* mixed forest and two *Pinus tabulaeformis* pure forests in Pingquan county. Besides, investigations on plant species diversity, forest crown density, incidence extent, gradient and exposure of three forest communities were taken, while dilution heat method and semi-micro Macro Kjeldahl method were used to determine the content of organic matter and nitrogen in soil(table32-36).

The result of principle component analysis shows that the growth status is main factor which influent genetic diversity of *D. punctatus tabulaeformis* populations. Besides, site conditions had some effects on it. An integrated effect was produced by all site conditions, so main factor could not be judged(table37).

Mixed forest has a great influence on gene flow among different populations of *D. punctatus tabulaeformis*, because gene flow between populations in mixed forest and in pure forests was lower than that of two pure forests. Gene flow between populations in pure forest with larger species abundance and in mixed forest was higher than between population in pure forest with lower species abundance and in mixed forest, which showed that the correlation between gene flow among different populations and species abundance of pine forests is negative.

Community	Tree height	Diameter at breast height	Crown density	Exposure	Gradient	Incidence extent
I	7.9	11.6	0.6	Ubac	31.7°	1
II	7.8	10.9	0.5	Adret	34.3°	2
III	8.5	13.5	0.5	Half adret	36.0°	3

Note: I,II: pure forests; III: mixed forest.

Table 32. Statistical results of stand condition investigation

Community	Sample plot	Species number	Individual number	Simpson s diversity index	Shannon-weaver diversity index
I	1	20	285	0.7962	2.9628
	2	16	327	0.8400	2.9936
	3	17	307	0.8619	3.1770
II	4	25	499	0.8722	3.3170
	5	15	728	0.8387	2.9772
	6	20	392	0.8184	2.9746
III	7	29	1021	0.8363	3.3473
	8	31	868	0.8627	3.4626
	9	29	949	0.8055	3.1790

Note: I,II: pure forests; III: mixed forest

Table 33. Species diversity index analysis of 3 populations in *D. punctatus tabulaeformis*

Community	Sample plot	$FeSO_4$ volume of using (mL)	Organic matter content ($g \cdot kg^{-1}$)	Average
I	1	12	20.12	32.70
	2	1	47.79	
	3	8	30.18	
II	1	13	17.61	22.64
	2	8	30.18	
	3	12	20.12	
III	1	3	42.76	32.28
	2	7	32.70	
	3	11.5	21.38	

Note: I, II: pure forests; III: mixed forest

Table 34. Soil organic matter content of 3 populations in *D. punctatus tabulaeformis*

Community	Sample plot	Acid standard solution volume of using (ml)	Soil total nitrogen content ($g \cdot kg^{-1}$)	Average
III	1	1.1	0.011	0.0107
	2	1.6	0.018	
	3	0.6	0.003	
II	1	0.5	0.002	0.0051
	2	1	0.009	
	3	0.7	0.005	
I	1	1.2	0.012	0.0049
	2	0.5	0.002	
	3	0.45	0.001	

Table 35. Soil total nitrogen content of 3 populations in *D. punctatus tabulaeformis*

Population numbers	II	I	III	Total
Polymorphic bands	107	99	91	124
Polymorphism(%)	73.29	67.81	62.33	84.93
Observed number of alleles	1.7329	1.6781	1.6233	1.8493
Effective number of alleles	1.4804	1.3694	1.3441	1.4371
Nei's gene diversity	0.2683	0.2127	0.2024	0.2561
Shannon's Information index	0.3935	0.3193	0.3046	0.3877

Table 36. The genetic diversity of 3 populations in *D. punctatus tabulaeformis*

Normalizing characteristic vector	Factor 1	Factor 2	Factor 3
Shannon-weaver index of diversity	0.2403	0.3895	-0.1527
Simpson diversity index	0.2218	0.3852	0.1777
Gradient	0.1933	0.0608	-0.4748
Exposure	0.4173	-0.1938	0.1688
Crown density	-0.1848	0.4354	-0.3303
Organic matter	-0.3535	-0.3378	-0.1414
Nitrogen	-0.3636	-0.1911	0.3595
Tree height	-0.0288	0.4434	0.4204
Diameter at breast height	-0.2028	0.216	0.4462
Incidence extent	0.4173	-0.1938	0.1688
Stand type	0.4173	-0.1938	0.1688

Table 37. Principle component analysis of 3 populations in *D. punctatus tabulaeformis*

3.2 The influence of ecological factors on the genetic diversity of *D. punctatus spectabilis*

Correlation analysis by SPSS software of 4 populations of *D. punctatus spectabilis* in different geographical areas shows a significant negative relation between genetic diversity and elevation, and the genetic diversity within populations was positively related with the annual temperature and moisture. In opposite, the genetic diversity was weakly related with latitude. Besides, there was a positive relation between the genetic distance and the distance of elevation, which shows that geographic isolation has obstruct effect on gene flow (table38,39). Generally, the genetic distance between groups in the same population is certainly related with the geographical distance and host($p<0.01$,high notable correlativity).

	QC~TC	TC~JC	JC~SC	SC~QC	TC~SC	QC~JC
Altitude distance (m)	247	40	520	717	480	197
Genetic distances	0.0720	0.0521	0.0500	0.0808	0.0733	0.0813
Relationship quotiety			0.2780			

Table 38. Correlation of the genetic diversitys within 4 populations of *D. punctatus spectabilis* and the ecological factors

Ecologocal variables	Genetic diversity estimated by Shannon index	Genetic diversity estimated by Nei index
Latitude	0.0920	0.0730
Altitude	-0.6250	-0.6380
Annual temperature	0.4800	0.4650
Annual moisture	0.5610	0.5640

Table 39. Relationship of genetic distances and geographical distances (altitude distances) between 4 populations of *D. punctatus* spectabilis

3.3 The influence of ecological factors on the genetic diversity of *D. punctatus* Walker

Three populations of *D. punctatus* Walker and three populations of geographic subspecies of *D. punctatus* Walker—*D. punctatus spectabilis* Bulter were examined and compared by RAPD. The influence of ecological factors on the genetic diversity is also discussed by the correlation analysis. The correlations between the genetic index and ecological and geographic factors are significant (table40,41).

Code	Location	Longitude and latitude	Altitude (m)	Annual Precipitation (mm)	Annual mean temperature (°C)	Annual mean moisture (%)
HTM	Huitong , Hunan	26° 40′N 109°26′E	260	1304.2	16.3	75
TDM	Tongdao, Hunan	26° 07′N 109°46′E	280	1480.7	16.6	75
YJM	Yujiang , Jiangxi	28° 11′N 116°54′E	310	1788.8	17.6	76
SYC	Shenyang, Liaoni	41° 11′ N 119°23′E	900	500	8.3	37
JZC	Jinzhou, Liaoning	40° 27′ N 119°51′E	380	640	9.1	55
QDC	Qingdao, Shandong	35°30′ N 119°37′E	183	800	12.2	73

Table 40. Origin and the ecological factors of the tested *D. punctatus* Walker materials

Ecological variables	*A*	*Ae*	*h*	*I*	*P*
Longitude	-0.6658	-0.7367	-0.7506	-0.7470	-0.6686
Latitude	-0.3489	-0.4182	-0.4048	-0.4151	-0.3499
Altitude	-0.3564	-0.3312	-0.3487	-0.3357	-0.3601
Annual precipitation	0.6324	0.9161*	0.8632*	0.8927*	0.6370
Annual temperature	0.7246	0.8507*	0.8554	0.8570*	0.7282
Annual moisture	0.6265	0.6034	0.6423	0.6212	0.6302

Note: * p<0.05, notable correlativity; ** p<0.01, high notable correlativity; *A:* Number of alleles per locus; *Ae:* Effective number of alleles per locus; *I:* The Shannon information index; *h:* Nei's genetic index *P:* Percentage of polyporphic loci

Table 41. Correlation of the genetic diversity and the ecological factors of 6 populations of *D. punctatus* Walker

3.4 The influence of ecological factors on the genetic diversity of 5 sub-populations of *D. punctatus tabulaeformis*

The population genetic variability and genetic structure of 5 sub-populations of *D. punctatus tabulaeformis* in Pingquan city, Hebei province were analyzed using SSR. The influence of ecological factors on the genetic diversity is also discussed by the correlation analysis. The genetic index has positive relation with ecological factors weakly. The genetic index has positive relationship with ecological factors but not significant(table42). Stand type, slope, aspect and altitude were the main traits account for the genetic diversity according to the Principal Component Analysis (PCA).

Ecological variables	*A*	*Ae*	*I*	*Ho*	*He*	*h*	*P*
Altitude (m)	0.8238	0.8552	0.8158	-0.1849	0.6753	0.6717	0.6023
Aspect	-0.7505	-0.3947	-0.3622	0.7219	-0.1426	-0.1371	-0.3294
Slope	0.8251	0.8348	0.8591	0.2665	0.8227	0.8216	0.5535

Note:*P＜0.05, **P＜0.01, ***P＜0.001 *A*: Number of alleles per locus; *Ae*: Effective number of alleles per locus; *I*: The Shannon information index; *Ho*: Observed heterozygosity; *He*: Expected heterozygosity; *P*: Percentage of polyporphic loci

Table 42. Correlation of genetic diversity and the ecological factors of five sub-populations of *D. punctatus tabulaeformis*

4. Conclusions

For *Dendrolimus*, beside of population control, there were a few evidences for genetic structure and population differentiation of them. For such aspect, this paper may be a more effective approach, and morphological diversities, allozyme, RAPD, AFLP, ISSR, mitochondrial DNA and SSR should be the best probe tools.

The results shows that a very high level of genetic differentiation and a very low gene flow among the species of populations of *D. punctatus* When $Nm < 1$, the mutational pressure is not strong enough to prevent that allele from reaching high frequency, gene flow can't counterbalance genetic drift, and genetic drift will result in substantial local differentiation. There are great differences on genetic diversity of *Dendrolimus* populations in different

forest stands and site conditions. The correlations between the genetic index and ecological and geographic factors are significant.

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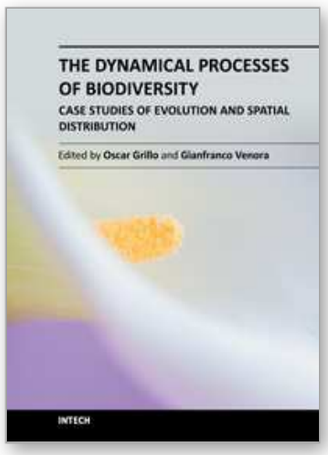

The Dynamical Processes of Biodiversity - Case Studies of Evolution and Spatial Distribution

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