Influence of long-period pesticide force on genetic polymorphism of wolf spider *Pardosa pseudoannulata* (Lycosidae : Araneae) *

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Accepted on March 13, 2007

Abstract Wolf spiders are predators in large quantities in the fields. How wolf spiders keep their dominant species status under long-period pesticide force? To answer this question, eight geographical populations of *Pardosa pseudoannulata* were used as materials to test the influence of geographical habitats on their genomic DNA polymorphism. The RAPD pattern showed polymorphic variations among and within different populations. Total 84 bands amplified by 10 random primers, of which 62 (73.81%) are polymorphic, were generated from 55 individuals of eight geographical populations. Meanwhile, Shannon's index (H_o = 0.5177) showed a rich genetic diversity of *P. pseudoannulata*, and most of the genetic variation (64.24%) was found within populations. Multiple regression analysis suggested that it is the climatic variation (such as annual average temperature etc.) that results in adaptive eco-geographic differentiation, and it is the long-period pesticide force that speeds up the genetic differentiation of *P. pseudoannulata* which changed the genetic diversity of the population.

Keywords: *pardosa pseudoannulata* (Lycosidae : Araneae), eco-geography, long-period pesticide force, genetic differentiation.

There are tremendous effects on agricultural ecological system produced by habitat fragment due to human activities[1]. Especially, spraying pesticide and single cultivating mode strengthen the pests' resistance to pesticides and sharply decrease the quantity of their natural enemies[2,3].

However, as one kind of the main enemies of insect pests, spiders still keep their dominant status in the fields. Therefore, it is important to explore population genetic dynamics under various stresses caused by environment and human activities[4—8].

Pardosa pseudoannulata (Lycosidae : Araneae), a common species of Lycosids, is distributed in China, India, Korea, Japan and other countries of East Asia[9]. It is an ideal material to study the relationship between the natural enemies and their habitats in the ecological system because it consumes more planthoppers and leafhoppers than other spider species, and it is important in controlling pests and developing non-pesticide agriculture[10—12].

Eight populations of *Pardosa pseudoannulata* distributed in the southern area of China were studied by RAPD[13—15] markers. The aim of this study is to determine the genetic variation within and among *P. pseudoannulata* populations and illustrate the molecular mechanism of forming this dominant spider species by the long-period pesticide force.

1 Materials and methods

1.1 Sampling

Fifty-five adult female samples of *P. pseudoannulata* were collected from eight populations in the southern area of China (Table 1), three populations in Yunnan Province, two in Hunan Province, three in Hainan Province. All the samples were collected during March to May in 2004.

1.2 DNA extraction and RAPD protocol

A modified DNA extraction was performed as previously reported[16,17]. Fifty RAPD primers were tested and selected on the basis of the reproducibility of the banding profiles. This procedure yielded 10 primers for the population genetic analysis (Table 2).

* Supported by National Natural Science Foundation of China (Grant Nos. 30370208, 30570226)

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Table 1. The natural habitats of eight populations of *P. pseudoannulata* in this study

Province	Sampling site	Abbr.	Collecting time	Altitude (m)	Latitude (N)	Longitude (E)	Annual precipitation (mm)	Annual average temperature (℃)
Hunan	Tianding Village	TD	March 2004	27	28°12′	112°59′	1361	17.2
	Leifeng Village	LF	March 2004	27	28°12′	112°59′	1361	17.2
Hainan	Nanxin Farm	NX	March 2004	20	18°14′	109°31′	1279	25.0
	Wuzhi Mt.	WZ	March 2004	728	18°46′	109°31′	1430	22.5
	Danzhou Town	DZ	March 2004	45	19°31′	109°34′	1766	24.0
Yunnan	Shangpa Village	SP	April 2004	1224	26°55′	98°52′	1380	19.0
	Yamu River	YM	May 2004	1788	27°13′	98°43′	1380	14.0
	Egong Village	EG	May 2004	1680	27°16′	98°37′	1380	15.0

Table 2. Arbitrary primers used in the study

Primer	Sequence	Primer	Sequence
S1	5′ GTTTCGCTCC3′	S62	5′ GTGAGGCGTC3′
S3	5′ CATCCCCCTG3′	S92	5′CAGCTCACGA3′
S21	5′CAGGCCCTTC3′	S112	5′ACGCGCATGA3′
S23	5′AGTCAGCCAC3′	S175	5′TCATCCGAGG3′
S60	5′ACCCGGTCAC3′	S179	5′AATGCGGGAG3′

RAPD protocol[18,19] was performed in a volume of 25 μL containing template DNA (200 ng/μL), 25 mmol/L $MgCl_2$, 10 mmol/L dNTP, 10 × buffer, *Taq* polymerase and the primers selected. Conditions for the PCR-reaction were 90 s at 95℃, followed by 40 cycles of 40 s at 94℃, 1 min at 36℃ and 90 s at 72℃. A final extension step lasted for 5 min at 72℃. Amplified DNA fragments were size-separated on 2% agarose gels and stained with ethidium bromide. The banding profiles were visualized under ultraviolet light and the gel image was saved in computer using the Tanon GIS-1000B software.

1.3 Data analysis

The fragments produced by each primer were treated as a character and numbered sequentially[20]. Genotypes were scored by the presence (1) or absence (0) of all polymorphic bands[21]. For each population and each selected random primer, the number of polymorphic loci and the percentage of polymorphic loci (P) at population and species level were calculated.

Shannon's index of phenotypic diversity (H_o) was estimated by the fomula below:

$$H_o = -\sum p_i \log 2 p_i \qquad (1)$$

where p_i is the frequency of the presence or absence of the band and used to quantify the degree of within-population diversity. H_{pop} is the average diversity over different populations and H_{sp} is the diversity calculated from the phenotypic frequencies p in all populations considered together $\left(-\sum p_i \log 2 p_i\right)$. So it is possible to calculate the proportion of diversity within (H_{pop}/H_{sp}) and among populations $[(H_{sp} - H_{pop})/H_{sp}]$. This procedure was performed by the software POPGENE1.32.

Genetic distance between all pairs of individual landraces was estimated by

$$GD = -\ln[2N_{ij}/(N_i + N_j)] \qquad (2)$$

where N_{ij} is the number of bands found in both landraces i and j, and N_i and N_j are the numbers of bands found in landrace i and j, respectively. A dendrogram was constructed based on genetic distance using unweighted pair group method average (UPGMA)[22,23]. The relationship between the index of genetic diversity of *P. pseudoannulata* and their environmental factors was investigated by means of a multiple regression test in SPSS13.0 and SAS6.12[24].

2 Results

2.1 Genetic diversity of *P. pseudoannulata*

Among all 55 individuals, RAPD analysis using ten random primers generated a total of 84 scorable fragments, of which 62 were polymorphic (73.81% polymorphism). The fingerprint patterns from various populations were very different from each other. The percentage of polymorphic loci within populations ranged from 17.7% to 38.7%, with the mean of 22.9% (Fig. 1). This uneven loci distribution in each geoecotype clearly indicated extensive genetic differentation among the individuals.

Table 3 shows that Shannon's index of phenotypic diversity of *P. pseudoannulata* (H_o) is 0.5177, and 64.24% of molecular diversity existed

within populations while 35.76% among populations.

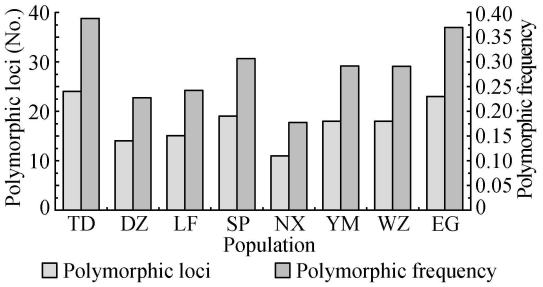

Fig. 1. Number of polymorphic bands generated from each primer and bands distribution in eight populations.

The phenotypic diversity of eight populations varied from 0.2698 (NX) to 0.3984 (TD) with an average of 0.3277. According to Shannon's index of phenotypic diversity and percentage of polymorphic loci, the order of the genetic diversity value of eight populations is as follows: TD>EG>YM>WZ>LF>SP>DZ>NX (Table 3). The effective number of migrants (N_m) among populations based on the Shannon's index ($F_{ST}=0.3670$) is 0.4312.

Table 3. Genetic diversity of within and among populations

Primer	Population								H_{sp} a)	H_{pop} b)	H_{pop}/H_{sp} c)	$(H_{sp}-H_{pop})/H_{sp}$ d)
	TD	LF	NX	WZ	DZ	SP	YM	EG				
S1	0.5802	0.4615	0.3579	0.3952	0.2781	0.3519	0.5181	0.5802	0.7287	0.4396	0.6034	0.3966
S3	0.7728	0.3325	0.2631	0.3781	0.1321	0.3688	0.4505	0.5728	0.6460	0.4088	0.6329	0.3671
S21	0.2157	0.2937	0.3198	0.2781	0.1321	0.2986	0.3038	0.2157	0.4174	0.2683	0.6428	0.3572
S23	0.3767	0.2599	0.3198	0.3412	0.4519	0.3505	0.3038	0.3767	0.4068	0.3568	0.8770	0.1230
S60	0.3727	0.1168	0.1321	0.1321	0.1321	0.2703	0.3662	0.3727	0.3713	0.2092	0.5634	0.4366
S62	0.3325	0.3783	0.2631	0.4952	0.2781	0.1038	0.3648	0.3325	0.4838	0.3393	0.7013	0.2987
S92	0.5482	0.2157	0.1321	0.2321	0.2781	0.3466	0.3519	0.5482	0.4761	0.2969	0.6236	0.3764
S112	0.2157	0.2937	0.3579	0.2781	0.2781	0.1519	0.2703	0.2157	0.4513	0.2688	0.5956	0.4044
S175	0.2599	0.2599	0.2198	0.3412	0.4102	0.1622	0.3406	0.2599	0.6971	0.3288	0.4717	0.5283
S179	0.3094	0.3251	0.3321	0.2724	0.3198	0.5128	0.4222	0.3094	0.4985	0.3550	0.7121	0.2879
Ave.	0.3984	0.2937	0.2698	0.3144	0.2710	0.291 7	0.3692	0.3784	0.5177	0.3277	0.6424	0.3576

a) H_{pop}: The average genetic diversity within populations; b) H_{sp}: total genetic diversity; c) H_{pop}/H_{sp}: proportion of genetic diversity within population; d) $(H_{sp}-H_{pop})/H_{sp}$: proportion of genetic diversity among population

2.2 UPGMA and Nei's analysis

The genetic distance of RAPD markers varied from 0.0753 to 0.3725, with the mean of 0.2426. The genetic distance between the NX and DZ populations is the nearest ($D=0.0753$), and the farthest genetic distance is between the EG and DZ populations ($D=0.3725$), suggesting that the genetic differentiation is obvious in all individuals. According to the genetic distance index, the results of UPGMA cluster analysis showed that the 55 individuals from eight populations are related to the geographic locations where they were collected. The individuals of Tianding population and Leifeng population were closely clustered together; the Shangpa, Yamu and Ega populations combined into one branch; and the other three populations clustered into one branch.

2.3 Correlation between genetic diversity and eco-geographic factors

For the eight populations, the main component analysis indicated that the genetic diversity within populations of *P. pseudoannulata* has a positive correlation with annual average temperature ($r=-0.8093$, $P<0.05$), and a negative correlation with latitude ($r=0.6326$, $P<0.05$) (Table 4). Multivariate recursive analysis ($a=0.05$) showed the regression coefficient of annual average temperature (r^2) is prominent among the four main physical factors ($r^2=0.5950$, $P<0.05$) listed in Table 4. It means that the annual average temperature is the key natural factor which influences populations' hereditary variety of *P. pseudoannulata*. In addition, we examined the differences between the expanded RAPD fragments in LF and TD populations with *t*-test. The results showed significant genetic differentiation exsited ($t=2.862>t_{0.05}$), as the Shannon's index of LF population (0.3984) is quite different from that of TD population (0.2937). We investigated their habitats and found that they differed from each other depending on whether they received long-term pesticide intimidating or not.

Table 4. Pearson correlation analyses for the relationship between Shannon's index within populations of *P. pseudoannulata* and climatic factors

Item	Correlation index (r)
Altitude	−0.4742
Latitude	0.6326*
Annual precipitation	−0.2846
Annual average temperature	−0.8093*

* means the correlation is prominent

3 Discussion

The eight geographic populations of *P. pseudoannulata*, distributed highly discontinuous and collected from the southern and southwest of China were analyzed to assess their genetic polymorphism under the long-period pesticide force. From the genetic parameters, *P. pseudoannulata* showed rich genetic diversity at the species level ($H_o = 0.5177$). The RAPD data showed that the intra-population genetic variation (64.24%) is greater than the inter-population (35.76%). According to Wright[25], F_{ST} values above 0.25 indicate substantial genetic differentiation. The mean value F_{ST}(0.3670) in our study was very high, so the genetic differentiation among populations was obvious. That is probably caused by insufficient gene flow rather than genetic drift. A moderate geographical barrier might significantly restrict the gene exchange among populations, which may lead local population diversity to co-evolve with its habitats[26]. Significant genetic differentiation was also found in *Pholcus phalangioides* and *Coelotes terrestris*[27,28]. Multiple regression analysis and RAPD polymorphism unrandom-distribution suggested that the climatic variation (such as annual average temperature etc.) results in adaptive eco-geographic differentiation, while gene transfer and drift are not dominant reasons for the differentiation[29−33].

It was found that long-period pesticide stress could change genetic diversity of *P. pseudoannulata*. Distance of the two habitats mentioned in this paper, Tianding Village and Leifeng Village, is shorter than 2 km and their climate factors are similar to each other. Tianding Village is an unpolluted rice cultivating base in Hunan Province, where pesticide has been forbidden for several decades, while organ phosphorous pesticides are used in Leifeng Village frequently. Due to the fact that there is no significant isolated patch between the two villages, we think that pesticide (human farming activities) should be responsible for the genetic differentiation of the *P. pseudoannulata* populations. In other words, long-period pesticide force has speeded up the genetic differentiation of *P. pseudoannulata* in paddy fields, which is more dominant than natural ecological factors.

In conclusion, our study suggests that intra-population differentiation of *P. pseudoannulata* is mainly determined by ecological factors and natural selection of climatic variation (mainly in annual average temperature) always results in the adaptive eco-geographic differentiation. But if in the same climate condition, long-period pesticide force will speed up the genetic differentiation faster than natural selection. Supplemented with the results of other techniques and studies, those findings might be of some importance for the further understanding of this spider species.

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