

Seasonal changes in mite allergen (*Der* I and *Der* II) concentrations in Japanese homes

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Background: There has been no report on seasonal changes in *Der* II allergen in floor and bedding dust.

Objective: The purpose of the study was to examine the extent of seasonal changes in *Der* I and *Der* II allergens in the floor and bedding dust found in houses.

Methods: We measured the absolute concentrations of mite allergens in dust collected monthly for 1 year from both the floors and bedding of eight houses in Tokyo. Dust samples were obtained from eight families without regard to their allergy histories. The concentrations of the *Der p* I, *Der f* I, and *Der* II allergens were measured by fluorometric sandwich ELISA.

Results: We found seasonal changes in the concentrations of these mite allergens. The highest concentrations of *Der* I (*Der p* I plus *Der f* I) and *Der* II (*Der p* II plus *Der f* II) were present from August to October, and the lowest ones from March to April. In floor dust, the mean highest concentrations of *Der* I and *Der* II (35.0 and 20.2 $\mu\text{g/g}$ of fine dust) were sevenfold and fivefold respectively, times the mean lowest concentrations. In bedding dust, the mean highest concentrations of *Der* I and *Der* II (51.3 and 29.6 $\mu\text{g/g}$ of fine dust) each were fourfold times the mean lowest concentrations.

Conclusions: The patterns of seasonal changes in *Der* II in floor and bedding dust were similar to those of *Der* I.

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INTRODUCTION

Allergy to house dust allergens is common among patients with bronchial asthma and allergic rhinitis. Two species of the mite genus *Dermatophagoides*, *D. pteronyssinus* and *D. farinae*, are important sources of house dust allergens.^{1–3} Two groups of major allergens, *Der* I (*Der p* I and *Der f* I) and *Der* II (*Der p* II and *Der f* II), have been purified from these *Dermatophagoides* species.^{4–8} Immunoassays have been used to measure the *Der* I and

Der II in studies done on the levels of mite allergens in indoor environments.^{9–11}

Previous studies have shown that there are seasonal variations in the number of mites in house dust.^{12–14} The primary determinant of these variations is humidity, and the optimum condition for mite growth appears to be an absolute indoor humidity of 7 g/kg.¹⁵ Successful culture of house dust mites in the laboratory occurs in a relative air humidity range of 70% to 85%.¹⁶ Temperature requirements for mite growth are not very strict, being between 15 and 32 °C with the optimum for the most rapid growth at 25 °C.¹⁶ Significant seasonal variations in the concentrations of mite allergen (*Der p* I) content also have been reported in dust samples^{17–19}; but there has been no report on seasonal changes in *Der* II allergen in house dust.

To determine the extent of seasonal changes in the *Der* I and *Der* II con-

tents in houses, we measured the amounts of these mite allergens in dust samples collected monthly for 1 year from the floors and bedding of eight houses in Tokyo.

MATERIALS AND METHODS

Dust Samples

Dust samples were obtained from the Tokyo homes of eight families without regard to the families' histories of allergies. Families A to D live in wooden houses, and families E to H in concrete apartment buildings. Sampling was conducted monthly from May 1989 through April 1990. Dust samples were collected with a vacuum cleaner (HC-V10, Panasonic, Osaka, Japan) for five minutes from about a 2-m² area on the living room floor and from the surfaces of futon (Japanese bedding filled with cotton wadding). Allergens were extracted from the dust as described elsewhere.^{20,21}

Reference Allergens and Their Antibodies

The allergens *Der p* I, *Der f* I, and *Der* II were purified as reported previously^{8,9} and used as references in the enzyme linked immunosorbent assay (ELISA). The preparation of rabbit antisera to *Der p* I, *Der f* I, and *Der* II has been described elsewhere.⁹ Biotinylation of the antibodies was performed as described previously.²²

Mite Allergen Quantitation

Der p I, *Der f* I, and *Der* II were quantitated using a fluorometric sandwich ELISA as described elsewhere.²³ The wells of flat-bottomed ELISA plates (Immulon 2; Dynatech, Alexandria, VA) were coated with 200 ng/0.1 mL of rabbit anti-*Der p* I IgG, anti-*Der f* I IgG, or anti-*Der* II IgG in 0.05 M carbonate-bicarbonate

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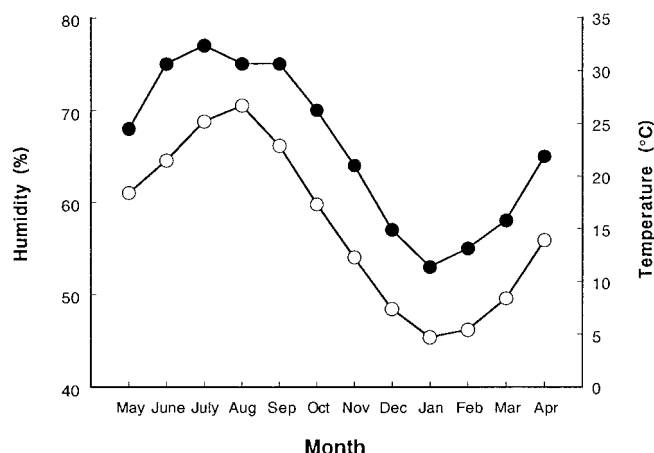


Figure 1. Monthly data for temperature and humidity in Tokyo. Outdoor temperature (○), relative humidity (●).

buffer, pH 9.6, and the plates kept for three hours at 37 °C. After three washes of the plates with phosphate-buffered saline containing 0.05% Tween 20 (PBST), the reference allergen or the diluted sample in PBST containing 1% bovine serum albumin (BSA) was added to the wells. After the plates had been incubated overnight at 4 °C, the wells were washed three times with PBST, and 25 ng/0.1 mL of biotinylated rabbit anti-*Der p* I

IgG, anti-*Der f* I IgG, or anti-*Der II* IgG was added. The plates then were incubated at room temperature for two hours. After additional washes, 0.1 mL of β -D-galactosidase conjugated streptavidin (Zymed Laboratories, San Francisco, CA) diluted 1/30,000 with BSA-PBST was added. The plates again were incubated at room temperature for two hours. They then were washed and 0.1 mL of 0.1 mM 4-methyl-

umbelliferyl- β -D-galactoside (Sigma Chemicals, St. Louis, MO) in 0.01 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl, 1 mM $MgCl_2$, 0.1% BSA, and 0.1% NaN_3 , was added to each well, after which the plates were incubated at 37 °C for two hours. The enzyme reaction was stopped with 0.1 M glycine-NaOH, pH 10.2, and the fluorescence intensity was read in the fluorescence units on a microplate fluorescence reader (Fluoroskan, Flow Laboratories, McLean, VA).

Outdoor Temperature and Humidity in Tokyo

The monthly mean values for outdoor temperature and humidity in Tokyo were obtained from the Japanese Meteorological Agency (Fig 1).

Statistics

The Wilcoxon signed-ranked test was used to compare the monthly concentrations of mite allergens. The Mann-Whitney U test was used to compare the concentrations of mite allergens between wooden houses and concrete apartment buildings.

Table 1. Monthly Measurements of Mite Allergens in Floor Dust of Living Rooms

Home	Allergens	Dust Allergens, μ g/g fine dust												Mean*
		May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
A	<i>Der I</i> †	2.68	4.06	1.80	11.17	10.34	9.63	7.67	5.37	3.81	1.03	1.21	0.90	3.54
	<i>Der II</i>	1.67	4.18	1.46	10.94	5.54	10.93	9.47	5.25	2.05	1.24	0.99	1.44	3.21
B	<i>Der I</i>	27.26	86.19	82.89	117.74	166.40	44.03	89.19	65.73	43.78	12.60	14.93	32.51	50.32
	<i>Der II</i>	13.02	41.23	42.96	54.89	59.89	69.36	46.62	30.54	15.35	10.64	12.41	10.78	27.19
C	<i>Der I</i>	3.14	11.76	15.79	33.88	58.85	14.66	14.30	7.14	7.42	2.71	3.11	17.55	10.56
	<i>Der II</i>	1.52	7.84	14.86	22.76	21.55	10.52	8.01	3.36	2.84	1.65	2.62	15.41	6.39
D	<i>Der I</i>	5.43	7.47	16.55	14.19	16.22	8.53	4.88	8.95	9.76	3.12	5.47	3.14	7.47
	<i>Der II</i>	5.23	7.19	17.42	15.72	19.80	15.12	6.57	10.37	9.90	5.87	7.18	14.05	10.18
E	<i>Der I</i>	12.91	16.04	19.38	49.05	38.51	15.65	13.24	17.36	11.51	3.56	2.91	3.14	12.13
	<i>Der II</i>	8.60	13.71	16.57	32.37	24.82	12.06	12.67	12.33	5.91	4.61	3.07	1.71	9.31
F	<i>Der I</i>	19.32	12.76	20.44	21.07	24.94	18.32	11.34	19.68	25.78	6.71	4.48	4.72	13.59
	<i>Der II</i>	10.57	7.49	11.48	1.87	19.00	15.39	6.96	7.76	8.49	5.14	2.35	1.84	7.46
G	<i>Der I</i>	42.07	40.00	46.68	112.75	73.17	49.89	54.48	46.33	18.68	20.38	15.48	22.51	38.56
	<i>Der II</i>	21.07	21.40	23.59	61.39	35.98	27.74	23.77	18.08	8.90	14.68	12.06	12.83	20.55
H	<i>Der I</i>	33.98	8.55	15.00	9.59	19.46	38.68	21.71	22.66	16.14	17.79	6.59	6.92	15.57
	<i>Der II</i>	13.74	4.51	11.23	5.37	11.37	21.97	12.93	11.62	6.66	5.77	3.37	3.96	8.03
Mean*	<i>Der I</i>	12.05	14.68	18.00	30.28	34.99	20.34	17.42	17.32	13.47	5.59	5.03	6.63	13.90
	<i>Der II</i>	6.80	9.95	13.00	19.93	20.16	18.48	12.58	10.14	6.35	4.75	4.02	5.20	9.48

* Geometric mean.

† *Der p* I + *Der f* I.

RESULTS

Monthly Measurements of Der I and Der II in Floor Dust of Living Room

Measurement of the monthly concentrations of *Der I* and *Der II* in floor dust showed significant seasonal changes in mite allergens in all the houses ($P < .02$) (Table 1). The highest values for *Der I* and *Der II* in each house were from July to October, and the lowest values from February to April. During the 1-year period of study, the highest concentrations of *Der I* concentrations in the floor dust from each house varied from 5-fold to 20-fold the lowest ones. Similarly, the highest concentrations of *Der II* concentrations varied from 4-fold to 19-fold the lowest ones. More than 10 μg of *Der I* per g of fine floor dust was found in all the houses in September, but in only two houses in March.

Figure 2a gives the geometric mean values of *Der I* and *Der II* in the floor dust. On the average, their respective highest values (35.0 and 20.2 $\mu\text{g}/\text{g}$ of fine dust) were in September and the lowest ones (5.02 and 4.02 $\mu\text{g}/\text{g}$ of fine dust) in March. These highest values for *Der I* and *Der II* respectively were 7-fold and 5-fold the lowest values.

Monthly Measurements of Der I and Der II in Japanese Bedding Dust

Monthly measurements of the concentrations of *Der I* and *Der II* in bedding dust (Table 2) also showed significant seasonal changes in all the houses ($P < .02$). The highest values for *Der I* and *Der II* were found from August to October, the lowest ones from January to May. During the study period, the concentrations of *Der I* varied from 3-fold to 17-fold, those of *Der II* concentrations from 2-fold to 19-fold. More than 10 μg of *Der I* per g of fine dust found in bedding of all the houses from July to September, but in only four houses in March.

The geometric mean values for *Der I* and *Der II* in the bedding dust are shown in Figure 2b. In general, their highest respective values (51.3 and 28.9 $\mu\text{g}/\text{g}$ of fine dust) were in August and the lowest values (12.3 and 7.62

$\mu\text{g}/\text{g}$ of fine dust) in April. These highest values were about 4-fold the lowest values.

DISCUSSION

We found seasonal changes in the concentrations of *Der I* and *Der II* in floor and bedding dust. An earlier study done in Japan reported distinct seasonal changes in the number of *Dermatophagoides* mites present in dust from all the houses surveyed in Saitama Prefecture, north of Tokyo.¹⁴ Great variations were seen in seasonal

changes in the presence of mite allergen in each home. The seasonal changes seen in the dust from floors and bedding were not significantly different for wooden houses and concrete apartment buildings. Linter et al reported that air-conditioning had a significant effect on suppression of mite populations and mite allergen production during the summer months.¹⁸ In this study, there was air-conditioning in all the homes, but it was not used continuously. Carpenter et al reported that air-conditioning markedly reduces

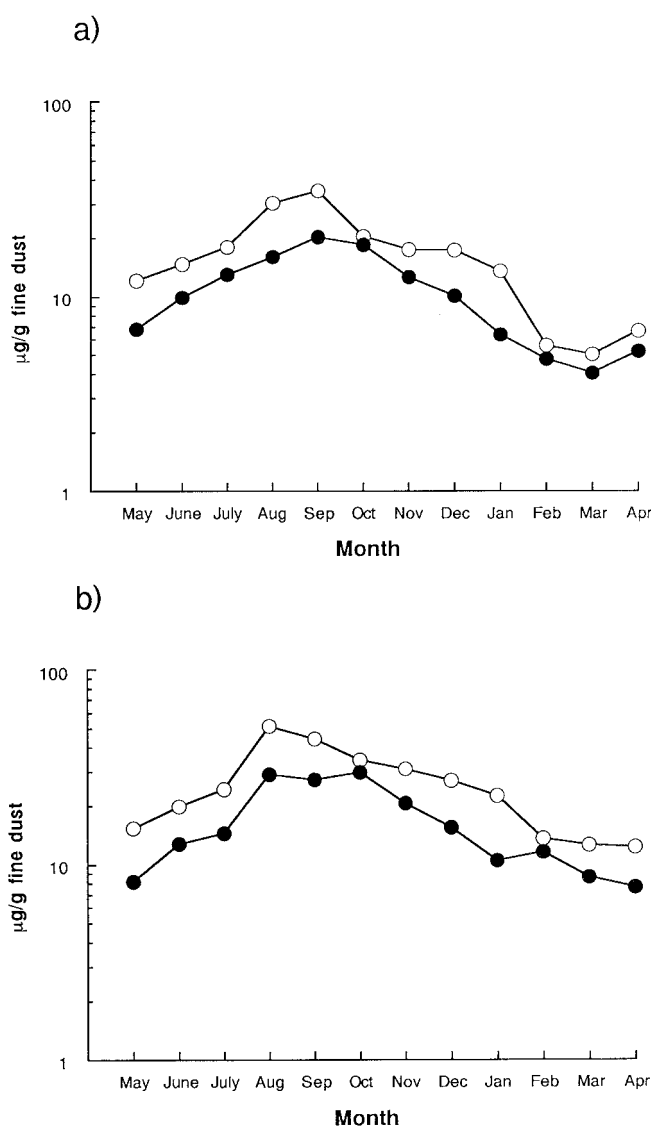


Figure 2. Geometric mean values of *Der I* and *Der II* in a) floor dust b) bedding dust. *Der I* (○), *Der II* (●).

Table 2. Monthly Measurements of Mite Allergens in Futon (Japanese bedding) Dust

Home	Allergens	Dust Allergens, $\mu\text{g/g}$ fine dust												Mean*
		May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
A	Der I†	26.38	20.36	20.33	45.57	28.79	8.45	15.26	19.78	8.11	9.88	9.05	8.12	15.65
	Der II	10.55	10.10	11.10	23.85	16.69	14.32	11.86	12.25	4.64	12.81	8.03	4.80	10.70
B	Der I	15.19	30.81	75.41	132.17	159.80	84.28	125.02	81.40	46.92	20.37	20.77	23.18	50.78
	Der II	8.48	23.50	49.68	76.11	78.76	54.22	69.24	43.05	18.35	15.47	14.45	15.39	30.33
C	Der I	7.21	10.85	11.69	16.89	21.50	13.42	16.18	18.89	14.05	10.45	9.81	9.93	12.81
	Der II	3.14	3.55	4.02	7.41	10.20	19.44	10.40	10.54	6.92	6.71	5.68	5.01	6.78
D	Der I	11.62	31.44	49.95	72.45	76.29	30.28	46.81	47.87	51.99	46.72	31.18	43.13	40.97
	Der II	9.84	32.05	38.09	52.06	61.23	34.11	37.03	32.04	29.08	43.39	25.96	22.40	31.96
E	Der I	10.83	10.98	10.72	29.79	29.18	33.52	17.68	12.84	10.76	11.91	7.28	5.50	13.69
	Der II	8.32	13.69	11.94	25.17	27.69	24.87	15.90	10.48	6.06	13.52	6.60	4.19	12.01
F	Der I	58.27	48.45	41.21	121.47	114.94	148.15	81.13	36.42	131.98	23.18	32.76	24.23	58.85
	Der II	19.64	24.94	21.69	54.33	59.42	56.26	44.10	16.94	46.29	18.28	15.36	15.85	28.58
G	Der I	24.68	35.35	30.08	57.69	21.29	35.61	41.18	40.25	34.66	13.11	20.53	18.90	28.87
	Der II	13.79	24.79	14.70	31.90	14.20	32.43	24.90	21.28	14.61	14.25	13.40	13.73	18.39
H	Der I	6.05	6.02	10.34	31.47	26.74	37.23	9.64	9.99	4.75	3.32	2.19	2.63	10.02
	Der II	3.15	3.22	5.74	15.65	15.84	24.95	5.84	4.93	2.10	1.59	1.30	1.52	4.50
Mean*	Der I	15.40	19.86	24.34	51.36	44.14	34.27	30.86	26.91	22.47	13.61	12.55	12.33	23.63
	Der II	8.16	12.83	14.39	28.84	27.25	29.56	20.59	15.49	10.50	11.58	8.61	7.63	14.54

* Geometric mean.

† *Der p* I + *Der f* I.

mite density if it is run constantly.²⁴ Why individual variations in the mite allergen level in this study occur has yet to be explained.

In floor dust, September peak of the mite allergen occurred 2 months after the mean outdoor relative humidity peak. The lowest mite allergen concentrations that occurred in March also were months after the lowest mean outdoor relative humidity. Seasonal changes in mite allergen concentrations in bedding dust showed a trend similar to that for floor dust. Platts-Mills et al reported similar findings for the USA; the levels of *Der p* I mite allergen in dust followed the pattern for humidity with a lag of about 1 to 3 months.¹⁷ Heide et al reported that highest absolute air humidity was during the months of July to September, just before the increase in the levels of *Der p* I allergen in floor dust samples.¹⁹ Further, the increase of the mite allergen levels in house dust promoted the level of airway hyper-responsiveness in patients with allergic asthma.¹⁹

We have confirmed our previous findings that high concentrations of

Der I and *Der* II are present in fine dust taken from bedding.²⁰ The warm, humid conditions that exist in bedclothes are ideal for mite replication.²⁵ High concentrations of *Der* I also have been found in fine dust from bedding.^{17,26} In previous studies, we reported that airborne concentrations of *Der* I and *Der* II are high during bed-making and sleeping, but are very low in living rooms.^{20,21} We speculated that exposure to mite allergens from bedding during sleep might be an important factor in the development of mite allergies.

Tateno reported that the washing of futons (Japanese bedding) with water is effective in reducing the concentration of mite allergens,²⁷ which we also confirmed.²⁸ Bedding should be washed frequently regardless of season because the mite allergen concentrations in bedding are high throughout the year.

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