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RESEARCH ARTICLE

Leaf developmental stability of *Platanus acerifolia* under urban environmental stress and its implication as an environmental indicator

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Abstract The developmental stability indices, leaf width based fluctuating asymmetry (FA1), and lateral vein length based directional asymmetry (RDA1) of *Platanus acerifolia* were studied. All the leaves were sampled from 14 sites that were categorized based on different urban environmental stress levels (UESL) in Shanghai metropolitan, China. Besides, foliar stomatal density and stomatal length were also studied as the subsidiary indices to test the availability of developmental stability indices as the indicator under a stressful environment. Results showed seasonal variation of FA1 and RDA1 existed among the 14 sites, but the data showed significant negative correlation between FA1 and UESL ($FA1 = 0.029 - 0.000\,9UESL + 0.000\,3UESL^2$, $r = 0.766\,5$, $P = 0.001\,4$). However, a similar trend was not found between RDA1 and UESL. Furthermore, the significant correlation among FA1 and leaf stomatal length and stomatal density implied they could be used as indicators of urban stress levels on a small scale. It seemed that RDA1 was possibly a normal parameter during leaf development but it was unavailable for use as an indicator of urban stresses.

Keywords Shanghai, *Platanus acerifolia*, developmental stability, urban environmental stresses

1 Introduction

Developmental stability reflects the ability of individuals with bilateral symmetry to buffer development against environmental and genetic stresses (Møller and Swaddle, 1997; Swaddle, 1999). Among current morphological traits used to measure developmental stability of organisms, fluctuating asymmetry (FA) is the most widely used metrics (Fig. 1). It is defined as small and random nondirectional departures from anticipated bilateral symmetry. Since FA is the variance in random deviations from perfect bilateral symmetry, it is usually expressed as Var (R_i-L_i) (Palmer and Strobeck, 1986; Polak and Trivers, 1994). Here, R_i-L_i refers to the difference of width or length on both sides of individuals with bilateral symmetry. FA has been measured to reveal the ability of organisms to minimize random deviation from ideal trajectory during developmental stages under particular environmental conditions (Graham et al., 1993a; Graham et al., 1993b; Graham et al., 1999; Møller and Shykoff, 1999; Hosken and Blanckenhorn, 2000). As manifested by many field observation and laboratory works, individuals and population with lower FA were more competitive in sexual selection, fecundity, success in being pollinated, and survival (Møller, 1998). Generally, in comparison to the other indicators, such as survival and productivity, FA is much easier to use as an indicator of the fitness of organisms (Graham et al., 1993; Leung and Forbes, 1997; Møller et al., 1999). Until now, many research efforts have been made to explore attractive aspects of FA. Thus, the fluctuating paradigm came into being (Simmons et al. 1998).

Over the past decades, developmental stability has been used to detect the possible response of organisms to environmental stresses of anthropogenic and natural sources. Historically, a large number of studies on FA were mainly focused on animals, and only a few studies were conducted on plants. Furthermore, previous studies on effects of environmental stresses on FA of plants mainly involved competition, elevation, radiation, pest, pathogen, and industrial pollution. However, studies on FA of urban plants under adverse stresses received less attention. Thus, in this paper the widely planted *Platanus acerifolia* in Shanghai was selected to detect whether there was FA; if there was, together with stomatal traits and physiological items, whether it was available for usage as an indicator of stress levels of the urban environment.

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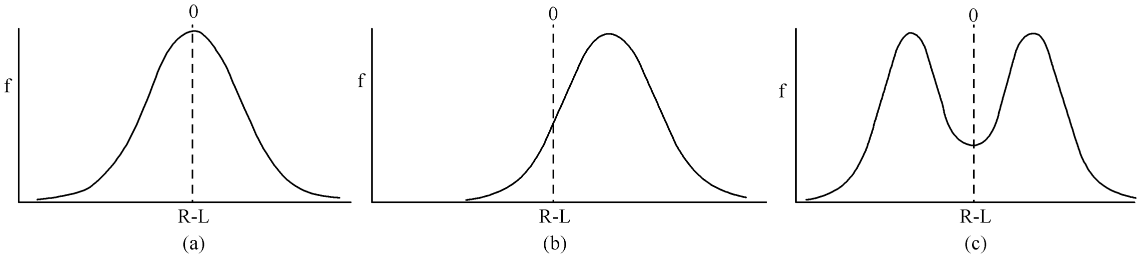

(a): Fluctuating asymmetry (FA); (b): Directional asymmetry (DA); (c): Antisymmetry (platykutic or biomodal) (Palmer, 1994)

Fig. 1 Frequency distribution of R-L in describing the mode of developmental stability

2 Study area and methods

2.1 Urban stress level and sample sites

Given the difficulties in quantitative assessment of urban environmental stresses, a semiquantitative assessment index, urban environmental stress levels (UESL) was constructed on the basis of field survey at 14 sample sites in Shanghai, China. The sites included Changfeng park (CF Park), Zhongshan park (ZS Park), Renmin park (RM Park), the Botanic garden, the Zoo, Wending road (WD Rd), Hunan road (HN Rd), Hongcao road (HC Rd), West Zhongshan road (West ZS Rd), Hutai Road (HT Rd), Jingshajiang Road (JSJ Rd), Fudan University (FD University), the No.3 Steel Factory (No.3 Steel), and Gaoqiao Chemical Plant (GQCP). Among these sample sites, the No.3 Steel Factory (No.3 Steel) and Gaoqiao Chemical Plant (GQCP) were typically heavy industrial areas with severe pollution, and Wending Road was a typical mixture of commercial area and residences. Whereas the sites at West Zhongshan road (West ZS Rd), Hutai Road (HT Rd), and Jingshajiang Road (JSJ Rd) were characterized with heavy traffic flow and subsequent severe air pollution, the sample site Hongcao road (HC Rd) was characterized with low traffic flow but traditional electronic industries, where air pollution was somewhat severe. Thus, in this study, UESL was determined with three major environmental factors: traffic flow level, industrial level, and impervious surface level (Zhang and Wang, 2004).

- Property values were assigned based on the following: traffic flow level (TRL) was numerically indexed 0 (average hourly flow less than 20); 1 (average hourly flow between 21—100); 2 (average hourly flow between 101—500); 3 (average hourly flow between 501—1000); 4 (average hourly flow between 1001—3000); 5 (average hourly flow more than 3000).
- Industrialized level (INL) was numerically indexed 0 (nonindustrial area nearly free from pollution); 1 (light industrial area with mediate pollution); 2 (heavy industrial area with severer pollution).
- Impervious surface level (ISL) was numerically indexed 1 (soft and unpaved land); 2 (unpaved land but hard to penetration of water); 3 (pavement with concrete). Thus, UESL was calculated using the formula UESL = TRL + INL + ISL. Here, UESL was used as an index to indicate the general stress level of urban environment (Fig. 2).

In this study, at each sample site, five trees were randomly selected and marked. The trees were sampled in the sunny days during early May, late September, and early November 2001, respectively.

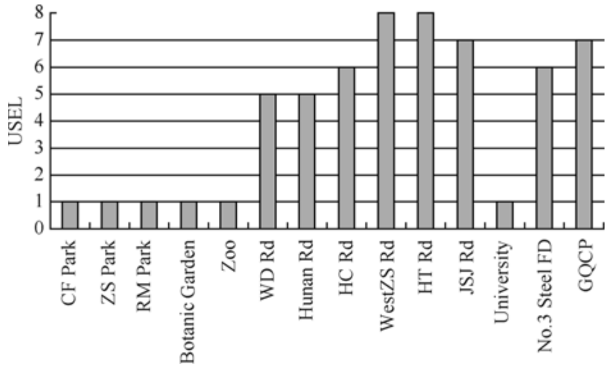

Fig. 2 Site-specific environmental stress levels at 14 sites within Shanghai urban area

2.2 Measurement of leaf stomatal traits

Twigs were haphazardly cut at the mid-canopy of the trees regardless of the direction of branches. At the top of the twigs, the fully expanded leaves between the third and fifth phyllotaxy were selected, and a leaf impression (about 1 cm^2) was made with clear nail oil at the adaxial surface of the uppermost fully expanded leaf. After 3—5 min, the drying imprint was peeled and carefully mounted on the glass slide of a microscope. For the imprints of leaf stomata of each site, a total of viewing fields were observed by using the micrometer. Stomatal density was counted by using the grid cell micrometer, the minimum rule of which was 0.5 mm. Within viewing fields of each leaf, under 400 × magnification 20 stomata were randomly selected to measure the maximum length by using the grid cell micrometer, the minimum rule of which was 0.1 mm. Finally, all the data of stomatal length were converted to the unit of μm.

2.3 Chlorophyll content and plasmalemma permeability

Twigs were kept in clear, cool plastic bags and quickly taken back to the laboratory. Fresh leaves were cut and mixed quickly, and then 50 mg leaf in 10 replications at each site were used to measure total chlorophyll concentration. Chlorophyll was extracted with N, N-Dimethylformamide (DMF) in the dark at 4ºC, and concentrations of chlorophyll a and b were determined in accordance with the formulations: $\mathrm{chl}a = 12.7 \times A_{664.5} - 2.79 \times A_{647}$, $\mathrm{chl}b = 20.7 \times A_{647} - 4.62 \times A_{664.5}$ (Inskeep and Bloom, 1985), where 664.5 nm and 647 nm were the wavelengths of maximum absorbance for chl*a* and chl*b*, respectively. Thus, concentration of total chlorophyll was determined and expressed in mg/g FW.

Prior to the measurement of relative electronic conductivity, fresh leaves were cut into circular leaflets of diameters 2 cm. The leaflets were fully mixed and about 1.00 g in 10 repeated samples at each site were used. Relative electronic conductivity was measured in accordance with the method proposed by The Shanghai Society for Plant Physiology (1988).

2.4 Measurement of leaf FA

In this study, ten fully expanded leaves were randomly picked from the mid-crown of each sampled tree. They were pressed and dried prior to the measurement of FA. In accordance to methods described by Møller (1998, 1999) and Rettig (1997), FA was measured as the difference of the perpendicular distance from the midpoint between the base and the tip of the leaf to the edges (to the nearest /mm). Besides, difference of the lateral vein length of the right and left halves was measured as an extra index of FA (Fig. 3).

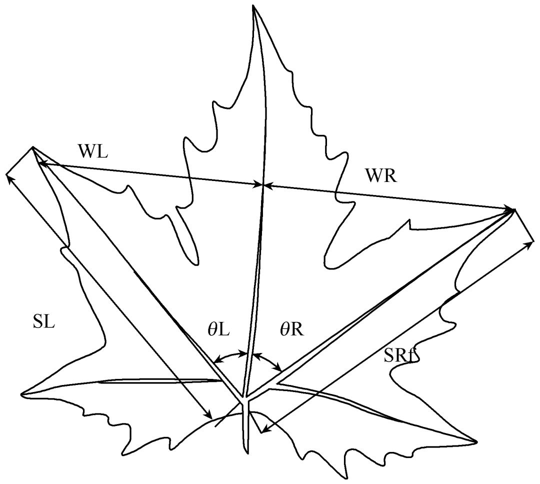

Fig. 3 Leaf metrical traits measurement of *Platanus acerifolia*

For the difference between leaf width of two sides, FA was expressed as D1, which was measured as the left leaf width minus the right leaf width. For the difference between lateral vein length of the two sides, FA was expressed as D2, which was measured as the left lateral vein length minus the right lateral vein length. D1 and D2 were calculated as follows: $D1 = L - R$ and $D2 = SL - SR$, where L and R were left leaf width and right leaf width, respectively, and *SL* and *SR* left lateral vein length and right lateral vein length, respectively. Besides, to check measurement errors, each leaf was measured again 30 min after the first measurement was completed. *Kolmogrov-Smirnov* test and one sample *t* test were used to check whether D1 and D2 of all the sample sites met the assumption of normal distribution with the mean value $\mu = 0$. The statistical analysis showed D1 did not significantly deviate from the characteristics of distribution of FA, and D2 did not significantly deviate from the characteristics of distribution of DA. Due to the complexity of computation of FA, the simple index of FA1 was adopted according to suggestions by Palmer and Strobeck (1986) and Palmer (1994). Herein, $FA1 = |L - R|$.

To minimize the effect of leaf size on FA and DA, leaf width (*LW*) and lateral vein length (*SL*) were adjusted as follows: $LW = (WL + WR) / 2$ and $SL = (SL + SR) / 2$, respectively. Furthermore, subsequent linear regression analysis showed there was significant effect of SL on D2. Thus, D2 was renamed as DA1. Another index of RDA1, calculated according to the formulation $RDA1 = |DA1| / SL$, was constructed to measure the DA of the lateral vein of the leaf. Besides, due to the disruption by climatic change, we failed to sample the leaves at the site of West ZS Rd in early March and GQ Petrochemical factory in early November. Therefore, nonequilibrium ANOVA was used to analyze the data further.

3 Results

3.1 Leaf FA as a response to urban stresses

As shown in Table 1 and 2, there was significant difference in leaf FA1 among sample sites due to interactions of seasons and sample sites as well as sample sites and trees. This could explain that significant difference in leaf FA1 among sample sites could be mainly attributed to site-specific environmental stresses, whereas the effect of various seasons did not solely show significant statistical level. However, there was significant difference in leaf RDA1 among sample sites due to season variation, interactions of seasons, and site-specific environmental stresses. This also indicated that RDA1 was subject to variation of seasons compared with FA1.

Figure 4 to 9 shows the variations of site-specific FA1 during three seasons. In general, at the sample sites of GQCP, the No.3 steel factory, HT Rd, and JSJ Rd, where the USEL were assessed at higher levels, higher leaf FA1 were

Table 1 Three-way ANOVA of site-specific leaf FA1 of *Platanus acerifolia*

Source of variation	DF	SS	MS	*F*	*P*
Seasons	2	0.002	0.001	1.300	0.273 1
Sites	13	0.045	0.003	4.560	<0.000 1
Seasons ×sites	23	0.053	0.002	3.060	<0.000 1
Trees	4	0.006	0.002	2.110	0.076 6
Seasons × trees	8	0.006	0.001	1.020	0.417 1
Sites × trees	52	0.056	0.001	1.430	0.025 6
Seasons × sites × trees	90	0.070	0.001	1.020	0.429 3
Error	1 718	1.302	0.001		

Table 2 Three-way ANOVA of site-specific leaf RDA1 of *Platanus acerifolia*

Source of variation	DF	SS	MS	*F*	*P*
Seasons	2	8.863	4.432	807.060	<0.000 1
Sites	13	1.072	0.082	15.020	<0.000 1
Seasons ×sites	23	2.192	0.095	17.360	<0.000 1
Trees	4	0.029	0.007	1.320	0.260 8
Seasons × trees	8	0.181	0.023	4.130	<0.000 1
Sites × trees	52	0.344	0.007	1.200	0.152 4
Seasons × sites × trees	90	0.690	0.008	1.400	0.009 6
Error	1 718	9.434	0.005		

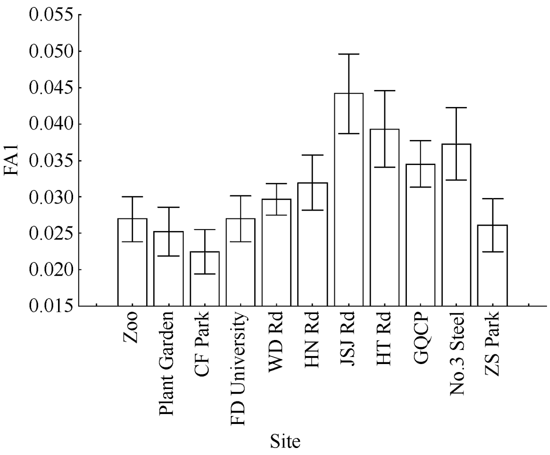

Fig. 4 Site-specific leaf fluctuating asymmetry (mean±SE) of *Platanus acerifolia* (March)

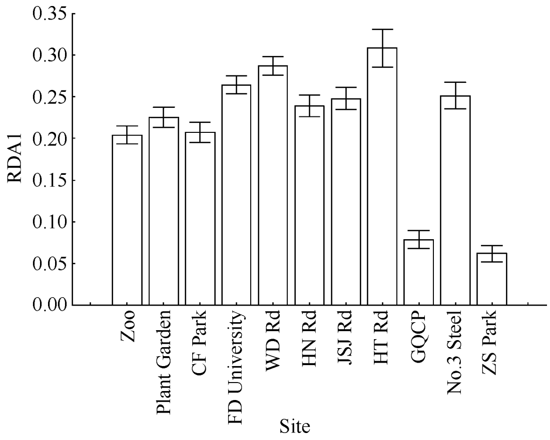

Fig. 5 Site-specific leaf directional asymmetry (mean±SE) of *Platanus acerifolia* (March)

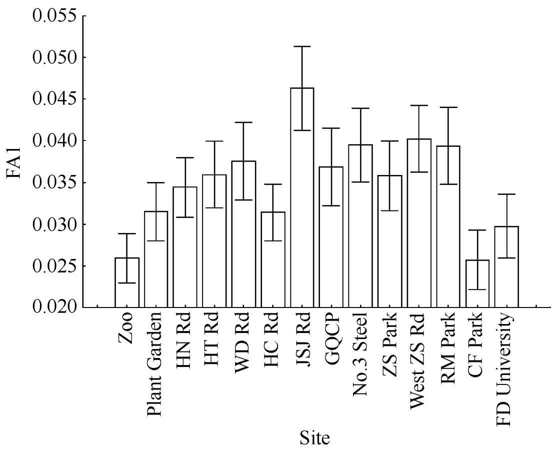

Fig. 6 Site-specific leaf fluctuating asymmetry (mean±SE) of *Platanus acerifolia* (September)

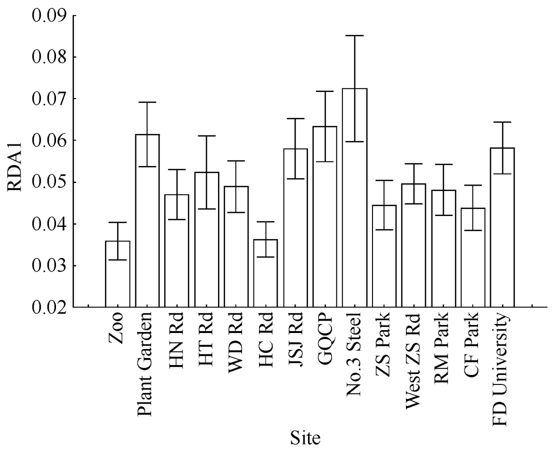

Fig. 7 Site-specific leaf directional asymmetry (mean±SE) of *Platanus acerifolia* (September)

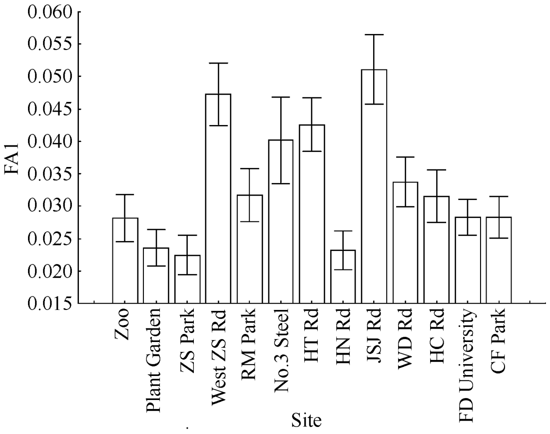

Fig. 8 Site-specific leaf fluctuating asymmetry (mean ± SE) of *Platanus acerifolia* (November)

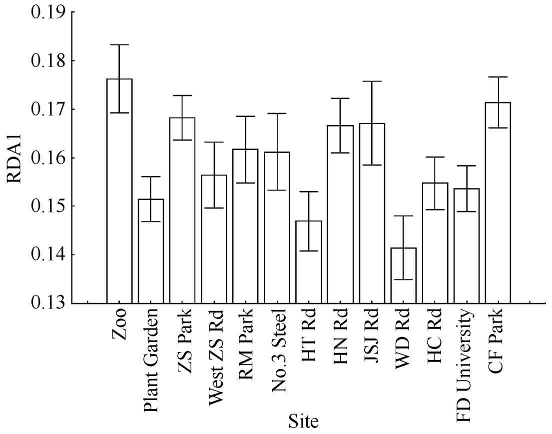

Fig. 9 Site-specific leaf directional asymmetry (mean ± SE) of *Platanus acerifolia* (September)

detected. The Results did not show significant difference in site-specific leaf FA1 among these sites. In contrast, at the sample sites of HN Rd, HC Rd, Botanical Garden, and Zoo, where the USEL were assessed at lower levels, lower leaf FA1 was detected. However, both at the sample sites with higher or lower USEL, significant seasonal variation of RDA1 showed somewhat puzzling results, which frustrated our effort in exploring the linkage between RDA1 and environmental stresses. In addition, based on all the data on nonlinear regression of the overall FA1, RDA1 and USEL are expressed by the following equations:

$$FA1 = 0.029 - 0.0009UESL + 0.0003UESL^2 \ (r=0.7665, P=0.0014)$$

$$RDA1 = -0.0024UESL^2 + 0.0201UESL + 0.1148 \ (r = 0.0212, P = 0.9426)$$

Here, it is very clear that leaf FA1 increased along the gradient of clean sites and USEL along heavily polluted sites. This tendency was similar to the findings by Freeman et al. (1993) and Kozlov et al. (1996), which, in turn, enforced the valid indication that increasing FA1 implied increasing USEL.

3.2 Correlation of FA and stomatal traits and common physiological indices

Table 3 shows significant correlation between stomatal traits and leaf FA1, especially among stomatal density, stomatal density, and FA1. There was no similar correlation between common physiological indices and FA1 except for the weak negative correlation between total chlorophyll content and stomatal density (p = 0.062). Thus, increased FA1 is associated with increased stomatal density and decreased stomatal length could be regarded as a complex response to environmental stresses (Zhang et al., 2004).

Table 3 Kendall's tau_b correlations among different indices of *Platanus acerifolia*

	SD	SL	FA1	DA1	TCHL	REC
SD	1.000					
SL	-0.581^{**}	1.000				
FA1	0.560^{**}	-0.603^{**}	1.000			
DA1	0.275	−0.067	0.143	1.000		
TCHL	−0.376	0.236	−0.309	−0.177	1.000	
RC	0.275	−0.089	0.231	0.429^{*}	−0.133	1.000

SD: stomatal density; SL: stomatal length; TCHL: total chlorophyll content;
REC: relative electronic conductivity;*: $P < 0.05$; **: $P < 0.01$

4 Discussion

So far, the mechanism of fluctuating asymmetry has still been unclear. It was argued that increasing FA occurred under environmental and genomic stress, which led to a reduction in developmental homeostasis. Experiments showed relatively severe stress, such as extreme temperature, caused increasing FA, so did genetic perturbations, which implied genomic stress, including certain specific genes, directional selection, inbreeding, and alternation of chromosome balance during trait ontogeny of organisms (Parsons, 1992). Based on field survey, Møller (1998) observed significantly higher FA of plant leaves at the deserted Chernobyl nuclear power station, where plants showed higher FA due to alternation of certain specific genes in contrast to the same species at the unpolluted sites. Besides, some studies demonstrated intraspecies hybrids have lower FA trait than interspecies hybrids, and the latter usually showed higher FA due to the increasing developmental instability (Freeman et al., 1995). Here, genomic stress was responsible for increasing developmental instability. However, some studies have documented developmental stability measured with increasing fluctuating asymmetry should mainly be attributed to environmental stresses (Freeman et al., 1993; Graham et al., 1998; Mpho et al., 2001; Wilsey et al., 1998; Wilsey and Saloniemi, 1999).

In this study, as a widespread ornamental tree in Shanghai, breeding of *Platanus acerifolia* was mainly in clone formation. Although it was very difficult to confirm whether the sample trees were from the same clone, this study's statistical results showed the contribution of genetic factors to the variation of FA was far from the significant level. As shown in the statistical results, environmental gradient mainly caused significant difference of FA among sample sites. Besides, environmentally induced rapid and uneven growth may cause increasing FA, though fluctuating asymmetry exists in normal leaves (Leung and Forbes, 1997). At the sample sites, such as industrial areas and main traffic roads, which were characterized with heavy air pollution, high level of nitrogen dioxide played an important role of an extra fertilizer in stimulating rapid growth and expansion of leaves. Consequently, increasing FA would possibly occur. On the other hand, major air pollutants, such as NO_X, PAH_S, particulate matters, TSP, etc., may cause adverse effects on leaves. For example, nitrogen dioxide absorbed into the inner part of a plant would cause potential harm, especially in the case of darkness that deactivates light-dependent enzymes and the increasing level of nitrite, which could harm plant tissues (The division two of Institute of Botany, the Chinese Academy of Sciences, 1978). Another example is the dust and bigger particulate matters that may deposit and cover leaf surfaces and stomatal apparatus. As a result, except for the decreasing area of effective photosynthesis, the plants are subject to the environmental stress of water loss (Ricks and Williams, 1974; Ricks and Williams, 1975). Nevertheless, trace elements, such as Ba, Cu, Mg, Ti, Fe, Zn, Mg, Al, and so on, in the dust and bigger particulate matters, which are mostly emitted by the vehicles, may possibly cause salt stress to the plants (Miguel et al., 1997; Morreno et al., 1999; Wróbel et al., 2000). In summary, the joint effects of environmental stresses may interrupt and break down the normal physiological process and disturb the signal transfer of the inner part of the tissues. Consequently, the deviation from normal distribution occurs as a general response of decreasing developmental stability, which is manifested by increasing FA under environmental stresses.

Some studies showed there was the tendency that fluctuating asymmetry would shift to directional asymmetry under the worst stress conditions (Graham et al., 1993; Graham et al., 1998). In this study, directional asymmetry (measured as RDA1 and DA2) of the lateral vein of *Platanus acerifolia* was possibly a normal formation during leaf development, which was not suitable for assessment of environmental stresses. As the common physiological indices, they are sensitive for indicating environmental stresses at local levels. However, these indices are also subject to disturbance and variation in the environment. In contrast, stomatal traits and fluctuating asymmetry characteristics are relatively stable in indicating environmental stresses, especially in the case of assessment of accumulative effects of environmental stress such as air pollution. Besides, it is easier to measure stomatal traits and fluctuating asymmetry characteristics than the common physiological indices in multisite field surveys. Thus, both economically and effectively, integrating multisite field data into spatial analysis based on GIS and RS will lead to better understanding of environmental stresses.

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