

Size distribution of allergenic Cry j 2 released from airborne *Cryptomeria japonica* pollen grains during the pollen scattering seasons

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Abstract Japanese cedar (*Cryptomeria japonica*) pollinosis is one of seasonal allergic rhinitis that mainly occurs in Japan. The pollinosis is caused by two main kinds of allergenic proteins called Cry j 1 and Cry j 2 which exist in *Cryptomeria japonica* pollen. In our previous study, we reported that the size-segregated of airborne fine allergenic Cry j 1 and morphological change of Cry j 1 due to the contact with rainfall. However, the study on airborne allergenic Cry j 2 in different particle sizes has not been identified until now. Therefore, the main aim of this study is to investigate the size distribution and scattering behavior of

allergenic Cry j 2. The Cry j 2 particles were collected and determined in different particle sizes at the urban sampling points during the most severe pollination season of 2012 in Saitama, Japan. After the size-segregated Cry j 2 allergenic particles were collected using an Andersen high-volume (AHV) atmospheric sample, the airborne Cry j 2 concentrations were determined with a surface plasmon resonance (SPR) method. At the same time, the airborne *Cryptomeria japonica* pollens were also counted by the Durham pollen sampler. The higher concentrations of the allergenic Cry j 2 were detected even in particle sizes equal to or less than 1.1 μm ($\text{PM}_{1.1}$) than other particle sizes. The airborne particles ranges from 0.06 to 11 μm were also collected by a low-pressure impactor (LPI) atmospheric sampler. After that, the concentrations of Cry j 2 allergenic particles in fine particle sizes were measured by the SPR method either. With the help of this study, we have confirmed the existence of fine daughter allergenic particles, which clearly differ from the parent pollen grains in size, especially after the rainy days. It is possible that the daughter allergenic species will be released from the fractions of cell wall and burst pollen grains. We concluded that rainwater was one of the important factors that affects the release of pollen allergenic proteins of both Cry j 1 and Cry j 2 from the parent pollen grains.

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1 Introduction

In Japan, *Cryptomeria japonica* (Japanese cedar) pollen is the most frequent cause of pollinosis because of its abundant pollination and widespread distribution (Yasueda et al. 2000). An epidemiological study conducted in 2008 indicated that the prevalence rates of Japanese cedar (*Cryptomeria japonica*) pollinosis have increased from 16.2 to 26.5 % during the past decade, and approximately 33 million patients were afflicted with this disease (Okamoto et al. 2009). In recent years, Japanese cedar pollinosis is called as “a folk disease” in Japan, due to the increase in the number of patients and increase in the related medical costs (Tanaka et al. 2012). Two major allergenic proteins of this pollen, *Cryptomeria japonica* 1 (Cry j 1) and *Cryptomeria japonica* 2 (Cry j 2), have been isolated (Yasueda et al. 1983). The allergenic Cry j 1 protein is a basic protein with molecular weight of 41 and 44 kDa, which exists in Ubisch bodies on the pollen wall. The allergenic Cry j 2 protein is also a basic protein with molecular weight of 37 kDa, which exists in the starch granule in pollen grain, and its content is one-tenth of Cry j 1 (Nakamura et al. 2004). It had been considered that the pollen grains as coarse particles about 30 μm only deposit in upper part of the respiratory system such as human nasal cavity and mouth, and originally, there was no inhalation to lower respiratory tract. However, the existence of airborne fine particles, such as $\text{PM}_{1.1}$ (suspended particulate matter below 1.1 μm) containing the *Cryptomeria japonica* pollen allergenic Cry j 1 protein, had been confirmed in our previous study (Wang et al. 2008). Other studies (Sugimura et al. 1996; Hashimoto et al. 1995) were conducted to evaluate the prevalence of sensitivity between Cry j 1 and Cry j 2 proteins. They measured specific IgE antibodies to these allergens in the sera of 145 patients. Furthermore, comparison of the sensitivity to Cry j 1 and Cry j 2 proteins was examined by the histamine release assay. More than 90 % of 145 patients had specific IgE antibodies to both allergens; the remainder had specific IgE to either one or the other (Hashimoto et al. 1995). In our previous study, the morphological observation of Ubisch bodies exfoliated from pollen and ruptured pollens was performed with a scanning electron microscope (SEM). Moreover, the existing evidences of visualized Cry j 1 allergenic particles in $\text{PM}_{1.1}$ were also sensitively found by an immunofluorescence

technique with a fluorescence microscope (Wang et al. 2008). The concentrations of Cry j 1 allergenic particles were observed during sunny days after rainfall. Therefore, rainwater (its pH is different before and after rain) was regarded to link with the release of fine allergenic particles (Wang et al. 2012b). Although there were some studies on Cry j 1 allergenic particles reported by our previous studies and other researches mentioned above, there were still no researches focused on the airborne behavior of Cry j 2 allergenic particles after rainfall. Therefore, the aim of the study is focused on the investigation of the size-segregated allergenic Cry j 2 and its scattering behavior during the *Cryptomeria japonica* pollen scattering seasons. We focused on the airborne tracer of *Cryptomeria japonica* pollen grains and its size-segregated allergenic particles located in the *Cryptomeria japonica* pollen within the pollen scattering seasons in 2012. It was suggested that meteorological factors effected on the scattering behavior of pollen counts and the release of allergenic Cry j 1 and Cry j 2 as the fine particles, and also suggested that the pollens and released allergenic particles are resuspended by the heavy traffic. Therefore, in this study, we investigated the relationships between airborne pollen counts and meteorological factors such as rainfall, wind speed and temperature. At the same time, we also investigated the relationships between the concentrations of allergenic particles and rainfall events. The atmospheric samples of size-segregated allergenic particles were also collected at the roadside point and the general urban atmospheric environment point. From the determination of the size-segregated concentrations Cry j 2 allergenic proteins, we can figure out the size-segregated allergenic Cry j 2 particles and the scattering behavior during the *Cryptomeria japonica* pollen scattering seasons.

2 Experimental design

2.1 Sampling methods of airborne pollen grains and suspended particulate matter

In order to grasp the airborne behavior of *Cryptomeria japonica* pollen grains and its size-segregated allergenic particles, we collected atmospheric samples of suspended particulate matter (SPM) within the pollen

scattering seasons. The atmospheric samples of size-segregated allergenic particles of *Cryptomeria japonica* pollen were collected at two sampling points in Saitama University, which is in the urban residential zone of Saitama City in the norther area of metropolitan Tokyo, Japan (35.86422N, 139.60787E). A roadside point refers to P1 located 2 m away from the vehicles pass in the national Route 463, in Saitama University. Another sampling point for collecting atmospheric particulate samples of the general urban atmospheric environment settled in the balcony of the tenth floor of the Building of Research and Project of Saitama University refers to P2, about 300 m away from the roadside P1 point, because it is less affected by automobile traffic. Thus, P2 point can be considered as an urban general atmospheric sampling point (Fig. 1).

In this study, we have used the following pollen sampler and air samplers for collecting the airborne behavior of particulate allergenic Cry j 1 and Cry j 2 in suspended particulate matter (SPM) at two sampling points (P1 and P2) of urban residential zones of Saitama City during the pollination period from February to March in 2012.

- (a) Firstly, in order to count pollen grains in atmosphere, airborne pollen grains were collected on a glass slide coated with white petrolatum by the Durham pollen sampler (model DK-1SA, Nishiseiki Co., Ltd., Japan), which is a standard method in Japan (Wang et al. 2012b).
- (b) The samples of suspended particulate matter were collected on the quartz fiber filters by an Andersen high-volume sampler (AHV, model AH-600, Shibata scientific technology Ltd). This air sampler can collect the atmospheric suspended particulate matter equivalent to the human respiratory system as five size-segregated particles (≤ 1.1 , 1.1–2.0, 2.0–3.3, 3.3–7.0, ≥ 7.0 μm) (Fig. 2a). Airflow of the sampler was 566 L/min. The filters were changed each 47 h.
- (c) For determining more detailed size distribution of particulate allergenic Cry j 2, a low-pressure impactor (LPI, model LP-20, Dylec Co., Ltd., Japan) was also used, by which the air samples were collected on the quartz fiber filters as thirteen size-segregated particles from 0.06 to

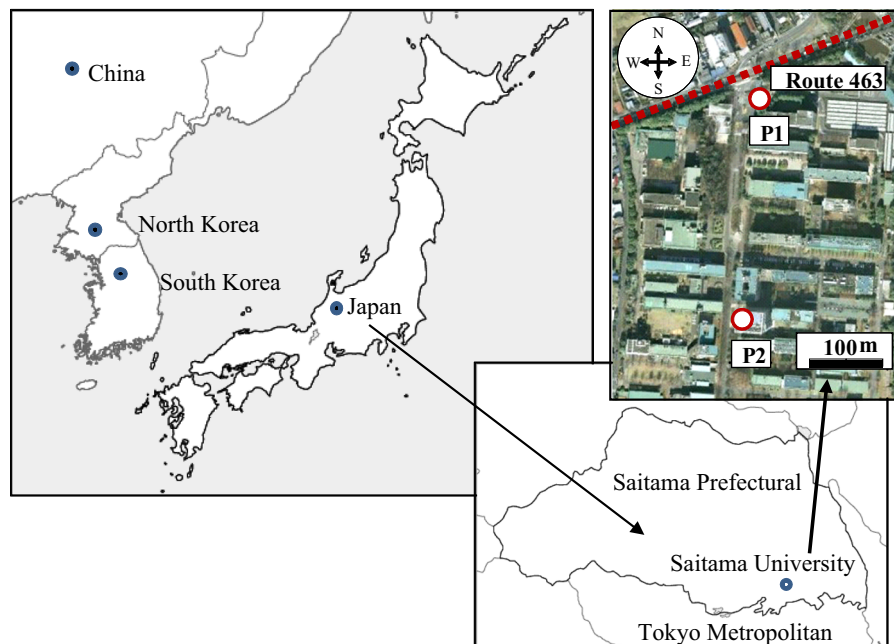


Fig. 1 Two sampling points at roadside of Route 463 (P1) and tenth floor of Research and Project Building (P2) of urban residential zones of Saitama City, Japan

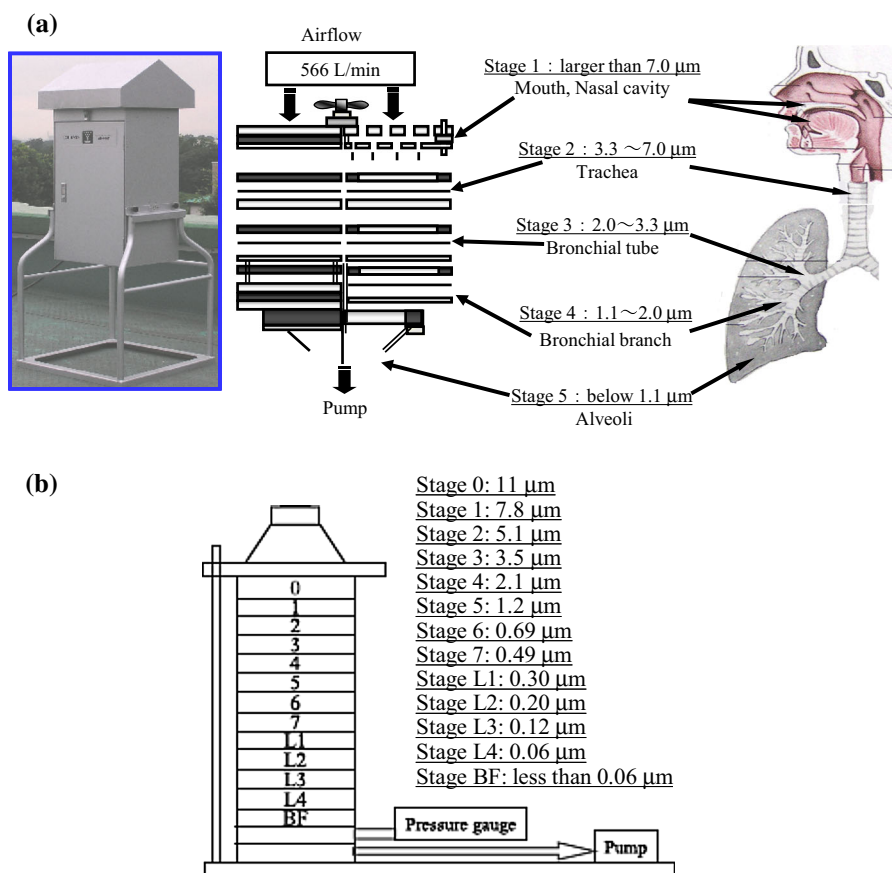


Fig. 2 **a** Principle diagram of an Andersen high-volume sampler (*left*) and sampling position of particulate matters which correspond to human airways (*right*); **b** principle diagram of a low-pressure impactor (LPI) atmospheric sampler

$11\ \mu\text{m}$ (≤ 0.06 , $0.06\text{--}0.12$, $0.12\text{--}0.20$, $0.20\text{--}0.30$, $0.30\text{--}0.49$, $0.49\text{--}0.69$, $0.69\text{--}1.2$, $1.2\text{--}2.1$, $2.1\text{--}3.5$, $3.5\text{--}5.1$, $5.1\text{--}7.8$, $7.8\text{--}11$ and $\geq 11\ \mu\text{m}$) (Fig. 2b). The particles with sufficient inertia cross the airflow streamlines and impact on the collection surface. The smaller particles, which have insufficient inertia, follow the streamlines into the next impactor stage. Airflow is $23.6\ \text{L/min}$. Because a LPI air sampler uses a low-pressure impactor with the volume of an airflow at $23.6\ \text{L/min}$, the detected limitation of Cry j 2 will be poor during a short time sampling. Basing on our previous study (Wang et al. 2012b), the Cry j 2 concentrations in the filter sample might not be detected if the sampling time is shorter than 167 h. Therefore, we only succeed to collect one filter sample by the LPI air sampler for 1 week (167 h). On the other hand, since an AHV air sampler is a

high-volume air sampler with the volume of an airflow at $566\ \text{L/min}$, the detected limitation of Cry j 2 is better even during a shorter time for each 2 days (48 h) sampling according to our field studies by an AHV air sampler. As we also have to change the filters for 1 h for each sampling operation, we set 47 h for each 2-day sampling operation.

2.2 Measurement methods of pollen grains and allergenic concentrations

The pollen grains were collected on a glass slide coated with white petrolatum by the Durham samplers. The pollen counts (pollens/ $\text{cm}^2/24\ \text{h}$) in the slide glass covered with cover glass, $1.8\ \text{cm} \times 1.8\ \text{cm}$, were carried out using the calculation of the pollen dyed by Phöbus Blackly distain solution which made from

0.6 mg of methyl violet 2B, 30 mL of phenol, 180 mL of glycerin, and 90 mL of ultrapure water and by an optical microscope (model GLB-B1500MBITaN, Shimadzu Co., Ltd., Japan) under 400 magnification (Wang et al. 2012b). Pollen grains on each slide were counted twice or more. Finally, the average pollen counts of the pollen grains and the standard deviations were calculated. The glass slide was changed each 1 day (24 h) of sampling operation; we can simply change the glass slide each time. The average pollen counts of 2 days (pollen counts/48 h) were also calculated for the further comparison with the concentrations of particulate allergenic Cry j 2 (ng/m³) collected by an AHV for each 2 days (47 h) of sampling operation.

The allergenic particle was extracted using following method. The filters were cut out (AHV: 8 mmφ, 30 pieces; LPI: one quarter of filter) and put into centrifuge tubes (polypropylene copolymer), followed by the addition of 2 mL of pollen extracting solution (0.125 M NH₄HCO₃, 150 mM NaCl, 3 mM EDTA, 0.005 wt % Tween 20, and 10 mM HEPES buffer solution) (Takahashi et al. 2001, 2002). After incubation of 24 h at 4 °C in the incubator (UNIMAX 2010, 25 Heidolph Co., Ltd., Japan), the centrifuge tubes were shaken at 192 rpm at room temperature for 1 h by vibrator, followed by centrifugation at 6000 rpm for 30 min at room temperature using the centrifuge (CN-1050, AS ONE Corp., Japan). After that time, the microtubes were centrifuged at 14,800 rpm for 10 min and the supernatants were pipetted. The supernatants were solvent exchanged for buffer solution (Hepes buffered saline with EDTA and surfactant P20 (HBS-EP, GE Healthcare Co., Ltd., Japan): 10 mM Hepes, 150 mM NaCl, 3 mM EDTA, 0.005 % surfactant P20) by the centrifugal filter units (Amicon Ultra-0.5, Millipore Co., Ltd., Japan). Solvent exchange was carried out according to the instructions of the centrifugal filter units. Five hundred microliters of supernatants were added in the filter device of Amicon Ultra-0.5. Subsequently, these were centrifugally separated at 14,800 rpm for 15 min. To recover the concentrated supernatants, the Amicon Ultra filter device was put upside down in a clean microtube and then centrifuged at 4000 rpm for 2 min in order to transfer the concentrated supernatants from the device to the tube. Concentrated supernatants were diluted by HBS-EP to a volume of 500 µl of the solution (Wang et al. 2012a, 2013a).

We determined particulate allergenic Cry j 1 or Cry j 2 concentrations by using a Biacore J system (GE Healthcare Japan Co., Ltd., Japan) based on a surface plasmon resonance (SPR) method. The SPR method was used to detect Cry j 1 or Cry j 2 concentrations by using allergen-specific monoclonal antibodies and the Biacore J system with the following modifications: Allergenic Cry j 1 or Cry j 2 concentrations in measured samples were investigated based on Cry j 1 monoclonal antibody (MAb) (clone: 013, Asahi Food & Healthcare Co., Ltd., Japan) or Cry j 2 MAb (clone: T27, 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. 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). Each filter sample was detected four times. And then, the average concentrations and standard deviations were calculated. Finally, the results were converted into the concentrations of the allergenic Cry j 1 or Cry j 2 (ng/m³) in atmosphere which can be calculated using the following formula (Wang et al. 2014)

$$C(\text{ng/m}^3) = (C_s - C_b) \times F/F_a \times V_e/V$$

Here C_s is concentration measurement (ng/mL), C_b blank solution concentration (ng/mL), F total area of the filter (mm²), F_a analyzed area of filter (mm²), V_e extraction solution (mL), and V total airflow (m³).

2.3 The information of meteorological data

In order to investigate the relationship between allergenic and meteorological conditions, we also refer to the meteorological data. Meteorological data (precipitation, wind speed and temperature) were determined by the Saitama Institute of Public Health and the Okubo purification plant located about only 1.0 km away from the sampling points and then downloaded from the official website (<http://www.data.jma.go.jp/obd/stats/etrn/index.php>).

2.4 Statistical analysis

The data were processed using Microsoft Office Excel 2003. Statistical analysis was performed by Spearman's test, using the SPSS 15.0 (SPSS Inc., Chicago,

IL) statistical package to establish the potential correlation between the pollens of two sampling points, and between pollen and the concentration of allergens.

3 Results

3.1 Daily variation in pollen counts during the pollen scattering spring seasons in 2012

The counts of scattering pollen grains at two sampling points (P1 and P2) are shown in Fig. 3a. As shown in Fig. 3a, we can find the counts of scattering pollen grains were higher at floor top of Research and Project Building of Saitama University, Japan, as a general atmospheric environment. The location is one of the typical urban areas in Japan. On the other hand, the sampling point was enough high from the ground level to avoid disturbance as a general atmospheric environment. The pollen grains of two sampling points were highly correlated ($r = 0.91$, $p < 0.001$, $n = 9$), although the counts of scattering pollen grains were higher at floor top of building.

Figure 3b shows the daily variation in wind speed, temperature and precipitation during the pollen scattering spring seasons in 2012. Peaks of airborne pollen grains appeared on March 12, 15, and 18 as shown in Fig. 3a. The higher average temperature from March 18 was observed comparing with those of previous days of March 16 and 17. On the other hand, airborne pollen grains were not observed on March 17, because it rained heavily on that day. Airborne pollen grain counts had to be decreased with the heavy rainfall events.

3.2 Size distribution of allergenic Cry j 2 in urban atmosphere

These pollen allergen particles appear to play a key role in the increasing incidence of asthma in the lower respiratory tract. In our opinions from this study, it is suggested that it is important to determine the size distribution of allergenic Cry j 2 particles released from airborne *Cryptomeria japonica* pollen grains same as allergenic Cry j 1 particles. The size distribution of the allergenic Cry j 2 particles at two sampling points are shown in Fig. 4. From Fig. 4, the high concentrations of allergenic Cry j 2 were detected especially in fine particle size ranges of 0.06 to 0.2 μm

Fig. 3 Comparing pollens between the roadside of Route 463 (P1) and tenth floor of Research and Project Building (P2) during the pollen scattering spring seasons in 2012. **a** The pollen counts of the two sampling points, **b** the daily variation in wind speed, wind direction, temperature and precipitation during the pollen scattering spring seasons in 2012

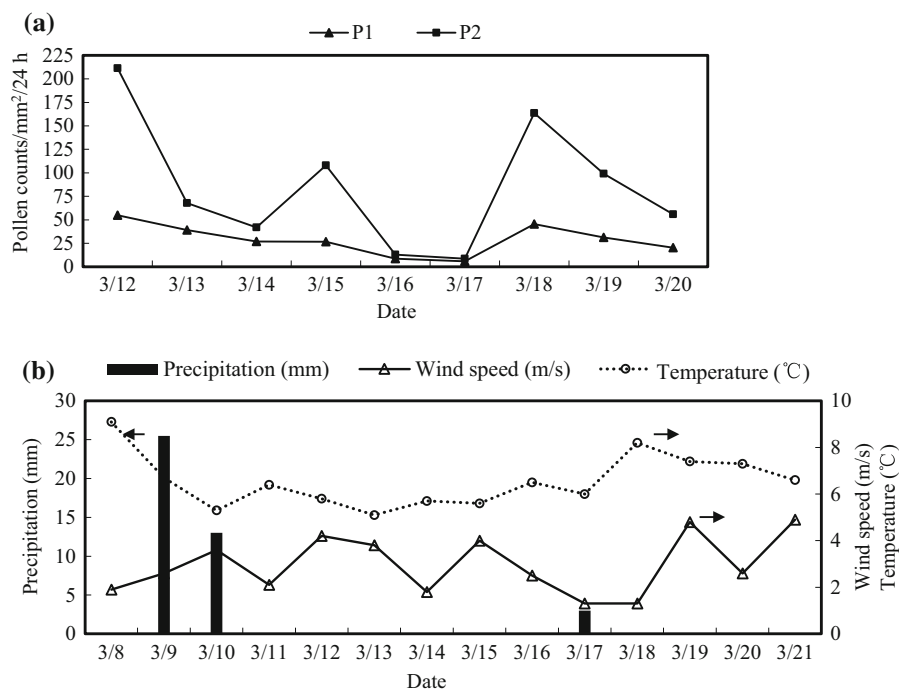
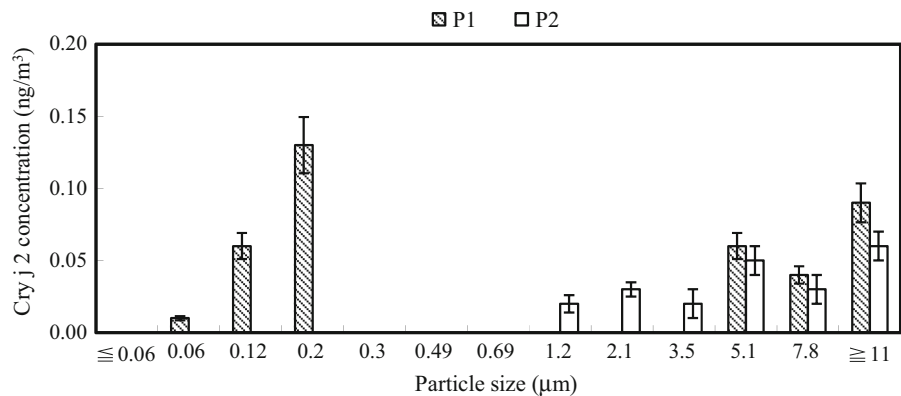


Fig. 4 Size distribution of allergen particles Cry j 2 at roadside of Route 463 (P1) and tenth floor of Research and Project Building (P2) by a LPI air sampler during March 11–17, 2012



of the roadside (P1). On the other hand, although the allergenic Cry j 2 particles that ranged from 1.2 to 11 μm were detected, the concentrations of the allergenic Cry j 2 particles were not detected in fine particle size ranges of 0.06 to 0.2 μm of the floor top of building as the general urban atmospheric environment. Although the counts of scattering pollen grains were higher at the floor top of Research and Project Building of Saitama University, Japan, as an urban general atmospheric sampling point (P2), the lower levels of allergenic Cry j 2 concentrations are shown in Figs. 3 and 4. On the other hand, the allergenic Cry j 2 concentration was determined in higher levels at the roadside (P1), but the counts of scattering pollen grains were lower. Based on these results, it can be considerable as the peculiar phenomenon caused from heavy vehicle travel at the roadside (P1).

3.3 Correlation between pollen counts and the size distribution of allergenic particles of Cry j 2

In our previous study (Wang et al. 2014), we have reported that the correlation between the size-segregated airborne allergenic Cry j 1 particles and pollen grain counts. However, there were no studies focused on the pollen counts and the airborne behavior of respirable particulate matter containing particulate allergenic Cry j 2 of *Cryptomeria japonica* pollen. Therefore, in this study, we focus on the investigation of pollen counts and the size distribution of pollen allergenic Cry j 2. As given in Fig. 5, it is indicated that the correlation exists between the pollen counts and concentrations of allergen particles Cry j 2 in suspended particulate matter by an AHV air sampler at roadside (P1) during the pollen scattering spring

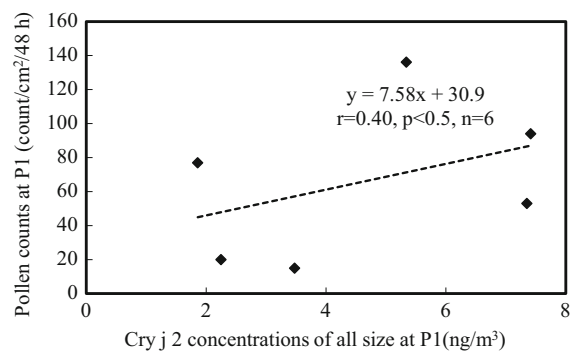


Fig. 5 Comparing pollen counts and concentrations of allergen particles Cry j 2 in suspended particulate matter by an AHV air sampler at roadside of Route 463 (P1) during the pollen scattering spring seasons in 2012

seasons in 2012. From the results, it could be found that there was poor correlation between the scattering pollen counts and Cry j 2 allergenic concentrations in all particle sizes ($r = 0.40$, $p < 0.5$, $n = 6$), although the pollen grains of two sampling points were highly correlated ($r = 0.91$, $p < 0.001$, $n = 9$).

3.4 Comparison of the behavior of particulate allergenic Cry j 1 and Cry j 2 with *Cryptomeria japonica* pollen grains in 2012

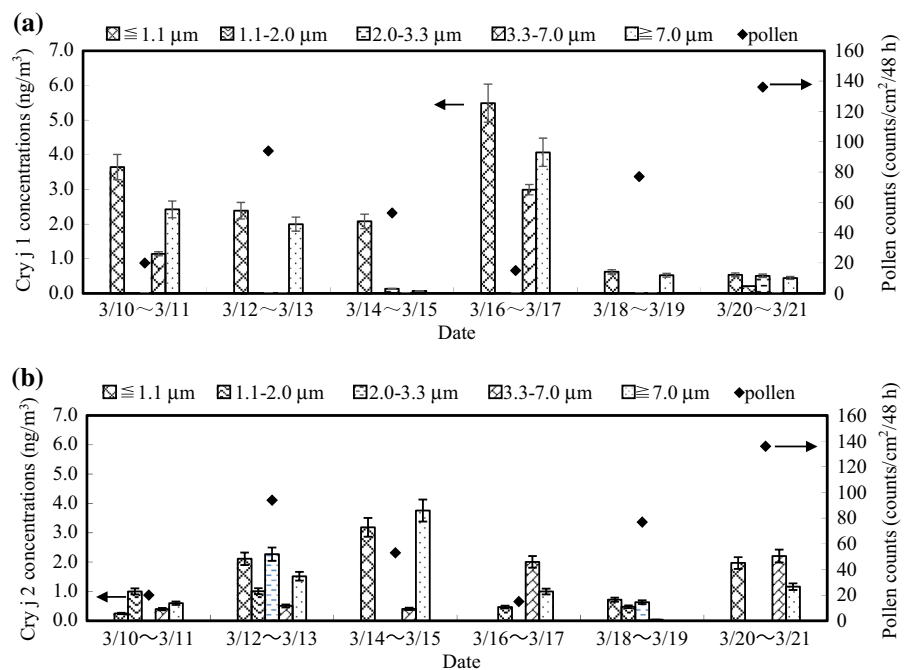
In this study, we found that the high concentrations of particulate allergenic Cry j 2 were detected especially in fine particle sizes ranging from 0.06 to 0.2 μm at the roadside, although the counts of scattering pollen grains were higher at top floor of building. Basing on these results, it is considerable that the peculiar phenomenon of higher concentrations of Cry j 2 allergenic fine or ultrafine particles may be resuspended due to the heavy

traffic density at the roadside. With the help of the results, it is suggested that we have to focus on the airborne allergenic Cry j 1 and Cry j 2 as the tracer of *Cryptomeria japonica* pollen allergen particles mainly at the roadside. The allergenic Cry j 1 and Cry j 2 in different airborne particles as PM_{1.1} to PM_{>7.0} (suspended particulate matter bigger than 7.0 µm) were collected at the roadside in urban residential zones of Saitama City. Figure 6a indicates the size-segregated allergenic Cry j 1 concentrations in 2012. From the investigation of the airborne behavior of the size-segregated allergenic Cry j 1, the higher concentrations of the allergenic Cry j 1 were detected in particle size as PM_{1.1} than other particle sizes. Figure 6b indicates the size-segregated allergenic Cry j 2 concentrations in 2012. We can make the comparison between the airborne behavior of the two major allergens of Cry j 1 and Cry j 2. As given in Fig. 6, we can find that the concentrations of the allergenic Cry j 2 were as high as that of Cry j 1. The higher concentrations of the allergenic Cry j 2 also were detected in particle size PM_{1.1} than in other particle sizes. Takahashi et al. (2001) reported that symptom of pollinosis appeared when reaching about 1–3 pg/m³ of *Cryptomeria japonica* pollen allergenic concentrations in the atmosphere. From the investigation of the airborne behavior of the size-segregated allergenic Cry j 1 and Cry j 2, the higher

concentrations of the allergenic Cry j 1 and Cry j 2 were measured in the fine particles as PM_{1.1} than in other particle sizes. The concentrations of allergenic Cry j 2 particles could not be ignored. From the determinations of Cry j 1 in all the particle sizes, it was found that allergenic Cry j 1 particles were mainly distributed in PM_{1.1} and PM_{>7} rather than other particle sizes. However, although the allergenic Cry j 2 particles were detected in all particle sizes, the concentrations and the size distribution of allergenic Cry j 2 particles seemed quite different in all samples.

Since the meteorological conditions may be the important factors for allergen particle releasing (Wang et al. 2012a, b), we did the detail investigation on meteorological conditions during the sampling periods. Figure 3b shows the daily variation in wind speed, temperature and precipitation during the pollen scattering spring seasons in 2012. From concentrations of the allergenic (Fig. 6) and meteorological data (Fig. 3b), we can find that Cry j 1 concentrations in PM_{1.1} were increased on sunny days after heavy rain (38.5 mm rainfall during March 9–10). Other rainy days included March 17 (3.0 mm rainfall). It could be seen that allergenic particles among fine particles were from resuspension on sunny days after rainfall. Cry j 2 concentrations were not increased at once on sunny day after rainfall, but increased after sunny days.

Fig. 6 Concentrations of **a** allergenic Cry j 1 and **b** allergenic Cry j 2 particles with the pollen counts at roadside of Rout 463 (P1) on March 10–21, 2012



4 Discussion

Differences in pollen grain counts may be dependent on the height and sampling points. From the results of this study, we can find that the counts of scattering pollen grains were higher at P2 than those at P1. The finding suggested that the pollen grains which fell on the surface of the roadside would rebound to the ambient air by wind movement. We also suggested that there is a secondary pollen source by heavy traffic of automobile transportation at the roadside.

In order to investigate the airborne behavior of pollen grains and allergenic Cry j 2, we focused on the determination of pollen counts and the concentrations of particulate allergenic Cry j 2 in this study. From the results, we can find that there were weak correlations between scattering pollen grain counts and the concentrations of allergenic Cry j 2 particles in respirable particulate matter with all particle sizes, although the pollen grains at two sampling points were highly correlated. It is clear that allergenic Cry j 2 proteins will release from *Cryptomeria japonica* pollen grains during their transportation. Bastl et al. (2014) proved that the impact on allergy sufferers during a pollen season was not correlated with the total pollen loads of three important pollen such as birch, grass and ragweed within the pollen seasons in Austria and Germany. It is important to determine allergenic concentrations, rather than the scattering pollen grain counts. Riediker et al. (2000) reported the weak correlation ($r = 0.57$, $p < 0.05$, $n = 11$) between *birch* pollen grain counts and allergenic concentrations of Bet v 1 in fine particles $< 1 \mu\text{m}$. As shown in Fig. 5, we can find that there was a weak correlation between scattering pollen count and all sizes of allergenic particles of Cry j 2 ($r = 0.40$, $p < 0.5$, $n = 6$), and the results from our study were in accordance with the studies on other pollen allergenic particles.

We also investigated the influence of weather condition on the airborne pollen grains and allergenic particulates. Concerning about the weather condition, we have examined the effects of pollen grain counts and the concentrations of the allergenic particles from three aspects of wind speed, temperature and precipitation. It was thought that strong wind speed in local scale may accelerate the scattering counts of the pollen grains. However, the temperature is the most important factor, because it

was found that the higher airborne *Cryptomeria japonica* pollens were counted after the higher temperature increased up to 20 °C. The pollen grains usually are scattered and released from the Japanese cedar tree in the mountain areas when the temperature is relatively higher and then transported the long distance from the mountains to the atmospheric sampling points with higher wind speed. Tamura et al. (1997) concluded that the highest temperature was statistically related to the maximum pollen counts of *Cryptomeria japonica* pollen in the air. Under appropriate conditions, i.e., humidity, gaseous and water-soluble compounds in SPM may induce local release of allergenic particles, resulting in either allergenic extrusion in particle sizes $< 1 \mu\text{m}$ followed by generation of bioaerosols or adsorption of pollen-derived proteins to airborne particulates. This view was also supported by findings of Behrendt et al. (1997). Schappi et al. (1997) examined *birch* pollen which had been in contact with rainfall and found the growth of pollen tubes and the release of starch granules containing allergenic contents Bet v 1. They identified Bet v 1 not only in large particles ($> 7.2 \mu\text{m}$), but also in fine particles ($< 7.2 \mu\text{m}$). It was concluded that the allergenic Bet v 1 concentrations in fine particles ($< 7.2 \mu\text{m}$) was higher during the days following light rainfall. Suphioglu et al. (1992) also found starch granules with Lol p IX antigens ($< 3 \mu\text{m}$ in particle sizes) released from *ryegrass* pollen in the air after rainfall. Based on our previous experiment (Wang et al. 2012b; 2013a), we have found different behaviors of allergenic Cry j 1 and Cry j 2 particles released from *Cryptomeria japonica* pollen grains with the time variations after contacting with the simulated air-polluted rainwater of Saitama urban area, Japan. It was found that the concentrations of Cry j 1 increased within about 4 h after contacting with the simulated air-polluted rainwater. However, the concentrations of Cry j 2 increased slowly after 1 day about 24 h by contacting with the simulated air-polluted rainwater. Since elution of Cry j 2 is induced by the burst of pollen grains in contact with basic solutions, it was thought that releasing of allergenic Cry j 2 proteins takes a longer time than the releasing of allergenic Cry j 1 proteins and is strongly dependent on solution ionic concentrations (Sagehashi et al. 2005; Wang et al. 2010; 2012b). Because the burst ratio of pollen grains will be increased with increasing solution ionic

components, it is thought that the burst ratio of pollen increased with increasing ionic components. Therefore, it is possible that rainfall may trap ionic air pollutants such as nitrates and sulfates, and then affect the release of allergenic proteins of Cry j 1 and Cry j 2 from the pollen grains. The two major daughter allergenic species were released from the pollen grains and then suspended as fine particles containing the pollen allergenic proteins. It was also reported that elution of allergenic Cry j 1 was induced by the alkaline solutions. The released allergenic particles concentrations from pollen were increased up to 60 % when the pollen grains were in contact with simulated Ca^{2+} containing alkaline rainwater (Wang et al. 2012b), especially during the Yellow Sand events within the pollen scattering seasons (Wang et al. 2013b, 2014). Moreover, pollen allergenic species released from the pollen grains easily become different particle sizes at roadside point in the urban areas, and the subsequent pollen grains resuspension by heavy traffic of automobile transportation. As the result, we have determined the higher concentrations of allergenic Cry j 1 and Cry j 2 fine particles at the roadside sampling point during our field investigation. Worsening asthmatic symptoms may associate with the generation of small-sized allergenic species induced by rainfall and exposed to polluted air. Differences between the average concentrations of Cry j 2 before and after rainfall could not be ignored.

From the investigation of the airborne behavior of the size-segregated allergenic Cry j 1 and Cry j 2, the higher concentrations of the allergenic Cry j 1 and allergenic Cry j 2 were measured in the fine particles as $\text{PM}_{1.1}$ than in other particle sizes. At the same time, we also detected high concentrations of Cry j 2, especially in fine particle size even in the fine particle sizes around 0.06 to 0.2 μm at the roadside. It is in good agreement with the observation of the pollen allergenic particles collected in urban areas compared with those collected in the suburban areas (Feng et al. 2011). Furthermore, Lu et al. (2014) reported that *Platanus* pollen grains become swollen and new particles could be found on the pollen surface after exposure to the mixture of gases (air pollutants), and suggested that more allergenic protein was released as fine particles with the higher allergenicity than the original allergenic protein. Therefore, it was thought that these modified Cry j 2 allergenic fine particles contributed to worsen

pollinosis symptoms in polluted urban areas (Wang et al. 2016), and the characterizations mentioned above are basically important used as the predictive information during the scattering seasons of *Cryptomeria japonica* (Japanese cedar) pollen.

5 Conclusion

In this study, we focused on the airborne allergenic Cry j 2 as the tracer of *Cryptomeria japonica* pollen allergenic particles within the pollen scattering seasons in 2012. The higher concentrations of Cry j 2 were detected, especially in fine particle sizes around 0.06 to 0.2 μm at the roadside. With the comparison of airborne behaviors of the concentrations of two major allergens of Cry j 1 and Cry j 2, we firstly found that the concentrations of the allergenic Cry j 2 were higher as those of Cry j 1. The higher concentrations of the allergenic Cry j 2 also were detected in particle size $\text{PM}_{1.1}$ than in other particle sizes. From the investigation of allergenic Cry j 2, we also can find that allergenic Cry j 2 fine particles were not increased on the sunny days after heavy rainfall, but increased on the sunny days after light rainfall. Since elution of allergenic Cry j 2 proteins is induced by the burst of pollen grains in contact with basic air-polluted rainfall, it was thought that releasing of allergenic Cry j 2 fine particles from the parent pollen grains takes a longer time than the releasing of allergenic Cry j 1 which may strongly dependent on solution pH and ionic concentrations. The allergenic Cry j 2 concentrations could not be ignored. Therefore, worsening asthmatic symptoms may associate with the generation of small-sized allergenic species. The experimental findings in the present study could be an evidence that the allergenic Cry j 2 fine particles may cause the adverse effects on the human respiratory system as same as the allergenic Cry j 1 fine particles.

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