

Citrus red mite (*Panonychus citri*) may be an important allergen in the development of asthma among exposed children

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Summary

Background Recent investigations have demonstrated that spider mites are important allergens in the development of asthma in fruit-cultivating farmers.

Objective The aim of this study was to evaluate the sensitization rate to the citrus red mite (*Panonychus citri*) in children living in rural areas, and to determine the allergenic cross-reactivity with other mites.

Methods A total of 7254 children (ages 7–15 years) living in rural areas were enrolled, and each subject was evaluated by a questionnaire and a skin prick test. Allergenic cross-reactivity was evaluated by ELISA inhibition tests.

Results The most common sensitizing allergens were house dust mites, followed by citrus red mite and cockroach. High serum-specific IgE bindings to the citrus red mite were detected in 21 of 100 randomly selected subjects. The prevalence of asthma was higher among those with positive skin responses to the citrus red mite than with negative skin responses to this mite. ELISA inhibition tests showed that IgE bindings to this mite were minimally inhibited with additions of domestic mites.

Conclusion Spider mites such as the citrus red mite may be important outdoor allergens among children living in rural areas, and spider mite-derived allergens have unique allergenic determinants compared with domestic mites.

Keywords: citrus red mite, children, asthma, allergy, sensitization, exposure, cross-reactivity

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Introduction

Allergic diseases due to domestic mites such as house dust mites and storage mites are a major health problem worldwide. House dust mites are important sources of allergens inside homes in humid areas worldwide [1,2].

Several studies conducted during the past few years have demonstrated that storage mites are major sensitizing allergens among farmers [3–5]. In addition, allergy to storage mites has been associated not only with occupational exposure, but also with damp housing conditions [6]. Because of the domestic exposure in some populations, the allergenic cross-reactivity between the domestic mites has been of great interest. Investigators have reported minimal or extensive allergenic cross-reactivity patterns [4,7–9].

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Spider mites (family *Tetranychidae*) form the family of phytophagous mites which have the severest economic effect on agriculture. Several of these mites are injurious to many crops all over the world [10]. The number of tetranychid species remained stable for 75 years and then increased, resulting in a more significant economic effect on agriculture. From 102 species listed by McGregor in 1950, the total number of species reached 204 in 1955 as recorded by Pritchard and Baker, and has increased to nearly 1200 species today [11].

Recently, citrus red mite (*Panonychus citri*, CRM)-induced asthma confirmed by specific bronchial challenge has been reported in citrus-cultivating farmers [12]. Subsequently, cross-sectional studies have demonstrated that spider mites such as the CRM, European red mite (*Panonychus ulmi*) and two-spotted spider mite (*Tetranychus urticae*) are important allergens in the development of work-related asthma and rhinitis symptoms [13,14]. All of the spider mites are outdoor phytophagous mites which damage fruit leaves throughout the world, and belong to the order Acari, to the suborder of Prostigmata, while domestic mites belong to the suborder of Astigmata [10,11]. However, allergenic cross-reactivity has previously been unknown between spider mites and domestic mites.

The aim of this study was to evaluate the sensitization rate to CRM in CRM-exposed children, and its allergenic cross-reactivity with spider mite *P. ulmi*, spider mite *T. urticae*, house dust mite *Dermatophagoides pteronyssinus*, and storage mite *T. putrescentiae*.

Methods

Study subjects

A total of 7950 children (ages 7–15 years) were enrolled in this study. They had been living in rural areas near citrus orchards since infancy and those recently moved from other areas were excluded. This study was conducted from 10 March 1998 to 30 June 1998. The response rate to the questionnaire was 91.2%; 7254 children responded. Among 6843 children who attended school of more than 100 school children, 6332 (92.5%) underwent skin prick tests to common aeroallergens and CRM. Sera from 100 randomly selected subjects aged 13–15 years were collected to measure serum-specific IgE to CRM and to perform ELISA inhibition tests. The parents gave written informed consent for the study, and the children agreed to participate with parental consent. The study protocol was approved by the Ethics Committee of Seoul National University Hospital.

Questionnaire survey

A questionnaire developed by the International Study of

Asthma and Allergic Diseases in Children (ISAAC) was used [15], and included demographic data, asthma symptoms, and environmental exposure to CRM. The questions on asthma symptoms concentrated on wheezing episodes, asthma diagnosed by a doctor, exercise-induced wheeze, and recurrent nocturnal cough. The questions on environmental exposure to CRM concerned frequency of visits to citrus orchards (< 1 –2/month, ≤ 1 /week, or > 1 /week) and the distance from the home to the citrus orchards (> 1 km, ≤ 1 km, ≤ 0.2 km, or ≤ 0.05 km).

Preparation of spider mites extracts

CRM, European red mite, and two-spotted spider mite were extracted as previously described [12,14]. In brief, the mites were mixed with modified Coca's solution in 1:5 w/v ratio. After incubation at room temperature for 24 h, the mixture was centrifuged at 3000 *g* for 30 min. The supernatant was dialysed (the cut-off molecular weight was 6000 D) against 4 L of distilled water for 48 h at 4 °C, and then lyophilized at -70 °C for the preparation of antigens used in ELISA and ELISA inhibition test. The same amount of glycerin was added with a final concentration of 1:20 w/v ratio, and this was used for the skin prick tests.

Skin prick tests

A panel of nine aeroallergens (Allergopharma Co., Hamburg, Germany) and CRM was used for the skin prick tests. None of the subjects had received anti-histamines orally for 5 days preceding the study. The commercially available panel included *D. pteronyssinus*, *D. farinae*, cockroach, cat fur, *Alternaria tenuis* and *Aspergillus fumigatus*, and pollens (grass pollen mixture, mugwort, and ragweed). A positive control of histamine (1 mg/mL, Allergopharma Co.), along with a negative control of diluent (Allergopharma Co.), was included in all tests. Fifteen minutes after the prick test, the mean diameter of the weal formed by the allergen was measured. If the diameter was the same or larger than 3 mm compared with that formed by a negative control (diluent), the skin response to an allergen was defined as positive [16].

ELISA for serum-specific IgE and ELISA inhibition tests

The presence of CRM-specific IgE antibodies was determined using ELISA as previously described [13]. All assays were performed in duplicate. The cutoff values (0.24) of serum-specific IgE levels to CRM were determined from the mean + the two-fold SD of the absorbance value from 30 CRM-unexposed non-atopic healthy controls.

Competitive ELISA inhibition tests were performed to

Table 1. Prevalence of asthma symptoms on a questionnaire

Age, years	7–9	10–12	13–15	χ^2 trend
Asthma symptoms	(n = 2087)	(n = 2137)	(n = 3030)	
Ever experiencing wheezing	17.1%	14.5%	11.4%	33.28**
Wheeze in last 12 months	11.5%	11.2%	9.4%	5.99*
Asthma diagnosed by doctor	7.2%	6.5%	3.0%	68.6**
Exercise-induced wheeze	7.4%	11.3%	13.2%	40.8**
Nocturnal cough	20.0%	16.8%	15.7%	37.6**

* $P < 0.05$; ** $P < 0.01$.

determine allergenic cross-reactivity between CRM and the other mites. The tests were performed using the pooled sera of five subjects who showed positive skin responses to CRM, but negative responses to *D. pteronyssinus*, and of five subjects with positive skin responses to both CRM and *D. pteronyssinus*. These pooled sera (50 μ L) were preincubated with five concentrations (0.001, 0.01, 1, 10 and 100 μ g/mL) of CRM extract, *D. pteronyssinus*, *T. putrescentiae* extracts (Allergopharma Co.), *P. ulmi*, and *T. urticae* extracts for 1 h at room temperature, and then incubated with the CRM-coated microtitre plate for 2 h. The same steps as in the ELISA were then followed. After studying the control samples in which equal volumes of PBS, pH 7.5 were preincubated instead of inhibitors, the inhibition of the specific IgE binding was expressed as [100 – (absorbance of samples preincubated with allergens/absorbance of samples preincubated with PBS) $\times$ 100 (%)].

Statistical analysis

The results were analysed and tested for statistical

significance by the calculation of χ^2 values, relative risk (RR), and the correlation coefficient. Cochran–Mantel–Haenszel statistics were applied to determine the multiple categorical variables. The results were considered statistically significant when $P < 0.05$.

Results

Responses to a questionnaire on asthma symptoms

The prevalence of asthmatic symptoms on the questionnaire is shown in Table 1. The prevalence of asthma diagnosed by a doctor was 7.2% among the children 7–9 years of age, 6.5% among those 10–12 years of age, and 3.0% among those 13–15 years of age. The prevalence of ever experiencing wheezing was 17.1% among those 7–9 years of age, 14.5% among those 10–12 years of age, and 11.4% among those 13–15 years of age. Of the three groups, 11.5%, 11.2%, and 9.4% had experienced wheezing during the previous 12 months, respectively. The prevalence of exercise-induced wheezing was 7.4% among those

Table 2. Skin responses to inhalant allergens

Age, years	7–9	10–12	13–15	χ^2 trend
Allergens	(n = 1699)	(n = 1731)	(n = 2902)	
<i>Dermatophagoides pteronyssinus</i>	22.7%	25.5%	27.7%	14.9**
<i>D. farinae</i>	20.4%	21.8%	23.8%	7.93**
Citrus red mite	8.8%	12.6%	18.6%	84.5**
Cockroach	9.6%	12.8%	16.7%	47.1**
Cat fur	2.4%	2.7%	2.8%	0.29
<i>Alternaria tenuis</i>	5.3%	5.9%	2.8%	20.5**
<i>Aspergillus fumigatus</i>	1.9%	1.7%	1.1%	4.61*
Grass pollen	1.6%	3.4%	2.2%	0.06
Ragweed pollen	1.6%	2.5%	1.3%	3.13
Mugwort pollen	1.4%	2.9%	2.7%	6.07*

* $P < 0.05$; ** $P < 0.01$.

Table 3. Relationship between skin responses to common sensitizing aeroallergens and asthma symptoms on a questionnaire

	Asthma diagnosed by doctor		Row Total	RR	95% CI
	Negative	Positive (row %)			
<i>Dermatophagoides pteronyssinus</i>					
Negative	4333	200 (4.41)	4533	1.0	
Positive	1453	127 (8.04)	1580	1.82*	1.47–2.26
<i>D. farinae</i>					
Negative	4533	204 (4.31)	4737	1.0	
Positive	1253	123 (8.94)	1376	2.08*	1.67–2.57
Citrus red mite					
Negative	4971	268 (5.12)	5282	1.0	
Positive	794	80 (9.15)	874	1.79*	1.41–2.20
Cockroach					
Negative	4988	294 (5.57)	5282	1.0	
Positive	798	33 (3.97)	831	0.71	0.50–1.01

RR (relative risk): * $P < 0.01$.

7–9 years of age, 11.3% among those 10–12 years of age, and 13.2% among those 13–15 years of age. Of the three groups, 20.0%, 16.8%, and 15.7%, respectively, experienced recurrent nocturnal cough without respiratory infections. As the children's ages increased, the prevalence of asthma diagnosed by a doctor, ever experiencing wheezing, recurrent nocturnal cough, and wheezing during the last

12 months showed a decreasing trend (χ^2 trend = 68.6, 33.3, and 37.6, $P < 0.01$, respectively; χ^2 trend = 6.0, $P < 0.05$). However, the prevalence of exercise-induced wheezing showed an increasing trend (χ^2 trend = 40.8, $P < 0.01$).

Skin response to aeroallergens including CRM

Table 2 shows skin responses to common aeroallergen and CRM. On skin prick tests, the most common sensitizing allergens among children of 7–9, 10–12 and 13–15 years of age were *D. pteronyssinus* (22.7%, 25.5%, and 27.7%), followed by *D. farinae* (20.4%, 21.8%, and 23.8%), CRM (8.8%, 12.6%, and 18.6%), and cockroach (9.6%, 12.8%, and 16.7%). The positive skin responses to these allergens showed an increasing trend as the children's ages increased. The statistical significance was the most marked for CRM (χ^2 trend = 14.9, $P < 0.01$; χ^2 trend = 7.93, $P < 0.01$; χ^2 trend = 84.5, $P < 0.001$; χ^2 trend = 47.1, $P < 0.01$, respectively). Similar trends were observed among both boys and girls (P -value of Cochran–Mantel–Haenszel statistics = 0.001, 0.005, 0.001, and 0.001, respectively).

Relationship between skin responses to aeroallergens and asthma

Among the 327 asthmatic children diagnosed by a doctor on the questionnaire, the most common sensitizing allergens were *D. pteronyssinus* (38.8%), followed by *D.*

Table 4. Relationship between environmental exposure and skin responses to the citrus red mite

	Skin response to CRM		RR	95% CI	χ^2 trend (P value)
	Negative	Positive			
Housing environment*	(n = 6195)				
No	2098	248	1.0		
Yes	3211	638	1.57	1.37–1.80	
Visiting frequency†	(n = 5825)				23.40 (0.0001)
≤ 1–2/month	3741	570	1.0		
≤ 1/week	913	181	1.04	1.01–1.07	
> 1/week	332	88	1.10	1.04–1.15	
Distance‡	(n = 5988)				49.36 (0.0001)
> 1 km	2092	248	1.0		
0.2–1 km	383	63	1.33	1.03–1.72	
0.05–0.2 km	405	77	1.51	1.19–1.91	
≤ 50 m	2243	477	1.65	1.43–1.91	

CRM, Citrus red mite; RR, relative risk.

*Housing near citrus orchards.

†Mean frequency of visits to citrus orchards.

‡Distance from house to citrus orchards.

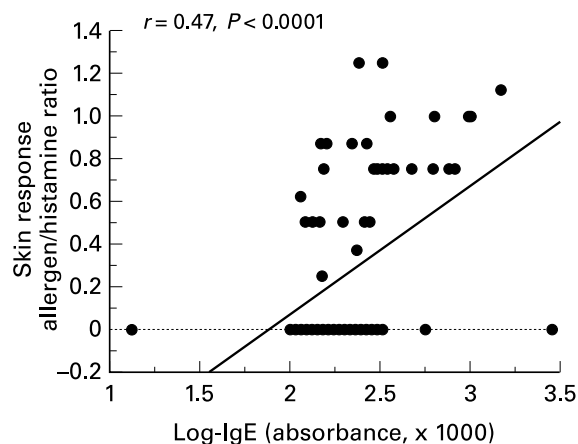


Fig. 1 Relationship between serum-specific IgE levels and skin responses to the citrus red mite

farinae (37.6%), CRM (24.7%), and cockroach (10.1%). Table 3 shows the relationships between skin responses to common sensitizing allergens and asthma diagnosed by a doctor on the questionnaire. The prevalence of asthma diagnosed by a doctor was higher among subjects with positive skin responses to CRM, *D. pteronyssinus*, or *D. farinae*, than among those with negative skin responses (9.2% vs. 5.1%, RR = 1.79, 95% confidence interval (CI)

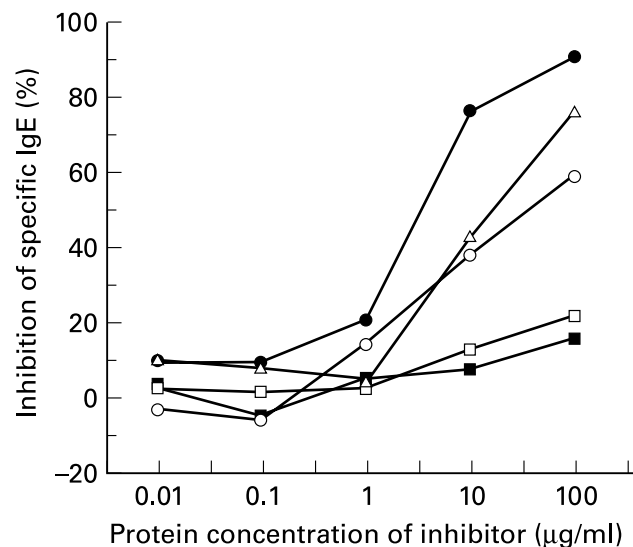


Fig. 2. Inhibition of citrus red mite-specific IgE with serial additions of *Dermatophagoides pteronyssinus*, *Tyrophagus putrescentiae*, citrus red mite, two-spotted spider mite, and European red mite using sera from five subjects with positive skin responses to the citrus red mite, but not to *D. pteronyssinus*. ■, *D. pteronyssinus*; □, *T. putrescentiae*; ●, citrus red mite; ○, two-spotted spider mite; Δ, European red mite.

1.41–2.20; 8.0% vs. 4.4%, RR = 1.82, 95% CI 1.47–2.26; 8.9% vs. 4.3%, RR = 2.08, 95% CI 1.67–2.57, respectively). However, with regard to skin responses to cockroach, no significant difference was observed (4.0% vs. 5.6%, RR = 0.71, 95% CI 0.50–1.01).

Effect of environmental exposure on the skin responses to CRM

Table 4 shows relationships between skin responses to CRM and environmental exposure. Positive skin responses to CRM were higher among children living near citrus orchards than among those living far from citrus orchards (19.9% vs. 11.8%, RR = 1.57, 95% CI 1.37–1.80). The positive rate was 11.9% among those living in a house > 1 km away from citrus farms, 16.4% among those 0.2–1 km away, 19.0% among those 0.05–0.2 km away, and 21.3% among those < 0.05 km away. These figures showed a reverse correlation between positive skin responses to CRM and housing distance from citrus orchards (χ^2 trend = 49.36, $P = 0.0001$). Of those subjects with a mean visiting frequency to citrus orchards of less than two times per month, 15.2% showed a positive response to CRM, as did 19.8% of those with a mean frequency of less than once per week, and 26.5% of those with a mean frequency of more than two times per week. This figure indicated a significant positive correlation between positive skin responses to CRM and a frequency of visits to citrus orchards (χ^2 trend = 23.40, $P = 0.0001$).

Serum-specific IgE to CRM, and its allergenic cross-reactivity with other mites

High serum-specific IgE bindings to CRM were observed in 21 (21%) of 100 randomly selected subjects. The relationship between serum-specific IgE levels and skin responses (allergen/histamine ratio) to CRM are illustrated in Fig. 1, which demonstrates a significant correlation between them ($r = 0.47$, $P < 0.0001$). Significant relationships were also observed between serum-specific IgE levels to CRM, and skin responses to *D. pteronyssinus* or *D. farinae*, although the statistical significance was less marked for CRM ($r = 0.35$, $P < 0.01$; $r = 0.37$, $P < 0.01$).

The homologous and heterologous inhibitions of specific IgE binding to CRM are shown in Fig. 2 and Fig. 3. In pooled sera from five subjects with positive skin response to CRM, but not to *D. pteronyssinus*, the inhibitory effects were significantly achieved at a higher concentration (100 $\mu\text{g/mL}$) of CRM (90.8%), *P. ulmi* (76.5%), and *T. urticae* (58.9%), but not of *T. putrescentiae* (21.3%) and *D. pteronyssinus* (15.6%) (Fig. 2). In pooled sera from five

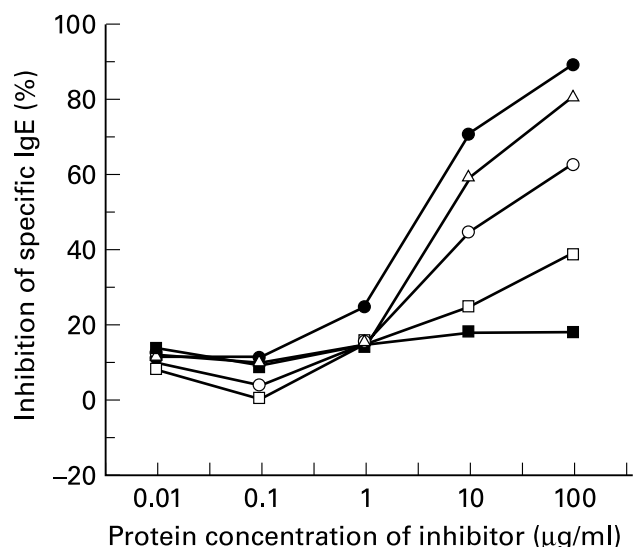


Fig. 3. Inhibition of citrus red mite-specific IgE with serial additions of *Dermatophagoides pteronyssinus*, *Tyrophagus putrescentiae*, citrus red mite, two-spotted spider mite, and European red mite using sera from five subjects with positive skin responses to the citrus red mite and *D. pteronyssinus*. ■, *D. pteronyssinus*; □, *T. putrescentiae*; ●, citrus red mite; ○, two-spotted spider mite; △, European red mite.

subjects with positive responses to both CRM and *D. pteronyssinus*, minimal inhibitions were also noted with the additions of 100 µg/mL of *P. pteronyssinus* (17.7%) and *T. putrescentiae* (38.7%). However, significant inhibitions were noted when 100 µg/mL of CRM (89.4%), *P. ulmi* (81.4%), and *T. urticae* (62.5%) were added (Fig. 3).

Discussion

Our results showed that the citrus red mite (CRM) is a common sensitizing allergen, next to house dust mites, among children who have been living in rural areas. Sensitization to CRM was significantly correlated with the prevalence of asthma on the questionnaire, and environmental exposure to CRM played an important role in the development of this sensitization.

The most common sensitizing allergens among Korean children living in urban areas were: *D. farinae* (30.9%), *D. pteronyssinus* (27.5%), cat fur (20.4%), and cockroach (11.8%) as a criterion of allergen/histamine ratio ≥ 1.0 [2]. The present study, however, showed that the positive skin responses to the house dust mites *D. pteronyssinus* and *D. farinae*, and cat fur among children from rural areas, were lower than among those from urban areas, despite more sensitive criteria of positive skin test responses (weal formed by allergen > 3.0 mm). Allergens are ubiquitous, but are present in different concentrations in different

environments. Features of modern-day housing, such as the trend towards houses that are sealed against cold winters and as a result are poorly ventilated, have led to increased exposure to house dust mites and allergens shed by animals [17,18]. The housing in rural areas in Korea is not modernized, and the outdoor air quality is very good. Most children in rural areas have lived there since infancy, rarely moving to other areas, and spending much of their time outdoors. These differences of environment and life-style could explain the lower sensitization to indoor allergens among children in rural areas.

A recent cross-sectional survey demonstrated that CRM was the most important allergen in the development of asthma and allergic rhinitis among citrus farmers [13]. Subsequently, another cross-sectional survey has demonstrated that spider mites such as *P. ulmi* and *T. urticae* are important allergens in the development of work-related asthma and rhinitis symptoms in apple farmers [14]. These studies suggest that outdoor spider mites play an important role in the development of respiratory symptoms in exposed individuals. The present study shows that CRM is a common sensitizing allergen, next to house dust mites, and sensitization to CRM was significantly correlated with the prevalence of asthma on the questionnaire. These findings suggest that outdoor spider mites such as CRM are important allergens in the development of asthma in children, as well as adults, exposed to CRM.

Although sensitization to a causative allergen requires exposure to only a small dose, it is possible that high doses may be associated with the greatest risk of sensitization to causative allergens. The relationship between environmental exposure and sensitization to indoor allergens has been studied. The results from a follow up of a cohort of children by Sporik *et al.* [19] showed that exposure to high levels of house dust mite allergen increased the risk of sensitization. In this study, we hypothesized that environmental exposure to CRM may determine the risk of sensitization to the spider mite. The present study demonstrated that children who had frequently visited citrus orchards, or had been living near citrus orchards, had enhanced skin responses to CRM. From these findings, we can assume that environmental exposure is a major determinant of the risk of sensitization to CRM.

In this study, serum-specific IgE responses to CRM were significantly correlated with skin responses to house dust mites *D. pteronyssinus* and *D. farinae*, as well as CRM. This finding suggests two possibilities: first, atopy may be a risk factor for sensitization to CRM, and second, positive IgE responses to CRM may reflect allergenic cross-reactivity to house dust mites. There is agreement that the closer the taxonomic relationship between the mites, the greater the level of cross-allergenicity [8,9,20]. Spider mites belong to the order Acari, to the suborder of

Prostigmatia, and to the family of spider mite Tetranychidae. CRM and the European red mite belong to the genus *Panonychus*, and the two-spotted spider mite to the genus *Tetranychus*. Domestic mites such as *D. pteronyssinus* and *T. putrescentiae* belong to the order Acari, but to the suborder Astigmatia. Because of domestic exposure in some populations, many investigators have evaluated the allergenic cross-reactivity between house dust mites and storage mites. Radioallergosorbent test (RAST) correlation and RAST inhibition studies have shown that the house dust mite *D. pteronyssinus* and the storage mite *Lepidoglyphus destructor* possess distinct allergenic determinants [7], but other investigators have reported a more extensive cross-reactivity [4,9]. However, few studies have been performed to determine the allergenic cross-reactivity between spider mites and domestic mites. Burches *et al.* [21] reported that all subjects sensitized to *T. urticae* were cross-reactive to house dust mites. In the present study, however, ELISA inhibition test showed that IgE bindings to CRM were inhibited minimally with additions of *D. pteronyssinus* and *T. putrescentiae* in sera from children with positive skin responses to both CRM and *D. pteronyssinus*, as well as to only CRM. However, the IgE bindings were inhibited significantly with the additions of spider mites *P. ulmi* and *T. urticae*. These results suggest the following: first, sensitization to CRM is influenced by a genetic predisposition to specific IgE responses to common aeroallergens; second, allergenic cross-reactivity is variable when sera from different populations are used; and third, the majority of allergenic determinants of CRM are unique to CRM-exposed children.

In conclusion, spider mites such as the CRM may be important outdoor allergens among children living in rural areas, and spider mite-derived allergens have unique allergenic determinants compared with domestic mites such as house dust mites and storage mites.

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