

Diurnal and Nocturnal Behaviour of Airborne *Cryptomeria japonica* Pollen Grains and the Allergenic Species in Urban Atmosphere of Saitama, Japan

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ABSTRACT

Japanese cedar (*Cryptomeria japonica*) pollinosis is the most popular pollinosis in Japan. It has been reported that *Cryptomeria japonica* pollen allergenic species are suspended as fine particles in the urban atmosphere. These allergenic fine particles are responsible for inducing asthma by breaking into the lower respiratory tract. It has also been found that pollinosis symptoms on the sufferers appear mainly at night-time by the results from epidemiological studies. However, the exact reason for these phenomena is not yet clarified. In this study, the diurnal and nocturnal behaviours of *Cryptomeria japonica* pollen grains and their allergenic species in the urban area of Saitama city of Kanto Plain were investigated. Airborne pollen grains and allergenic Cry j 1 concentrations in total suspended particulate matter (TSP) were investigated at two sampling sites, a heavy traffic road (roadside site) and at the balcony of the 10th floor of the Building of Research and Project of Saitama University (general urban site). The latter sampling site where located about 300 m away from the roadside site was used as a general urban site unaffected by automobile traffic. The airborne pollen counts were measured with a real-time pollen monitor. Cry j 1 particles were collected with two high volume air samplers, and these concentrations were measured by surface plasmon resonance method with a Biacore J system. The diurnal variation of the airborne pollen counts was similar to the trends of temperature and wind speed during the day-time; whereas its tendency with wind speed trend was not observed during the night-time. Airborne pollen counts were lower with northern wind than with southern wind because the pollen comes from the mountainous areas, and the mountains in the south

are closer, about half the distance to the northern mountains. It is suggested that the peaks of airborne pollen counts during night-time in the sampling site occurred by transport of pollen grains released during day-time in the mountainous forest areas, located c.a. 100 km away from the sampling site. On the roadside site the allergenic Cry j 1 concentrations were higher than at the general urban site, nevertheless pollen grains counts were lower. These results suggested that worsening of pollinosis symptoms during night-time in urban area was caused by transport of pollen grains during day-time in the mountainous forest areas. Moreover, pollen allergenic species become different morphology from pollen grain at roadside site, and the subsequent pollen grains re-suspension by automobile traffic.

Key words: *Cryptomeria japonica* pollen, Diurnal behaviour, Allergenic Cry j 1, Suspended particulate matters, Urban atmosphere

1. INTRODUCTION

Japanese cedar (*Cryptomeria japonica*) trees had been afforested in mountainous areas from mid-1940s to 1950s for post-war lumber demand in Japan (Okuda *et al.*, 2006). Currently, these trees have grown up over 30 years and heavily release pollen grains (Kitamura *et al.*, 2005). The prevalence of Japanese cedar pollinosis had become over 26.5% in Japan, especially it had become higher than 30% in the urban areas in Kanto (Murayama *et al.*, 2010). The forest cannot be seen in urban areas of Kanto Plain, Japan. The airborne *Cryptomeria japonica* pollen grains in the urban areas are transported from the mountainous forest areas, several hundred kilometres away (Kanazashi and Suzuki, 2010). Moreover, since pollinosis symptoms on the

sufferers are reported mainly at night-time (Maeda *et al.*, 2008), it suggests that airborne pollen grains and allergenic species may be higher during night-time.

Cryptomeria japonica pollen has two main allergenic species, so-called Cry j 1 and Cry j 2, which are responsible for pollinosis. These allergenic species are located in the pollen wall and ubiquitin bodies on pollen surface and starch granules in the pollen grains, Cry j 1 and Cry j 2 respectively (Nakamura *et al.*, 2004). Because of the high prevalence of pollinosis in urban areas, air pollutants and automobile traffics are considered to produce synergetic effects on the allergens (Karle *et al.*, 2012; Rogerieux *et al.*, 2007; Behrendt *et al.*, 1997). Especially, symptoms of asthma on patients induced by *Cryptomeria japonica* pollen allergenic species have been observed (Maeda *et al.*, 2008), and worsening during pollen scattering season (Taylor and Jonsson, 2004). It is suggested that this asthma is caused by the breaking of small-sized *Cryptomeria japonica* pollen allergenic species into lower respiratory tract. In contrast particle size of the pollen grains is *c.a.* 30 μm , it had been reported that small-sized Cry j 1 species were suspended as fine particles which particle size were below 1.1 μm in urban atmosphere (Wang *et al.*, 2008). It is also thought that particles containing small-sized allergenic species and pollen grains deposited onto the paved asphalt road materials are re-suspended by automobile traffic.

The behaviour of *Cryptomeria japonica* pollen grains and of its allergenic species in the urban atmosphere

has been studied in a few reports. It has also been found that pollinosis symptoms on the sufferers appear mainly at night-time by the results from epidemiological studies. However, actual cause of these phenomena is not yet clarified. Moreover, since allergenic particles were collected over several days in these reports, diurnal behaviour of these allergenic particles was also unknown. In this study, the diurnal behaviours of *Cryptomeria japonica* pollen grains and their allergenic species in the urban of Saitama city of Kanto Plain, Japan were investigated.

2. MATERIALS AND METHODS

2.1 Location of Sampling Site

Monitoring of airborne pollen counts and the collection of particles containing allergenic Cry j 1 were performed in Saitama University, where is located in the urban area of Kanto Plain, Japan (35.86° N and 139.61° E) (site IV in Fig. 1(a)). The forests of *Cryptomeria japonica* are around mountainous areas of Chichibu and neighbour prefectures, several hundred kilometres away from the sampling site. Most *Cryptomeria japonica* pollen grains in the sampling site were transported from those circumjacent mountainous areas. The others observation sites in Fig. 1(a) observe pollen counts as part of monitoring network system of the Ministry of the Environment (<http://kafun.taiki.go.jp/>; 22 Oct. 2012).

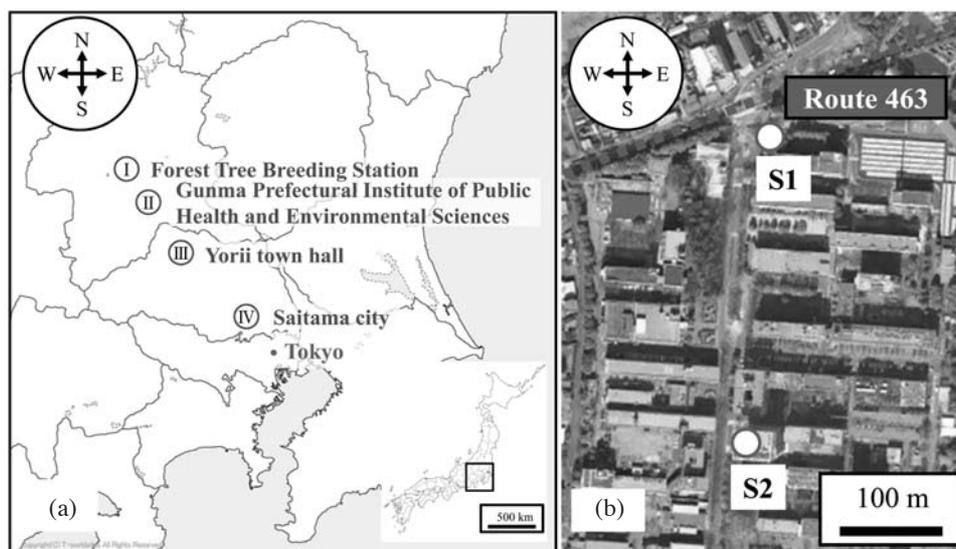


Fig. 1. Sampling location. (a) Site I: Forest Tree Breeding Station, Gunma; Site II: Gunma Prefectural Institute of Public Health and Environmental Sciences, Gunma; Site III: Yorii town hall, Saitama and Site IV: Saitama city hall and Saitama University, Saitama, Japan. (b) The sampling sites in Saitama University (S1: 2 m near roadside, S2: 300 m away from the roadside, the red dashed line indicates National road 463).

The diurnal air samples were taken at two sampling sites in Saitama University (Fig. 1(b)). A roadside site (S1) where is located 2 m away from the vehicles pass in the National Route 463, in Saitama University. Another sampling site is settled in the balcony of the 10th floor of the Building of Research and Project of Saitama University (S2) (here after described as “general urban site”), about 300 m away from the roadside site (S1), as less effect site of automobile traffic. The diurnal air samples were collected during 12-hours sampling period (the day-time; 8:00-20:00 and the night-time; 20:00-8:00) from 26 March to 8 April, 2011.

2.2 Monitoring of Diurnal Airborne Pollen Counts

The diurnal airborne pollen counts were monitored and calculated with a real-time pollen monitor (Model KH3000-01, Yamatronics Co., Ltd., Japan) operated at a flow rate 4.1 L/min. The pollen monitor counts particles which have characteristics of previously memorized optical scatter pattern of 28 to 35 μm of diameter and spherical shape, such as the pollen grains, using optical system by forward and side scatter with a laser diode (Seno *et al.*, 2002). The pollen counts of monitor are well correlated with those of the Burkard pollen sampler which is used as the standard pollen sampler in European countries (Takahashi *et al.*, 2001a). The results of airborne pollen counts were converted into 12-hours average counts of the pollen per 1.0 m³ volume (counts/m³).

2.3 Investigation of Allergenic Cry j 1 Concentrations in Airborne Particles

High-volume air samplers (Model HV-1000R, Shibata Scientific Technology Co., Ltd., Japan) were used to collect atmospheric particles. Total suspended particulate matter (TSP) including *Cryptomeria japonica* pollen grains and their allergenic fine particles were collected on quartz fibre filters (Tokyo Dylec Co., Ltd., Japan).

Allergenic Cry j 1 species were extracted using the following method: A circular area with a diameter of 47 mm was cut from the quartz fibre filters, this area was added 2 mL of Cry j 1 extraction solution (150 mM of NaCl, 0.125 M of NH₄CO₃, 3 mM of EDTA, 0.005 wt% of Tween 20 and 10 mM of HEPES buffer solution) (Wang *et al.*, 2010) and left overnight at 4°C, and then shook at 192 rpm for 1 hour. Afterwards the samples were centrifuged for 30 min at 6,000 rpm, and the supernatant solutions were picked up. Finally, allergenic Cry j 1 concentrations on these supernatants were measured by a Biacore J system (GE Healthcare Co., Ltd., Japan) based on the surface plasmon resonance

(SPR) method.

The SPR method was used to detect Cry j 1 concentrations by using allergen-specific monoclonal antibodies and the Biacore J system, as previously described (Wang *et al.*, 2012; Takahashi *et al.*, 2001b) with the following minor modifications: Allergenic Cry j 1 concentrations in measured samples were determined based on Cry j 1 monoclonal antibody (MAb) (clone 013, Asahi Food & Healthcare Co., Ltd., Japan) immobilized on a CM5 sensor chip (GE Healthcare Co., Ltd., Japan) by using the SPR method with the Biacore J system. Approximately 700 μg of Cry j 1 MAb was immobilized on the chip. The regeneration and dissociation of antigen-antibody complex reaction on the CM5 sensor chip were performed by 70 μL of pH 2.0 glycine-HCl (GE Healthcare Co., Ltd., Japan). Finally, the results were converted into Cry j 1 concentration (ng/m³) in the urban atmosphere (Wang *et al.*, 2012).

2.4 Meteorological Data during the Sampling Periods

Meteorological data were supplied from the Saitama Institute of Public Health and the Okubo Purification Plant which only located around 700 meters from our air sampling sites. Data on precipitation, wind speed, wind direction and temperature were acquired.

3. RESULTS AND DISCUSSION

3.1 Diurnal and Nocturnal Behaviour of the Airborne Pollen Grains

Fig. 2 indicates the diurnal and nocturnal variations of meteorological data (temperature, precipitation (Fig. 2(a)), wind speeds and wind direction (Fig. 2(b)) and the airborne pollen counts and allergenic Cry j 1 concentrations at the roadside site and the general urban site from 26 March to April 8 in 2011 (Fig. 2(c)). In both sites, the highest airborne pollen counts were 764 counts/m³, and average pollen counts were 180 counts/m³. The highest airborne Cry j 1 concentrations were 50 ng/m³, and average Cry j 1 concentrations were 18 ng/m³ during the observation period. It has been reported that the symptoms of pollinosis appear when reaching a few pg/m³ of allergenic Cry j 1 concentrations in the atmosphere (Takahashi *et al.*, 2001b). Thus, the airborne Cry j 1 particles suspended above the threshold level that triggers pollinosis symptoms during the observation.

The diurnal variation of the airborne pollen counts showed good correlation with temperature, and this is in good agreement with the report by other researchers (Bartkova-Scevkova, 2003). At the latter air sampling period, the airborne pollen grain counts notably incr-

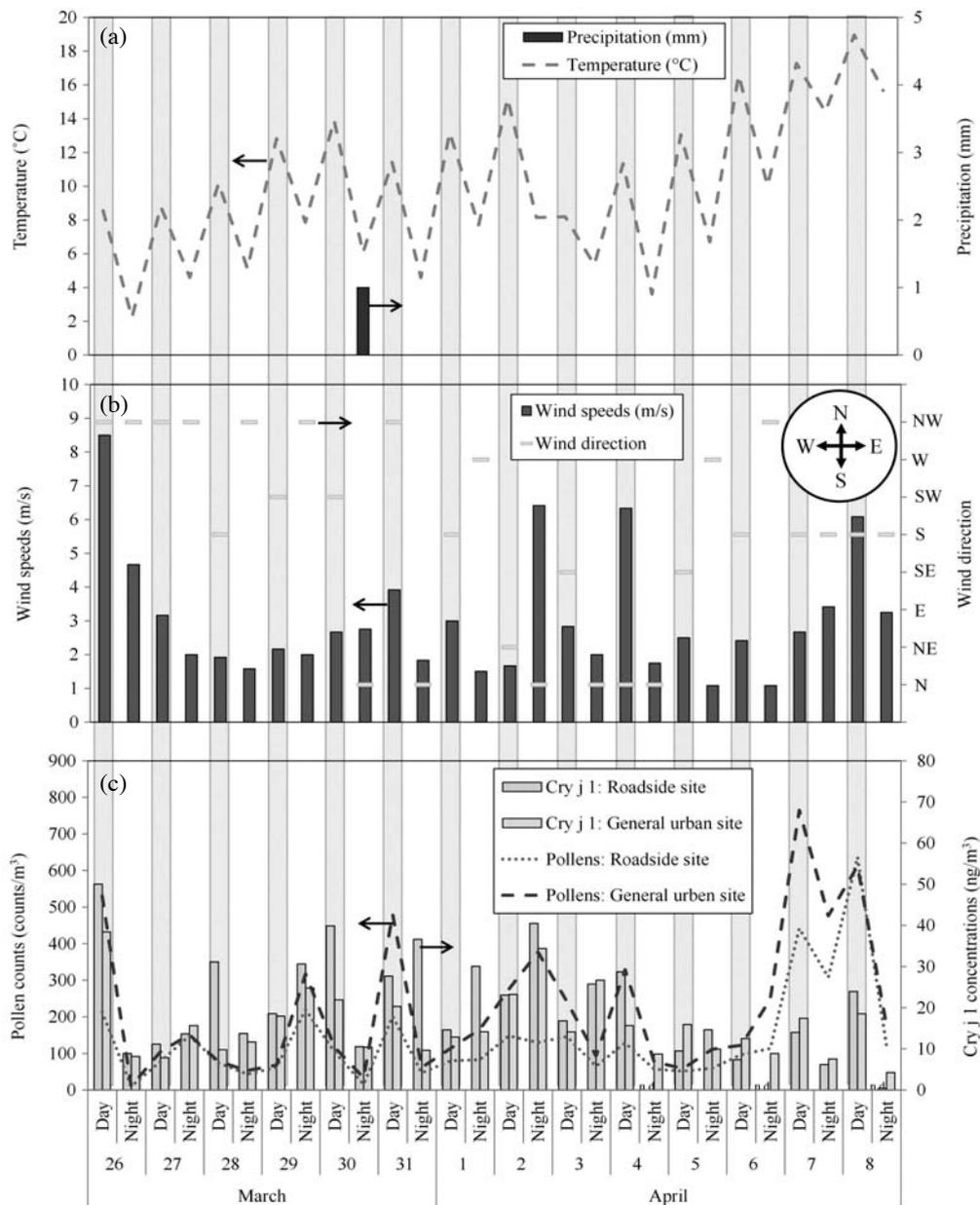


Fig. 2. Diurnal variation of temperature, precipitation (a), wind speed, wind direction (b), pollen counts and allergenic Cry j 1 concentrations (c) at the roadside site and the general urban site. Meteorological data were provided by the Saitama Institute of Public Health and the Okubo Purification Plant.

eased with increasing temperature. It has reported that pollinosis suffers are induced symptoms by temperature above 15°C (Dejima *et al.*, 1992). Therefore, it is thought that the airborne pollen grains are also increased by high temperature.

The trend of the airborne pollen grains was similar to the trend of wind speed during day-time; whereas this similarity was not observed during night-time. It is thought that the airborne pollen counts were related

to wind speed, because under stronger winds more pollen grains are released from the source trees. During night-time, it was also thought that only a few pollen grains are released from the original trees of emission sources, since male flowers which contain pollen grains close under dark conditions.

During night-time in 30 March, the airborne pollen grains and Cry j 1 concentrations decreased after 1 mm of rain precipitation. Airborne particles disappeared

Table 1. Hourly data of wind direction during night-time of 2 Apr. and day-time of 7 Apr. at Saitama, Saitama.

At night-time of 2 Apr.		At day-time of 7 Apr.	
Observation time	Wind direction	Observation time	Wind direction
20	NNW	8	WNW
21	N	9	N
22	NNW	10	NNE
23	NNW	11	SW
24	NNW	12	SSW
1	NNW	13	S
2	NNW	14	SSE
3	NNW	15	S
4	NNW	16	S
5	NNW	17	S
6	NW	18	S
7	NNW	19	SSW
8	NNW	20	S

from the atmosphere by trapping into rainwater, due to the so-called wash-out phenomenon. The airborne pollen grains and particles containing allergenic Cry j 1 also disappeared by trapping into rainwater.

Airborne pollen counts were lower with northern wind than with southern wind. The mountains in the south are closer, about half the distance to the northern mountains. Temperature also increased with southern wind. It is thought that pollen counts depend more on temperature rather than on wind speed. On the other hand, pollen counts are strongly affected by wind speed when northern wind blows, because of the longer transportation distance.

The airborne pollen counts peaks were observed when winds blew mostly from northwest and southwest. During the night-time of 2 April, wind direction in sampling sites was kept northwest (Table 1). Observation sites of Maebashi and Yorii, near the site II and site III in Fig. 1, where windward of sampling sites were also kept northwest wind (Data not shown). Thus, it was estimated that the airborne pollen grains were transported from mountainous areas of Gunma prefecture located c.a. 100 km away from the sampling site. At day-time of 7 April, winds blew from northwest till 10 a.m., subsequently southern wind blew till night-time. Thus, it was estimated that the airborne pollen grains were mostly transported from mountainous areas to the west of Tokyo metropolitan area and located c.a. 50 km away from the sampling site.

3.2 Airborne Pollen Counts Peaks during the Night-time in Urban Areas of Kanto Plain

Fig. 3 shows the tracking data of the airborne pollen peaks at night-time of 2 April using monitoring network

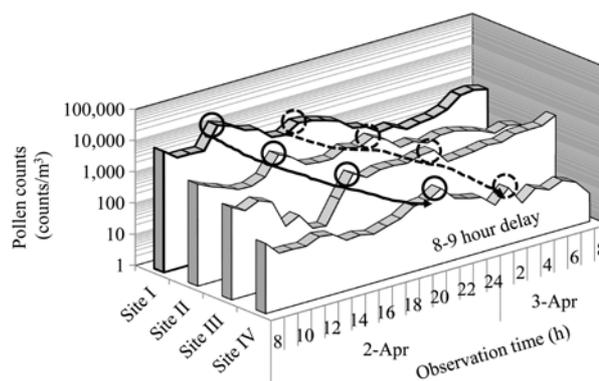


Fig. 3. Time variation of airborne pollen counts at 2 April to 3 April in 2011. About 8 to 9 hour peak delays of airborne pollen counts were observed at site IV of the urban compared with the site I of original emission sources. Here, site I: Forest Tree Breeding Station, Gunma; site II: Gunma Prefectural Institute of Public Health and Environmental Sciences, Gunma; site III: Yorii town hall and site IV: Saitama city hall, Japan; Data were downloaded from the Ministry the of Environment (<http://kafun.taiki.go.jp/>; 22 Oct. 2012).

system of the Ministry of the Environment (<http://kafun.taiki.go.jp/>; 22 Oct. 2012). Observation sites I, II, III and IV are related to each observation site of Fig. 1(a).

The Forest Tree Breeding Station as the observation site I is located nearest mountainous area of original emission sources of pollen grains. The pollen peak appeared at site I at the noon on 2 April were moving to the southern observation site with decreasing pollen counts by diffusion, since wind blew from north at night-time of 2 April. The pollen peak went through to site II at 14:00, site III at 17:00 and site IV, where located near our sampling sites, at 21:00 on 2 April.

Another pollen peak appeared in site I at 18:00 also went through site IV at 2:00 on 3 April. The airborne pollen grains arriving to our urban sampling site need 8 to 9 hours, which is about 8 to 9 hour delay shown in Fig. 3 after being released from the Japanese cedar (*Cryptomeria japonica*) forest as the original emission sources. Thus, the pollen grains from original emission trees in the forest in the mountainous areas, released during the day-time, were observed at our urban sampling site during the night-time. The transportation times were consistent with the average wind speed, 2 m/s at the day-time of 2 April. As the result, it was thought that appearance of peaks of the airborne pollen counts during the night-time in urban area of Kanto Plain was produced by transportation of pollen grains released from trees in mountainous areas during the day-time.

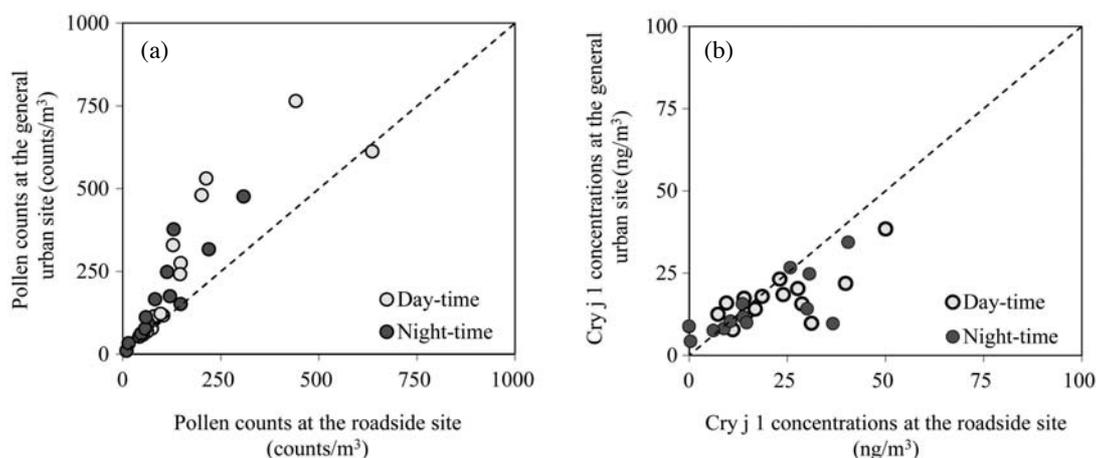


Fig. 4. Correlation between the roadside site and the general urban site in (a) pollen counts and (b) allergenic Cry j 1 concentrations.

3.3 Comparison between the Airborne Pollen Counts and Allergenic Cry j 1 Concentrations

The correlations about pollen counts and allergenic Cry j 1 concentrations between the roadside site and the general urban site are shown in Fig. 4(a) and Fig. 4(b) respectively. Cry j 1 concentrations between the roadside site and the general urban site indicated similar behaviour in common with pollen counts. Cry j 1 concentrations at the roadside site were higher than at the general urban site; whereas pollen counts were lower. Differences of these behaviours between day-time and night-time were not observed.

It is thought that the airborne pollen grains in the sky of urban areas keep their morphology, since these airborne pollen grains are just transported from releasing forest sources in mountainous areas, and not affected by air pollutants and automobile traffic. In the urban atmosphere, the airborne pollen grains underwent morphological change such as burst of pollen grains and adhesion of other suspended particulate matter (Okuyama *et al.*, 2007), and small-sizing of particles containing allergenic Cry j 1 (Wang *et al.*, 2008). We presume that pollen grains were more suspended on the roadside site, since there were more pollen grains transported and re-suspended. However, the results were different from our thinking. For the results, it is suggested that morphological change of pollen grains including small-sizing is enhanced at roadside by heavy traffic.

It may be thought that during the deposition process of transported pollen grains on the asphalt, their counts would decrease by deposition on trees. However, the effect of dry deposition was low, since Cry j 1 concentrations on the roadside site were higher than ones at the general urban site. The results suggested that the

airborne pollen grains in the urban atmosphere include morphological changed pollen grains were uncounted by the pollen monitor device using optical systems such as the monitor of KH-3000-01. This device is widely used at the monitoring network system of the Ministry of the Environment, Japan (<http://kafun.taiki.go.jp/>; 22 Oct. 2012). It is possible that the real airborne pollen counts in urban atmosphere are underestimated and this may lead to serious damages on pollinosis sufferers.

4. CONCLUSIONS

In this study, it was investigated the diurnal and nocturnal behaviour of *Cryptomeria japonica* pollen grains and its allergenic species in urban area in Kanto Plain, Japan. Aims of this study were to gain knowledge about the causation of worsening of pollinosis symptoms during night-time and formation of fine particles containing allergenic Cry j 1 in urban area. Peaks of pollen grains were observed during night-time in the sampling sites. Since the pollen counts were higher than during the day-time in the peaks, it is suggested that appearances of the pollen peaks during night-time in urban area are caused by transport and deposition of pollen grains. Allergenic Cry j 1 concentrations at roadside site were higher than ones at the general urban site, nevertheless pollen grains counts were lower at roadside site. These results suggested that worsening of pollinosis symptoms during night-time in urban area was caused by the transport of pollen grains during day-time from the original forest of mountainous areas. Moreover, pollen allergenic species become different morphology from pollen grain at roadside site, and the subsequent pollen grains re-suspension by heavy

automobile transportation.

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