Journal of Environmental Sciences Vol. 16, No. 3, pp. 454—457, 2004

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2004)03-0454-04 **CLC number**: X503.23 **Document code**: A

Effects of traffic pollution on the genetic structure of *Poa annua* L. populations

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Abstract: The genetic composition *of Poa annua* L. populations with a series of traffic pollution was studied by starch electrophoresis. Five enzyme systems were stained. The results showed that: (1) Traffic pollution can dramatically change genotypic frequencies at some loci of *P. annua* populations. Significant deviations from Hardy-Weinberg equilibrium were observed on loci *Fe*-1 and *Me* due to the excess of heterozygotes in some populations. (2) The effective number of alleles per locus and the observed and expected heterozygosity were higher in the pollution series than in the clear control site (Botanic Park population), but the increase was not related with the pollution extent. (3) Most genetic variation was found within populations, and only 6.21% was among populations of the polluted series. Slightly higher differentiation (F_{ST} = 7.98%) was observed when the control population was included. (4) The calculated gene flow (Nm) is 2.8841 per generation. The mean of genetic identity is 0.9864 and the genetic distance average to 0.0138.

Keywords: genetic composition; traffic pollution; allozyme analysis; *Poa annua* L.

Introduction

Urban environments are specific habitats dominated by human activities. Species surviving there suffered from various stresses. Therefore, population structure, morphology, physiology of plant species may show different patterns from those of their counterparts in natural ecosystems.

One significant stress in cities is environmental pollution. As a selective factor, environmental pollution can bias the genetic composition of populations, as observed in many plant species in the field or under control fumigation experiments (Mueller-Starck, 1985; Bergmann, 1987; Prus-Glowacki, 1999; Chen, 2000; 2003; Longauer, 2001). However, this effect depends on the selection coefficient (s). When s is small, environmental pollution may increase habitat heterozygosity and subsequently does benefits to the maintenance of genetic variation. Pollution with high s will lead the loss of sensitive alleles/genotypes, therefore decrease allelic diversity and eventually lead to local extinction of sensitive species.

Under selection, changes in genetic composition of plant populations also depend on the number of generations. More generations experienced, more significant the change is. Therefore, annuals are more suitable to detect such genetic changes than perennials, because they have passed more generations than perennials do under certain period (Chen, 1999).

Poa annua is a very successful colonizer, found in both disturbed and undisturbed sites. The most common chromosome count is $2n = 28$ and it is fully self-compatible (Warwick, 1979). Plants are normally self-pollinated, with 0% to 15% outcrossing in natural populations depending on environmental and population conditions (Mengistu, 1999). It is an ideal species for detecting the effects of air pollution on genetic structure due to its short life-span, moderate sensitivity to air pollution, such as SO_2 (Chen, unpublished data). In gaseous pollution sites, *P. annua* was among the few remaining plant species though the density was very low sometimes, for example, in heavily polluted sulfur acid factory sites (personal observation). In previous studies, we found that effective allele number per loci, and expected and observed heterozygosity in populations within and near a sulfur acid factory were lower than those in populations at relatively clear sites (Chen, 2000), and clinal changes of genetic composition along an gradient of organic pollution (Chen, 2003).

Although traffic is a critical pollution source in cities, hardly any information of effects of traffic pollution on genetic structure of plant populations was available. In the present paper, we investigate the genetic structure of *Poa annua* populations along a traffic pollution gradient and compare with the control.

1 Materials and methods

1.1 Studied sites

Traffic pollution is a mixed pollution of mobile exhaust and dusts, which are related to traffic flux. Therefore, we use traffic flow as a representative of traffic pollution. Four sites in western Shanghai were chosen to represent a series of

Foundation item: The National "211" Program and the Shanghai Priority Academic Discipline Program; * Corresponding author

traffic pollution according to the traffic flow(Table 1).

Table 1 Traffic flux of four sites of traffic pollution series(traffics per hour, April 2, 2001. Monday)

Population code	Location	Number of driveways	Normal (14:00—15:00)	Rash hour (16:30—17:30)	Mean
T-1	Zhongshan Road (W.)	8	7476	9497	8486
T-2	Tianshan Road	4	1473	1876	1674
T-3	Yangliuqing Road	2	639	698	669
T-4	Friendship Road	1	9	12	10

We also collected samples from Shanghai Botanic Park as a control population. Shanghai Botanic Park is sited on the southern part of Shanghai district. No distinct pollution source was found around the park, and this district was relatively clear.

1.2 Sampling and electrophoresis analysis

At each sampling site, leaves of *P. annua* were collected with > 0.5 m distance from each other to avoid collecting ramets of the same clone. Tissue homogenates were prepared by grinding finely cut leaf samples in a tris-HCl extract buffer(0.05 mol/L, pH 7.5, containing 5% sucrose, 1.25% and 4% polyvinylpyrrolidone). The cellular extract was absorbed onto 3 × 6 mm wicks and stored at −70℃ until isozyme analysis. Electrophoresis was performed using 11% starch gels(8% hydrolyzed potato starch(S5651) of Sigma, 3% potato starch and 4% sucrose). TVB gel and electrode buffers and staining procedures of 5 enzyme systems(Table 2) followed the methods described previously(Chen, 2003).

Table 2 Summary of enzyme systems used in starch gel electrophoresis of *Poa annua* leaf samples

Abbreviation	Enzyme system	Enzyme code	Noumber of loci
Sod	Superoxide dismutase	E.C.1.15.1.1	2
Me	Malic enzyme	E.C.1.1.1.40	1
Mdh	Malate dehydrogenase	E.C.1.1.1.37	1
Fe	Fluorescent esterase	E.C.3.1.1.-	2
Pgd	6-phosphogluconate dehydrogenase	E.C.1.1.1.44	1

1.3 Statistic analysis

In both the previous and the present study, zymographs of the studied allozyme expressed a pattern of co-dominant inheritance mode (Chen, 2003). Therefore, isozyme phenotypes were interpreted genetically according to standard principles (Pasteur, 1988). Popgene 1.31, a software package designed for use with electrophoretic data, was used to analyze the data(Yeh, 1999). The mean number of alleles per locus(A), the mean effective number of alleles per locus (A_e), and the mean percentage polymorphic loci(P) were calculated at the population level. Genotype frequencies of individual loci for each population were compared to the expected Hardy-Weinberg proportions by chi-square analysis to test deviations that could be caused by inbreeding or other violations of the Hardy-Weinberg model. Nei' genetic identity coefficients and distances were calculated for all pairs of populations(Nei, 1972). Inbreeding coefficient(F) was calculated by $1 - Ho/He$, and was chi-square tested using the following formula: $\chi^2 = nF^2$, where n is the sample size and the degree is 1(Workman, 1970).

2 Results

Allelic frequencies are shown in Table 3. Except loci *Sod*-2, *Mdh*, other five loci are polymorphic at least in one population and the percentage of polymorphic loci is 71%. Narrow ranges of allelic frequencies are observed across the five populations, except locus *Fe*-1 whose allele *B*, the commonest, has a range of 0.6190—0.9634(Table 3). Chi-square test shows four loci, *Fe*-1 in T-1 and T-3, *Fe*-2 in T-3 and *Me* in T-4 populations, are significantly biased from the expects of Hardy-Weinberg equilibrium. All loci in the Botanic Park are in Hardy-Weinberg equilibrium.

Table 3 Allelic frequencies of nine *Poa annua* populations (Numerials in brackets are sample size)

Locus	Allele	T-1 (42)	T-2 (41)	T-3 (41)	T-4 (42)	Botanic Park (41)
Sod-1	*A*	0.8929	0.8780	0.9390	0.8929	0.9634
	B	0.1071	0.1220	0.0610	0.1071	0.0366
Sod-2	*A*	1.0000	1.0000	1.0000	1.0000	1.0000
Me	*A*	0.1492	0.2195	0.1463	0.0952	0.0732
	B	0.8571	0.7805	0.8415	0.8571	0.9146
	C			0.0122	0.0476	0.0122
Mdh	*A*	1.0000	1.0000	1.0000	1.0000	1.0000
Pgd	*A*	1.0000	1.0000	1.0000	0.9762	0.9756
	B				0.0238	0.0244
Fe-1	*A*	0.3810	0.0854	0.3537	0.2262	0.0366
	B	0.6190	0.9146	0.6463	0.7738	0.9634
Fe-2	*A*			0.2805		
	B	1.0000	1.0000	0.7195	1.0000	1.0000

Mean of percentages of polymorphic loci is 51.43% with a range of 42.86% to 57.14%. Numbers of alleles per locus average to 1.60(1.4286—1.7143). Mean of expected heterozygosity is 0.1159(0.0495—0.1297) and the observed heterozygosity average to 0.1435 with a range of 0.0453 to 0.2439 (Table 4). No significant difference is observed among populations of pollution series. All the indices of genetic variation of the control population are lower than those of populations of the traffic pollution series, except P and A.

Table 4 Genetic variation of populations of pollution series and the control

Population	P	A	Ae	He	Ho	F	I
T-1	42.86	1.4286	1.2077	0.1297	0.1803	-0.3901**	0.2022
T-2	42.86	1.4286	1.1398	0.1019	0.1220	-0.1973	0.1698
T-3	57.14	1.7143	1.2884	0.1780	0.2439	-0.3702**	0.2790
T-4	57.14	1.7143	1.1663	0.1203	0.1259	-0.0466	0.2127
Botanic Park	57.14	1.7143	1.0556	0.0495	0.0453	0.0848	0.1079
Mean	51.43	1.6000	1.1716	0.1159	0.1435	-0.1839	0.1943
Total	71.43	1.8571	1.1700	0.1260	0.1435	-0.1839	0.2226

Notes: ** $P < 0.05$

All the observed heterozygosities are higher than the expected ones in populations of pollution series, indicating excess in heterozygotes. The inbreeding coefficients(F) are smaller than zero in pollution series, and Fs of T-1 and T-3 are significantly biased from 0($P < 0.05$). F of the Botanic Park population is higher than zero, indicating a deficit of heterozygotes, but not significantly.

The total heterozygosity is 0.1260. F-statistics reveal 6.21% of the genetic variation among populations of the pollution series. However, slightly higher differentiation(F_{ST} = 7.98%) is observed when the control population is included. The calculated gene flow (N_m) is 2.8841 per generation. Nei's genetic distance and identity confirm above conclusions. The mean of genetic identity is 0.9864 and the genetic distance average to 0.0138(Table 5).

Table 5 The genetic identity and genetic distance of *Poa annua* populations

	T-1	T-2	T-3	T-4	Botanic Park
T-1		0.9850	0.9866	0.9956	0.9808
T-2	0.0151		0.9745	0.9948	0.9958
T-3	0.0135	0.0258		0.9838	0.9726
T-4	0.0044	0.0052	0.0163		0.9940
Botanic Park	0.0194	0.0043	0.0278	0.0061	

3 Discussion

As the development of urbanization, traffic pollution becomes a serious environmental problem in developing countries. The present study indicates that traffic pollution can affect the genetic composition of *Poa annua*. Several loci in populations of pollution series show biased genetic composition from the Hardy-Weinberg expectation, whereas all loci of the Botanic Park population are in Hardy-Weinberg equilibrium. Excess of heterozygotes was observed in populations of pollution series, although most loci were in Hardy-Weinberg equilibrium. Biased genetic composition has been observed in a few plant populations(Bergmann, 1989; Bakhatiyarova, 1995; Wojnicka-Poltorak, 1997; Prus-Glowacki, 1999; Longauer, 2001).

Indicies of genetic diversity showed that populations of pollution series have higher genetic variation than those of the control. They indicated that traffic pollution can maintain high genetic variation to some extent, but no distinct trend was observed. There are several reasons can explain this pattern. First, in the field experiments, the stress is often complex(Longauer, 2001), especially for traffic pollution. Traffic pollution is a mixture of complex compounds, such as CO, NOx, hydrocarbon, dusts, etc. Plants are not sensitive to CO and NOx concentration has been decreased as the improvement of gasoline. Other pollutants by traffic are not high enough to lead to serious damage to *P. annua*.

Secondly, founder effect and gene flow may affect the genetic consequences of traffic pollution on *P. annua* populations. *P. annua* suffered habitat fragmentation in urban areas. In such habitats, founder effect led by colonization, inbreeding and subsequent inbreeding depression, and random drift will decrease genetic diversity. However, *P. annua* is a wide spread weed, and usually high gene flow can be found among populations. Traffic can also increase the gene flow. Furthermore, human activities can also increase the gene flow. For example, seeds of *P. annua* can migrate to other populations via greening in cities (Chen, 2003). Actually, high gene flow(N_m = 2.8841) was found in the present study. Effects of increased gene flow and fragmentation as well as inbreeding and drift have diverse consequences on genetic structure of plant populations and output of their mixture increases the uncertainty under traffic pollution.

Thirdly, other abiotic factors, such as other air pollutants emitted from factory, soil pollutants, drought, individual or in combination may also be driving force of selection(Longauer, 2001) and affect the genetic composition

of *P. annua* populations. Therefore, no distinct trend can be observed under such complex conditions without dominant pollutant.

Effects of traffic pollution on the genetic composition of *P. annua* populations are different from those of other pollutants. Under the stress of ambient sulfur oxidants, significant decreased genetic diversity was observed and the genetic composition was biased from those in clean sites (Chen, 2000). However, heterozygosity and allelic frequencies at some loci showed clinal changes along an organic pollution gradient. At some loci, all individuals were heterozygous at the most heavily polluted site(Chen, 2003).

Diverse impacts of different pollutants on the genetic composition of plant populations were also observed in other species. For example, genetic diversity in two damaged-degrading *Pinus sylvestris* populations, near a chemical enterprises producing mineral fertilizers, plastics and organic dyes, were lower than in three populations of the background and not polluted zone in Ukraine (Korshikov, 2002). However, increased genetic diversity was observed in population polluted by heavy metal and gaseous pollutants (Prus-Glowacki, 1999).

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(Received for review May 15, 2003. Accepted June 24, 2003)