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# Longitudinal Evaluation of Allergen and Culturable Fungal Concentrations in Inner-City Households

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*To characterize seasonal variation of three allergens (dust mite, cat, and cockroach) and total culturable fungi and to explore whether residential characteristics were associated with the concentrations of these agents, floor dust was collected from 47 inner-city homes in Minneapolis, Minnesota, over a 1-year period. A longitudinal analysis of allergen and fungal concentrations was carried out using mixed-effect models. Overall, relative humidity was a better predictor of allergen concentrations over time than indoor temperature. Seasonal variation of cat and cockroach allergens was negligible compared with the variability associated with residential characteristics such as race/ethnicity, family income, and the presence of cats. Fungal concentrations showed significant seasonal variation that outweighed the variability associated with residential characteristics. Less than 30% of the dust mite allergen and cockroach allergens concentrations were above limits of detection. Observed cockroach allergen concentrations were higher in Spanish- and Somali-speaking households than in English-speaking households, while English-speaking households had significantly higher cat allergen concentrations compared with the other language groups. The ratios of within-home to between-home variance for total culturable fungi, dust mite, cockroach, and cat allergen concentrations were 2.54, 1.91, 0.55, and 0.24, respectively. This ratio is used to predict the number of repeated measurements of each allergen required to robustly estimate long-term exposure estimates such that exposure misclassification bias is kept within acceptable limits. It is not clear whether repeated measurements of dust mite and cockroach allergens are required for long-term average exposure because of the large fraction of nondetects. It is concluded that a single measurement of cat allergen is a reasonable surrogate for long-term average exposure, since repeated measurements over time were highly correlated. Total culturable fungi will require greater than nine repeated measurements for robust assessment of long-term exposures because of low correlations in fungal measures over time.*

**Keywords** allergen, cat, cockroach, dust mite, fungi, longitudinal study

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## INTRODUCTION

Allergic sensitization and respiratory symptoms are increasing in low income and/or minority populations living in inner-city households in the United States.<sup>(1–3)</sup> Exposures to indoor allergens such as dust mite (Der f 1), cat (Fel d 1), and cockroach (Bla g 1), and total culturable fungi in house dust have been associated with the development of childhood asthma or allergic symptoms in young adults.<sup>(4–14)</sup>

High cockroach allergen concentrations have been found in less educated, lower income minority households. In contrast, cat and dust mite allergens are more common in households of higher socioeconomic status and white Americans.<sup>(1–3)</sup> Allergen concentrations are also associated with household characteristics such as housing type, relative humidity, construction year, and with deteriorated housing.<sup>(15–21)</sup> Therefore, low-income minority people can have a different profile of allergen exposure due to a combination of environmental and socioeconomic factors.

Furthermore, indoor allergen and total culturable fungal concentrations appear to vary substantially by geographic location and season.<sup>(16,17,22–27)</sup> As a result, the exposure measurement strategy is a key to estimating the true exposure to indoor allergens and the relationship between the exposure and allergic sensitization or respiratory symptoms in epidemiologic studies. An imperfect measure of exposure can lead to a bias away from the true exposure in a regression model, which is a function of two variance components: within- and between-home variability.<sup>(28,29)</sup>

In addition, the two variance components affect the magnitude of correlation between repeated measurements of allergens, which determines the number of repeated measurements of indoor allergens and total culturable fungi needed to estimate the true long-term exposure. However, few studies have been conducted to characterize within-home and between-home variance components of allergen concentrations and total culturable fungi over time, and their findings and conclusions are not consistent.<sup>(23,24,30–32)</sup> Heinrich et al.<sup>(23)</sup> reported that

ratios of within- and between-home variance of Der p 1, Der f 1, and Fel d 1 were below 1. It was concluded that a single measurement of dust mite and cat allergens was a good proxy for the true exposure over time, while repeated measurements of total culturable fungi were needed.

Chew et al.<sup>(24)</sup> reported similar findings that between-home standard deviation for Der f 1, Der p 1, and Fel d 1 in floor dust were much larger than within-home standard deviation. In contrast, Hirsch et al.<sup>(30)</sup> concluded that repeated measurements were recommended for Der p 1 and Der f 1 even though within-home variance of these agents was much smaller than between-home variance. Giovannangelo et al.<sup>(31)</sup> reported that within-home variance of Der p 1 and Fel d 1 was smaller than between-home variance, but for Der f 1, larger within-home variance was observed. Topp et al.<sup>(32)</sup> concluded that a single measurement of Der p 1, Der f 1, and Fel d 1 was not a good proxy for the long-term exposure. Several investigators have concluded that further detailed studies were needed to investigate variation of these agents over time.

The aim of this study was to assess the seasonal variability in levels of common indoor allergens such as cat, cockroach, and dust mite and total culturable fungi, and to examine the longitudinal variability of allergen levels for different residential characteristics—race/ethnicity, family income, housing type, and indoor climate—in an inner-city population. A longitudinal analysis of allergen and total fungal concentrations expressed as both concentrations (per g dust) and loading (per m<sup>2</sup>) was performed. Concentrations per unit mass that represent allergen and fungal content in floor dust are considered to be suitable exposure metric for epidemiologic studies, while allergen loading (the amount of allergen per unit area) has been used for assessing allergen and fungal levels in intervention studies.<sup>(33,34)</sup>

## METHODS

### Subjects

As a priority, this study enrolled families who had participated in a previous population-based random sample (the SHIELD study)<sup>(35)</sup> and who still lived at the same address. Recruiting beyond this initial target population involved a progressive, tiered approach to reach the desired recruitment goal of 60 families by targeting (a) new residents of previous SHIELD household addresses, (b) SHIELD families who moved within the South Minneapolis area, and (c) non-SHIELD residents of the Lyndale, Whittier, and adjacent neighborhoods in South Minneapolis. The study was designed such that each participating household would be sampled six times over a 1-year period. At the end of the study period, 47 households that had at least four visits were included for longitudinal analysis. Informed consent was obtained in person before the start of the study. This study was approved by the University of Minnesota Research Subjects' Protection Program Institutional Review Board: Human Subjects Committee.

## Study Design

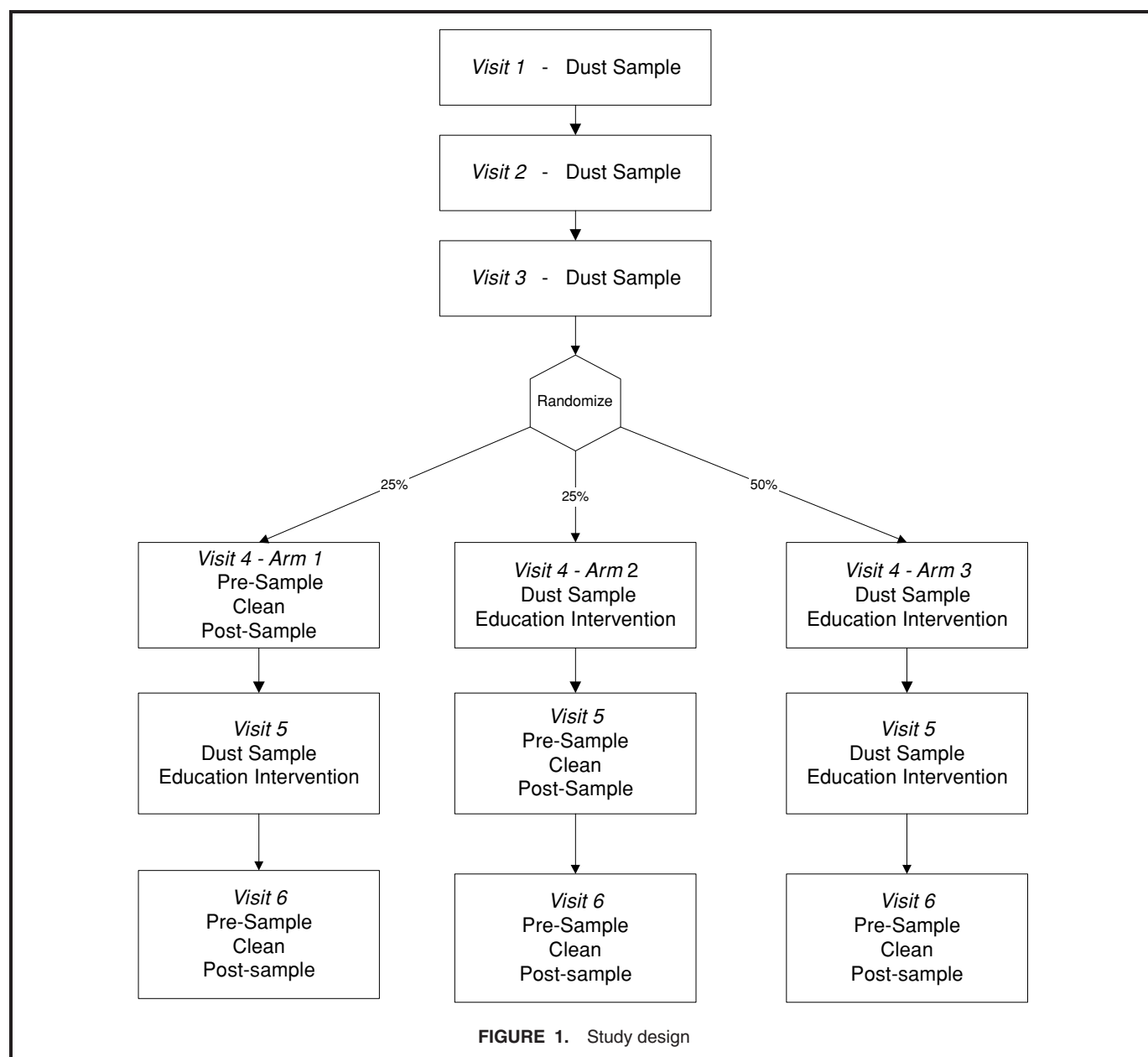
This was a longitudinal allergen exposure assessment study designed to evaluate the effect of two interventions—cleaning and education—on reducing the levels of allergens and to characterize the variability of these agents in residential households as shown in Figure 1. While the effectiveness of the two intervention strategies is reported in a separate article,<sup>(36)</sup> this article focuses on longitudinal variability in allergen levels. After completion of the first three visits, households were assigned to three different intervention groups—Arm 1, Arm 2, and Arm 3—based on allergen concentrations through Visit 3. In Arm 1, the cleaning intervention was carried out at Visits 4 and 6 and an education intervention at Visit 5. In Arm 2, an education intervention was completed at Visit 4, and cleaning interventions at Visits 5 and 6. In Arm 3, the education intervention was carried out at the Visits 4 and 5 with a cleaning intervention at Visit 6. To control for the effect of the two types of intervention on allergen concentrations in longitudinal analysis, each visit was classified into four classes depending on the type of intervention at the previous visits: no prior intervention, prior cleaning, prior education, and prior cleaning and education.

## Dust Collection

Dust samples for allergen measurement were collected six times from households between June 2004 and July 2005. To ensure that the same rooms and locations were sampled in subsequent visits, maps were drawn of the general household layout with more detail provided for rooms where samples were obtained indicating the exact sample locations. There was a prescribed order of priority for sampling locations for each allergen. Fungal samples were collected from the living room floor or upholstered furniture. Dust mite allergen samples were collected from bedroom floors, living room floors, or upholstered furniture. Cat and cockroach allergen samples were collected from kitchen floors, dining room floors, living room floors, or upholstered furniture.

Dust samples were collected using a microvacuum sampler (Gilian AirCon 520AC Air Sampling Pump; Gilian Instrument Corp., Wayne, N.J.) that has a sampling flow rate of 15 L/min. The dust was collected on 0.8  $\mu$ m pore size polycarbonate filters (model 738 PC; Zefon Analytical Accessories, St. Petersburg, Fla.) that were placed in 37-mm filter cassettes. The samplers were calibrated against a precision rotameter (Gilian Bubble Generator model 800285; Gilian Instrument Corp.) that had a range of 2–30 L/min and a precision of  $\pm 0.5\%$ .

A minimum of three nonadjacent square feet was selected for sampling. Each square foot was sampled using a 1 ft<sup>2</sup> (0.098 m<sup>2</sup>) template at least four times: vertically, horizontally, diagonally, and with a circular motion. Dust collection continued until the mass of collected dust was at least  $\sim 100$  mg. Filter cassettes were stored at  $\sim 40^\circ\text{F}$  refrigeration for up to 3 weeks before shipping to laboratory for analysis. Temperature and relative humidity were measured at the surface of two rooms, the sample bedroom floor and the living room floor,



using a Q-TRAK indoor air quality monitor (TSI, Shoreview, Minn.).

### Questionnaires and Checklists

A questionnaire about sociodemographic information and household characteristics (translated into Somali and Spanish for those households) was administered by a trained bilingual interviewer at the first and sixth visits, and a home assessment checklist was conducted at every home visit. Five residential characteristics derived from the questionnaire were used for further analysis (Table I). A housing disrepair index on a scale of 0 to 5 was created by summing the frequency of observation of five measures of disrepair found in the home assessment checklist: visible mold, leaking pipes/faucets, cracks/holes in walls, peeling paint, and visible floor wear.

### Laboratory Analyses

Dust samples sent to the laboratory were sieved through a 355- $\mu$ m pore mesh and divided into 100-mg aliquots of dust. These aliquots were extracted in 2.0 mL PBS-T (0.05% Tween 20 in phosphate-buffered saline, pH 7.4) and clarified by centrifugation. Supernatants for allergen analyses were decanted and stored at  $-30^{\circ}\text{C}$ . Concentrations of the allergens such as *Dermatophagoides farinae* (Der f 1), *D. pteronyssinus* (Der p 1), *Felis domesticus* (Fel d 1), and *Blattella germanica* (Bla g 1) were measured separately with monoclonal-antibody-based enzyme-linked immunosorbent assays (ELISAs), which were commercially available from Indoor Biotechnologies, Charlottesville, Va.<sup>(10,37,38)</sup> Fungal samples were plated on MEA and incubated at  $25^{\circ}\text{C}$ , and then fungal colonies were counted according to analytical procedures for culturable fungi

**TABLE I. Residential Characteristics of Study Subjects (N = 47)**

|                            | No. of Subjects (%) |
|----------------------------|---------------------|
| Race/Ethnicity             |                     |
| Somali immigrant           | 13 (27.6)           |
| Hispanic                   | 15 (31.9)           |
| White                      | 10 (21.3)           |
| Other <sup>A</sup>         | 9 (19.2)            |
| Language                   |                     |
| English speaking           | 19 (40.4)           |
| Somali speaking            | 13 (27.7)           |
| Spanish speaking           | 15 (31.9)           |
| Family income <sup>B</sup> |                     |
| <\$20,000                  | 19 (40.4)           |
| ≥\$20,000                  | 24 (51.1)           |
| Housing type               |                     |
| House                      | 21 (44.7)           |
| Apartment                  | 26 (55.3)           |
| Presence of cat            |                     |
| Yes                        | 9 (19.1)            |
| No                         | 38 (80.9)           |

<sup>A</sup> American Indian, Asian, Black.<sup>B</sup> Four subjects did not report household income.

at the laboratory. The results were reported in micrograms of allergen per gram of dust for Der p 1, Der f 1, and Fel d 1, in units per gram for Bla g 1, and in colony forming units (CFU) per gram for culturable fungi.

Reference dust samples obtained from the DACI laboratory (Johns Hopkins University, Baltimore, Md.) were used for quality control of laboratory performance and consisted of two dust batches. The reference values and coefficients of variation (CV) showed that Batch I contained high levels of Der f 1 (3.46 µg/g, CV = 26%), Der p 1 (46.7 µg/g, CV = 21%), and Fel d 1 (1,700 µg/g, CV = 21%) and low levels of Bla g 1 (<0.4 U/g), while Batch II contained high levels of Bla g 1 (22.8 U/g, CV = 73%) and low levels of Der f 1 (0.46 µg/g, CV = 30%), Der p 1 (0.17 µg/g, CV = 18%), and Fel d 1 (45.9 µg/g, CV = 19%). Thus, the reference lab values themselves had substantial CVs. A total of 40 reference dust samples (20 samples from each batch) were analyzed over a 1-year period of study.

The measurements by the laboratory used in this study showed that the arithmetic mean (%CV, % Recovery) of Der f 1 in Batch I was 3.88 µg/g (27%, 112%), and the majority of analytical results were within ±1 SD of the reference mean. The mean of Der p 1 in Batch I was 26.04 µg/g (23%, 56%) and the results were consistently between -1 SD and -2 SD of the reference mean. For Bla g 1, the mean of measured levels from Batch II was 10.88 U/g (48%, 48%) and the analytical results showed consistency within -1 SD of the reference mean. For Fel d 1, the means of measured levels from two Batches (I and II) were 1,715 µg/g (37%, 101%) and 44.28 µg/g (48%, 96%), respectively.

In general, laboratory performance fluctuated within ±2 SD of the reference means. There was a consistent underestimation of dust mite and cockroach allergen levels relative to reference concentrations during the study, but this underestimation is based on a comparison of the analysis of reference samples made by the authors' lab and the reference lab. The reference values were much higher than the values observed in the homes in the present study, and it is not clear if this underestimation will also hold for the lower values observed in this study.

### Statistical Analysis and Related Considerations

Concentrations of allergens were right-skewed and were transformed by natural logarithm for the analysis. For dust mite and cockroach allergens, more than 70% of dust samples were nondetectable. For dust samples with allergen concentrations below analytical detection limits, concentration values were imputed using half the method quantitation limits (MQL), which were obtained from laboratory analyses. In statistical analysis, allergen and fungal concentrations, indoor temperature, and relative humidity were treated as continuous variables. Family income, presence of cats, housing type, language, and season were coded as categorical variables for the purpose of modeling.

Participants were categorized into three ethnic groups based on the languages they spoke in their homes: English, Somali, or Spanish-speaking (Table I). Sampling periods were categorized into four seasons corresponding to climate conditions in Minneapolis (average outdoor temperature: 54°F, 70°F, 37°F, and 19°F for spring (March 20 to June 20), summer (June 21 to September 22), fall (September 23 to December 21), and winter (December 22 to March 19), respectively).

To examine whether residential characteristics such as language, family income, presence of cat, housing type, temperature, and relative humidity are associated with allergen concentrations, mixed-effects models with a random home effect and the other variables being modeled as fixed effects were fit to log-transformed allergen concentrations while considering the correlation between dust samples at different time points within the same household. Each model to estimate effects of covariates was adjusted for confounding variables. Mixed-effects models with a random home effect and fixed effects for season and intervention type were also used to characterize the seasonal variation of allergen concentrations.

Within- and between-home variability of allergen concentrations were also estimated using mixed-effect models, adjusted for season and intervention type. The ratio of the between-home variance to the sum of both variance components was used to estimate the correlation between repeated measurements, after adjusting for season and intervention type. As validation of the procedures of imputation for values below the detection limit, model sensitivity analyses were carried out for four cases. Concentrations below detection limits were assigned a value equal to 100% of MQL, 10% of MQL, 1% of MQL, and the half of values assigned to 100% of MQL and the other half of values to 1% of MQL. All statistical analyses

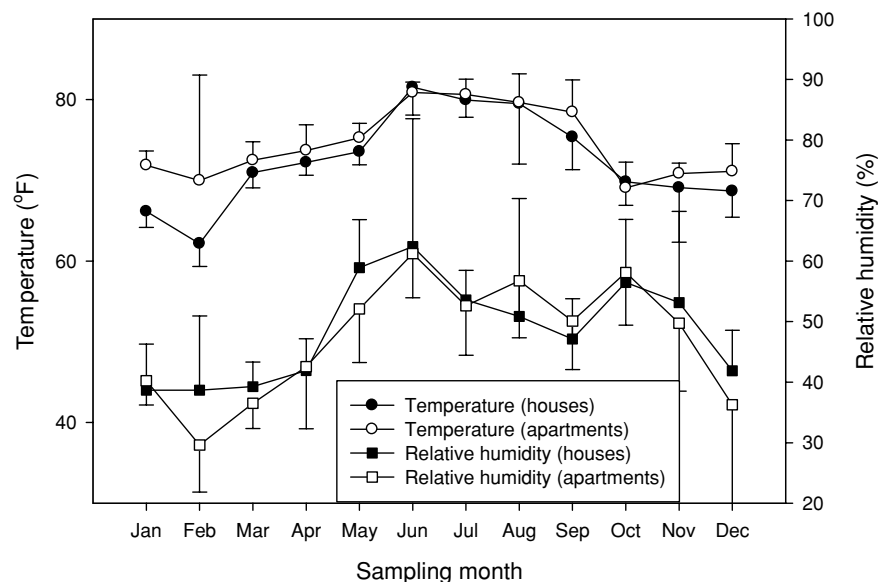


**FIGURE 2.** Indoor temperature and relative humidity level measurements from the bedroom floors in homes

were performed using SAS Version 9.1 (SAS Institute, Cary, N.C.).

## RESULTS

### Study Population and Allergen Distributions

Dust samples, indoor temperature, and relative humidity were collected from 47 residential households up to six times over a 1-year period in a diverse population of low-income

families. The population was predominantly non-English-speaking, a majority lived in apartments, and 40% reported a family income of less than \$20,000 per year (Table I). Temperature and relative humidity from bedroom floors in houses were not significantly different from those in apartments and the bedroom mirrored the living room values (Figure 2).

Table II presents the distributions of allergen concentrations expressed as concentrations (per g dust) as well as loading (per m<sup>2</sup>). Less than 30% of dust samples for total dust mite

**TABLE II.** Distribution of Allergen and Fungal Concentrations in Settled Dust (N = 262)

|                                    | Percent > LOD <sup>A</sup> | Quantile |       |        |        |        |         |
|------------------------------------|----------------------------|----------|-------|--------|--------|--------|---------|
|                                    |                            | Minimum  | 25th  | Median | 75th   | 90th   | Maximum |
| Per g dust                         |                            |          |       |        |        |        |         |
| Der f 1+Der p 1 (μg) <sup>B</sup>  | 25.6                       | <LOD     | <LOD  | <LOD   | 0.37   | 2.60   | 26.0    |
| Fel d 1 (μg) <sup>C</sup>          | 60.3                       | <LOD     | <LOD  | 0.54   | 4.0    | 125.2  | 2700.0  |
| Cat homes                          | 96.3                       | <LOD     | 40.0  | 120.1  | 440.0  | 810.0  | 2700.0  |
| No cat                             | 51.0                       | <LOD     | <LOD  | 0.11   | 1.2    | 3.3    | 110.0   |
| Bla g 1 (U)                        | 27.1                       | <LOD     | <LOD  | <LOD   | 2.0    | 42.0   | 130.0   |
| Fungi (10 <sup>3</sup> × CFU)      | 100.0                      | 2.58     | 40.01 | 13,531 | 37,111 | 73,863 | 237,500 |
| Per m <sup>2</sup> sampled surface |                            |          |       |        |        |        |         |
| Der f 1+Der p 1 (μg)               |                            | <LOD     | <LOD  | <LOD   | 0.02   | 0.77   | 10.71   |
| Fel d 1 (μg)                       |                            | <LOD     | <LOD  | 0.12   | 1.95   | 63.44  | 532.70  |
| Cat homes                          |                            | <LOD     | 8.13  | 49.54  | 152.93 | 391.63 | 532.70  |
| No cat                             |                            | <LOD     | <LOD  | 0.02   | 0.63   | 1.92   | 229.21  |
| Bla g 1 (U)                        |                            | <LOD     | <LOD  | <LOD   | 0.55   | 4.49   | 323.24  |
| Fungi (10 <sup>3</sup> × CFU)      |                            | 0.97     | 13.3  | 71.5   | 183.7  | 406.7  | 20,998  |

<sup>A</sup>Limit of detection.

<sup>B</sup>Total dust samples = 254.

<sup>C</sup>Total dust samples from cat and no cat homes: 54 vs. 208.

**TABLE III. Seasonal Differences of Allergen and Fungal Concentrations in Settled Dust**

|                                     | Geometric Mean Ratio <sup>A</sup> |        |        |        | Overall p-value |
|-------------------------------------|-----------------------------------|--------|--------|--------|-----------------|
|                                     | Fall                              | Winter | Spring | Summer |                 |
| Per g dust                          |                                   |        |        |        |                 |
| Der f 1 + Der p 1 ( $\mu\text{g}$ ) | 0.91                              | 0.6*   | 0.58*  | 1      | 0.01            |
| Fel d 1 ( $\mu\text{g}$ )           | 1.36                              | 0.91   | 0.72   | 1      | 0.16            |
| Bla g 1 (U)                         | 1.15                              | 1.14   | 1.36   | 1      | 0.38            |
| Fungi (CFU)                         | 1.14                              | 0.46*  | 1.87*  | 1      | <0.001          |
| Per m <sup>2</sup> sampled surface  |                                   |        |        |        |                 |
| Der f 1 + Der p 1 ( $\mu\text{g}$ ) | 1.05                              | 0.83   | 0.62   | 1      | 0.58            |
| Fel d 1 ( $\mu\text{g}$ )           | 1.38                              | 0.86   | 0.69   | 1      | 0.27            |
| Bla g 1 (U)                         | 1.51                              | 1.35   | 1.44   | 1      | 0.32            |
| Fungi (CFU)                         | 1.14                              | 0.61   | 1.64   | 1      | 0.02            |

<sup>A</sup>Antilog of least squares mean using mixed-effects models with a random home effect and a fixed effect of season, after adjusting for intervention, compared to reference group (summer).

\*p < 0.05.

(Der f 1 + Der p 1) and cockroach (Bla g 1) allergens were detectable. Statistical analysis for these allergens was carried out after imputing the missing values. Cat allergen (Fel d 1) was detectable in 96% of dust samples from households with cats and 51% of households with no cats. Total culturable fungi were detectable in all dust samples.

### SEASONAL VARIATION OF ALLERGENS: DUST MITE, CAT, COCKROACH, AND FUNGI

Dust mite allergen concentrations were highest (geometric mean, GM: 0.43  $\mu\text{g/g}$ ) in dust samples at Visit 1 (June to September 2004) and lowest (GM: 0.02  $\mu\text{g/g}$ ) at Visit 4 (February to March 2005). After accounting for within-home correlation and adjusting for the effect of intervention, a significant seasonal effect was observed for dust mite allergen, which was lower in winter and spring compared with summer (p < 0.05) (Table III). Cat allergen concentrations showed a bimodal distribution depending on the presence of cats in households. Unadjusted for correlation within homes, cat allergen concentrations were highest (GM: 2.38  $\mu\text{g/g}$ ) in dust samples at Visit 3 (November 2004 to January 2005) and lowest (GM: 0.34  $\mu\text{g/g}$ ) at Visit 5 (April to May 2005); however, seasonal variation was not significant in mixed-effect models after adjusting for intervention.

The concentrations of cockroach allergen were highest (GM: 2.1 U/g) in dust samples at Visit 3 and lowest (GM: 1.42 U/g) at Visit 5; however, seasonal variation was not significant in mixed-effect models after adjusting for intervention. The concentrations of fungi peaked (GM: 193,588 CFU/g) in dust samples of Visit 2 (September to November 2004) and was lowest (GM: 89,505 CFU/g) at Visit 3 (Figure 3); in addition, seasonal variation was significant in mixed-effects models, which were higher in spring and lower in winter compared with summer (p < 0.05). In sensitivity analyses, dust mite

allergen and fungal concentrations showed seasonal variation but cat and cockroach allergens did not.

### Within- and Between-Home Variability of Allergen Concentrations

Table IV shows within- and between-home variance, variance ratios, and the within-home correlation for allergens expressed as concentrations (per g dust) and loading (per m<sup>2</sup>). These were estimated using mixed-effects models with a random home effect and a fixed season effect. The models were further adjusted for the effect of intervention (no prior intervention, prior cleaning, prior education, and prior cleaning and education) because households were assigned to three different intervention groups as shown in Figure 1. For total culturable fungi, season-adjusted within-home variance components were larger than between-home variance

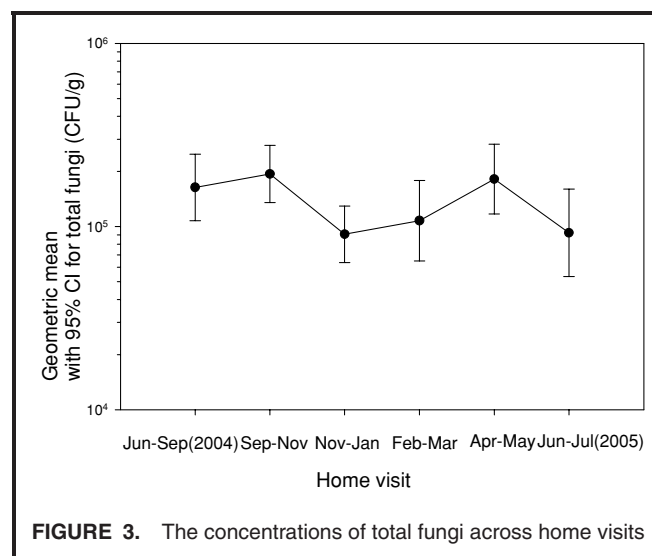


**TABLE IV. Within-Home ( $\sigma_w^2$ ) and Between-Home Variance ( $\sigma_b^2$ ) Components of Indoor Allergens and Total Fungi**

|                                    | $\sigma_w^2$ | $\sigma_b^2$ | Variance Ratio ( $\sigma_w^2/\sigma_b^2$ ) | Correlation Over Time <sup>A</sup> |
|------------------------------------|--------------|--------------|--------------------------------------------|------------------------------------|
| Per g dust                         |              |              |                                            |                                    |
| Der f 1+Der p 1 ( $\mu\text{g}$ )  | 1.13 [1.12]  | 0.59 [0.60]  | 1.91 [1.85]                                | 0.34 [0.35]                        |
| Fel d 1 ( $\mu\text{g}$ )          | 1.43 [1.68]  | 5.90 [5.70]  | 0.24 [0.30]                                | 0.81 [0.77]                        |
| Bla g 1 (U)                        | 0.75 [0.76]  | 1.36 [1.35]  | 0.55 [0.57]                                | 0.64 [0.64]                        |
| Fungi (CFU)                        | 1.47 [1.49]  | 0.58 [0.56]  | 2.54 [2.65]                                | 0.28 [0.27]                        |
| Per m <sup>2</sup> sampled surface |              |              |                                            |                                    |
| Der f 1+Der p 1 ( $\mu\text{g}$ )  | 3.42 [3.67]  | 1.75 [1.85]  | 1.96 [1.99]                                | 0.34 [0.34]                        |
| Fel d 1 ( $\mu\text{g}$ )          | 2.38 [2.78]  | 5.67 [5.48]  | 0.42 [0.50]                                | 0.70 [0.67]                        |
| Bla g 1 (U)                        | 1.75 [1.77]  | 1.10 [1.12]  | 1.58 [1.58]                                | 0.39 [0.39]                        |
| Fungi (CFU)                        | 2.00 [2.01]  | 0.75 [0.79]  | 2.68 [2.58]                                | 0.27 [0.28]                        |

Note: Values in brackets before adjusting for the effect of intervention.

<sup>A</sup>Ratio of the between-home variance to the sum of within- and between-home variance estimated using a mixed-effects model with a random home effect and fixed effects of intervention (no prior intervention, prior cleaning, prior education, and prior cleaning and education) and season: spring (March 20–June 20); summer (June 21–September 22); fall (September 23–December 21); winter (December 22–March 19).

components. Repeated measurements of these agents over time were weakly correlated (below 0.5). Taken together, this suggests that repeated measurements of total culturable fungi over time are needed for more accurate long-term exposure assessment. For cat allergen, season-adjusted within-home variability was smaller than between-home variability, and repeated measurements of this agent over time were highly correlated.

Variance ratios for allergen loading were relatively inflated when compared with those for allergen concentrations; in particular, variance ratios of cat and cockroach allergens increased markedly due to an increase of within-home variance component. This may be driven by the variability in floor dust loading (mass of dust per unit area) from one location to another within each home. Nonetheless, season-adjusted within-home variance of cat allergen loading was still smaller than between-home variance.

As described in the next section, the total variability of cat allergen over time was driven primarily by residential characteristics, such as language and presence of cats and mostly contributed to between-home variability. Language and family income, however, only slightly affected the total variability of cockroach allergen. This may explain partly why the variance ratio of cat allergen loading is still low.

In addition, after further adjustments for intervention, within-home correlations were not improved compared with those before adjustment, except for cat allergen. A sensitivity analysis was conducted to explore the effect of the percentage of samples above the detection limit on within-home correlations for dust mite, cockroach, and cat allergens were 0.21–0.54, 0.63–0.65, and 0.73–0.81, respectively. However, when the half of values below the detection limit were assigned to 100% of MQL and the other half of values to 1% of MQL, sensitivity analyses for these allergens resulted in lower correlations of 0.15, 0.31, and 0.42, respectively compared with 0.34, 0.64, and 0.81 as given in Table IV.

### Residential Characteristics Associated with Allergen Concentrations

Allergen concentrations were fit using mixed-effect models that take into account a random home effect and correlation within homes. English-speaking households had higher cat allergen concentrations than Spanish-speaking households, while cockroach allergen concentrations were higher in Spanish- and Somali-speaking households than in English-speaking households. Overall, relative humidity showed an inverse relation to allergen loading, and indoor temperature was not associated with observed allergens expressed as concentrations or loading after adjustment for language, family income, housing type, temperature, and season. In addition, no considerable bias was found in sensitivity analyses compared with those in Tables V–VII.

### Dust Mite Allergens

When dust mite allergens were expressed as concentrations ( $\mu\text{g/g}$  dust) or loading ( $\mu\text{g/m}^2$ ), race/ethnicity was not significantly associated with between-home differences in dust mite allergens ( $p > 0.05$ ) (Table V). Dust mite allergen concentrations were 3.7 (2.7 based on allergen loading) times higher in houses than apartments, adjusted for language, family income, and the presence of cats ( $p < 0.05$ ). Dust mite allergen concentrations were 2.1 (10.5 based on allergen loading) times lower in households with cats compared with no-cat households, adjusted for language, family income, and housing type ( $p < 0.05$ ). When dust mite allergens were expressed as allergen loading ( $\mu\text{g/m}^2$ ), relative humidity was inversely associated with dust mite allergen loading, and there was no significant association with temperature.

### Cat Allergen

All households with cats were English speaking. As a consequence, when cat allergen was expressed as concentrations ( $\mu\text{g/g}$  dust) or allergen loading ( $\mu\text{g/m}^2$ ), English-speaking

**TABLE V. Predictors of Dust Mite Allergen (N = 254)**

| Predictor <sup>A</sup>   | Ratios of Estimates (95% CI) <sup>B</sup> |                                         |
|--------------------------|-------------------------------------------|-----------------------------------------|
|                          | (Based on $\mu\text{g/g}$ of Dust)        | (Based on $\mu\text{g/m}^2$ of Surface) |
| Language                 |                                           |                                         |
| English speaking         | 0.81 (0.44–1.5)                           | 1.6 (0.53–4.81)                         |
| Somali speaking          | 0.6 (0.3–1.18)                            | 1.5 (0.44–5.08)                         |
| Spanish speaking         | Reference                                 |                                         |
| Income                   |                                           |                                         |
| <\$20,000                | 0.75 (0.37–1.55)                          | 1.52 (0.44–5.24)                        |
| ≥\$20,000                | Reference                                 |                                         |
| Housing type             |                                           |                                         |
| House                    | 3.65 (1.95–6.83)*                         | 2.71 (0.88–8.39)                        |
| Apartment                | Reference                                 |                                         |
| Cat in home              |                                           |                                         |
| No                       | 2.09 (0.99–4.42)*                         | 10.49 (2.73–40.36)*                     |
| Yes                      | Reference                                 |                                         |
| Temperature per 1°F      | 1.01 (0.98–1.05)                          | 0.98 (0.92–1.04)                        |
| Relative humidity per 1% | 1 (0.99–1.01)                             | 0.97 (0.94–0.99)*                       |

<sup>A</sup>Each mixed-effects model was adjusted for the following confounding variables: income controlled for language; housing type controlled for language, income, and presence of cat; presence of cat controlled for language, income, and housing type; temperature controlled for language, income, housing type, presence of cat, and season; relative humidity controlled for language, income, housing type, presence of cat, temperature, and season.

<sup>B</sup>Values back-transformed to original scale.

\* $p \leq 0.05$ .

households had 15 (21.4 based on allergen loading) times higher cat allergen than Spanish-speaking households ( $p < 0.05$ ) (Table VI). Family income and housing type were not significantly associated with cat allergen, after adjusted for confounders such as language and the presence of cats. Cat allergen concentrations were 72.8 (36.7 based on allergen loading) times higher in households with cats compared with no-cat households, adjusted for language, family income, and housing type ( $p < 0.05$ ).

### Cockroach Allergen

Spanish-speaking and Somali-speaking households had higher cockroach allergen concentrations compared with English-speaking homes ( $p < 0.05$ ) (Table VII). Cockroach allergen was 2.2 times higher in households with family income of <\$20,000 compared with households  $\geq$  \$20,000, adjusted for language ( $p < 0.05$ ). Although the majority of low income families lived in apartments and high income families lived in houses, housing type was not associated with cockroach allergen concentrations. In addition, there was no significant association between a housing disrepair index (on a scale of 0 to 5) and cockroach allergen concentrations in floor dust (data not shown). This suggests that there may be other residential characteristics that explain the effect of family income on cockroach allergen concentrations.

**TABLE VI. Predictors of Cat Allergen**

| Predictor <sup>B</sup>   | Ratios of Estimates (95% CI) <sup>A</sup> |                                         |
|--------------------------|-------------------------------------------|-----------------------------------------|
|                          | (Based on $\mu\text{g/g}$ of Dust)        | (Based on $\mu\text{g/m}^2$ of Surface) |
| Language                 |                                           |                                         |
| English speaking         | 14.96 (4.26–52.66)*                       | 21.39 (6.3–72.58)*                      |
| Somali speaking          | 0.28 (0.07–1.13)                          | 0.32 (0.1–1.47)                         |
| Spanish speaking         | Reference                                 |                                         |
| Income                   |                                           |                                         |
| < \$20,000               | 0.38 (0.09–1.58)                          | 0.38 (0.1–1.51)                         |
| ≥\$20,000                | Reference                                 |                                         |
| Housing type             |                                           |                                         |
| House                    | 1.75 (0.83–3.7)                           | 2.05 (0.87–4.82)                        |
| Apartment                | Reference                                 |                                         |
| Cat in home              |                                           |                                         |
| Yes                      | 72.8 (30.5–170.82)*                       | 36.66 (13.37–100.53)*                   |
| No                       | Reference                                 |                                         |
| Temperature per 1°F      | 1.0 (0.96–1.03)                           | 1 (0.95–1.04)                           |
| Relative humidity per 1% | 0.98 (0.97–1)*                            | 0.97 (0.95–0.99)*                       |

Note: Total samples = 262.

<sup>A</sup>Values back-transformed to original scale.

<sup>B</sup>Each mixed-effects model was adjusted for the following confounding variables: income controlled for language; housing type controlled by language, income, and presence of cat; presence of cat controlled for language, income, and housing type; temperature controlled for language, income, housing type, presence of cat, and season; relative humidity controlled for language, income, housing type, presence of cat, temperature, and season.

\* $p < 0.05$ .

### Fungi

When total culturable fungi were expressed as concentrations (CFU/g dust), there was no significant difference of fungal concentrations between English-speaking households and Spanish-speaking households. However, English-speaking households had 2 times higher fungal loading (CFU/m<sup>2</sup>) than Spanish-speaking households ( $p < 0.05$ ) (Table VIII). Somali-speaking households had similar fungal loading to Spanish-speaking households. Family income and housing type were not associated with total culturable fungi expressed as concentrations or loading.

### DISCUSSION

This study found that total culturable fungi showed seasonal variation and within-home variance of this agent was much larger than between-home variance. There was no significant seasonal variation of cat allergen, and within-home variance was smaller than between-home variance. These results imply that a single, one-time measurement for total culturable fungi does not provide precise information on the true long-term exposure, and that a single measurement is likely a good proxy for a long-term exposure to cat allergen. The large percentage of nondetects for dust mite and cockroach

**TABLE VII. Predictors of Cockroach Allergen**

| Predictor <sup>B</sup>   | Ratios of Estimates (95% CI) <sup>A</sup> |                                         |
|--------------------------|-------------------------------------------|-----------------------------------------|
|                          | (Based on $\mu\text{g/g}$ of Dust)        | (Based on $\mu\text{g/m}^2$ of Surface) |
| Language                 |                                           |                                         |
| Spanish speaking         | 5.57 (2.83–10.96)*                        | 5.61 (2.85–11.05)*                      |
| Somali speaking          | 2.25 (1.1–4.56)*                          | 2.25 (1.11–4.59)*                       |
| English speaking         | Reference                                 |                                         |
| Income                   |                                           |                                         |
| < \$20,000               | 2.22 (1.1–4.48)*                          | 1.74 (0.79–3.86)                        |
| ≥\$20,000                | Reference                                 |                                         |
| Housing type             |                                           |                                         |
| House                    | 1.14 (0.58–2.25)                          | 1.38 (0.65–2.96)                        |
| Apartment                | Reference                                 |                                         |
| Temperature per 1°F      | 1.02 (0.99–1.05)                          | 1.02 (0.98–1.06)                        |
| Relative humidity per 1% | 1 (0.99–1.01)                             | 0.98 (0.97–0.99)*                       |

Note: Total samples = 262.

<sup>A</sup> Values back-transformed to original scale.

<sup>B</sup> Each mixed-effects model was adjusted for the following confounding variables: income controlled for language; housing type controlled for language and income; temperature controlled for language, income, housing type, and season; relative humidity controlled for language, income, housing type, temperature, and season.

\* $p < 0.05$ .

**TABLE VIII. Predictors of Total Fungi (N = 262)**

| Predictor <sup>B</sup>   | Ratios of Estimates (95% CI) <sup>A</sup> |                                         |
|--------------------------|-------------------------------------------|-----------------------------------------|
|                          | (Based on $\mu\text{g/g}$ of Dust)        | (Based on $\mu\text{g/m}^2$ of Surface) |
| Language                 |                                           |                                         |
| English speaking         | 1.24 (0.67–2.29)                          | 2.08 (1.02–4.25)*                       |
| Somali speaking          | 0.88 (0.44–1.74)                          | 1.3 (0.59–2.86)                         |
| Spanish speaking         | Reference                                 |                                         |
| Income                   |                                           |                                         |
| < \$20,000               | 0.92 (0.47–1.79)                          | 1 (0.46–2.18)                           |
| ≥\$20,000                | Reference                                 |                                         |
| Housing type             |                                           |                                         |
| House                    | 1.43 (0.76–2.68)                          | 1.26 (0.6–2.66)                         |
| Apartment                | Reference                                 |                                         |
| Temperature per 1°F      | 1 (0.97–1.04)                             | 1 (0.96–1.04)                           |
| Relative humidity per 1% | 0.99 (0.98–1)                             | 0.98 (0.96–0.99)*                       |

Note: Total samples N = 262).

<sup>A</sup> Values back-transformed to original scale.

<sup>B</sup> Each mixed-effects model was adjusted for the following confounding variables: income controlled for language; housing type controlled for language and income; temperature controlled for language, income, housing type, and season; relative humidity controlled for language, income, housing type, temperature, and season.

\* $p < 0.05$ .

allergens precludes definitive recommendations for the number of repeated measurements for these allergens.

### Seasonal Variation of Allergens: Dust Mite, Cat, Cockroach, and Fungi

Seasonal variation of dust mite allergen concentrations was observed in households and it may be closely related to conditions that promote the growth of dust mites.<sup>(16,39,40)</sup> This study shows that mean indoor temperature and mean indoor relative humidity (RH) of study households are lowest (63°F and 34%, respectively) in February and highest (82°F and 59%, respectively) in June. Seasonal variation of dust mite allergen (Der f 1 or Der p 1) was also observed in other studies.<sup>(22–24,26)</sup> However, housing type outweighed the seasonal effect on dust mite concentrations, which was consistent with one previous study.<sup>(24)</sup> As for cat and cockroach allergen concentrations in house dust, seasonal differences are negligible compared with residential characteristics such as race/ethnicity, family income, and the presence of cats. These results are different from other study results that showed significant seasonal effects of cat and cockroach allergen concentrations.<sup>(23,24,41)</sup> Fungal concentrations showed seasonal variation consistent with other studies.<sup>(23,41)</sup>

### Implications of Within- and Between-Home Variability of Cat Allergen and Total Culturable Fungi over Time for Epidemiologic Study Design

Repeated measurements were highly correlated for cat allergen concentrations (0.81) but were weakly correlated for total fungi concentrations (0.28). Season-adjusted within-home variance for cat allergen was smaller than between-home variance but was larger than between-home variance for total culturable fungi.

Other studies have also analyzed variance components and correlations for cat allergen and total culturable fungi expressed as both concentrations (per g dust) and loading (per m<sup>2</sup>) using two repeated measurements of living room floor dust over a time period of several years.<sup>(23,31,32)</sup> The three studies showed that within-home variance for Fel d 1 was much smaller or no larger than between-home variance, and two repeated measurements of this agent based on concentrations or loading were moderately (0.49–0.52) or highly correlated (0.74–0.75). For total culturable fungi, however, correlations of two repeated measurements expressed as concentrations and loading were 0.15 and 0.42, respectively. The results of the present study and other studies suggest that factors affecting concentrations for cat allergen within a home over time influence the total variability less than residential factors affecting between-home variability, and the converse is true for total culturable fungi.

In addition, the variance components can be used to guide exposure assessment for epidemiologic studies that link exposure to response.<sup>(43)</sup> Under a classical error model, the bias between the true regression coefficient ( $\beta$ ) and the estimated regression coefficient of an imperfectly measured

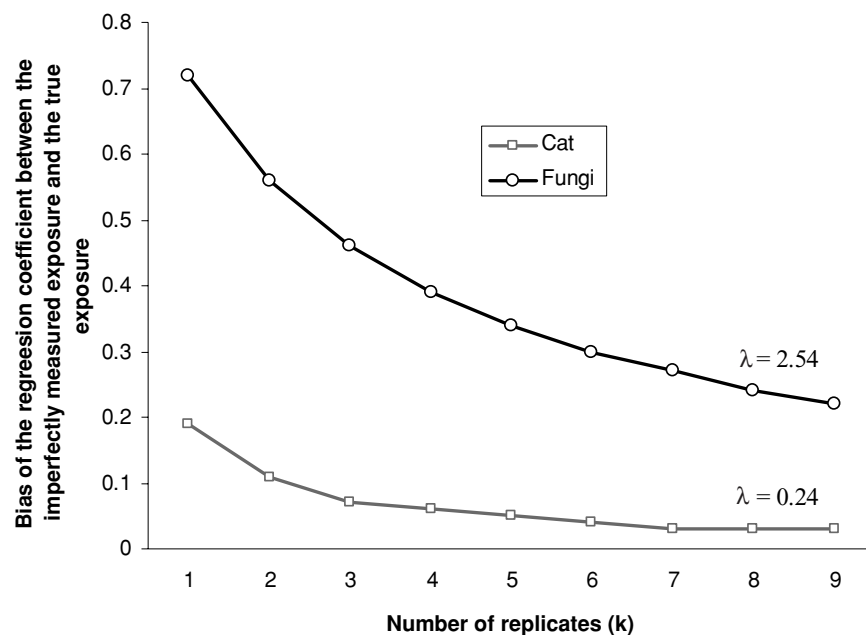


**FIGURE 4.** Illustration of the number of replicate dust samples required to reduce the bias depending on the variance ratio ( $\lambda$ ) of cat allergen and total fungi estimated using the mixed-effects models in this study

exposure variable ( $\beta^*$ ) relates to the ratio of within-group to between-group variance ( $\lambda$ ) and can be reduced by repeated measurements ( $k$ ) as indicated in the following empirical equation:<sup>(29,42)</sup>

$$\beta^* = \beta \left( 1 + \frac{\lambda}{k} \right)^{-1} \quad (1)$$

where  $\beta^*$  is the estimated regression coefficient of the imperfectly measured exposure in an exposure-response relationship;  $\beta$  is the true regression coefficient of the true exposure;  $\lambda$  is the ratio of within-group to between-group variance;  $k$  is the number of repeated measurements within groups. Equation 1 can be used to generate bias curves that can be useful in determining the number of repeated measurements needed to limit the amount of bias to some acceptable level (Figure 4). For example, given  $\lambda = 0.24$  for cat allergen in this study, a single measurement might be sufficient to have a bias less than 20%, while two repeated measurements might reduce the bias to  $\sim 10\%$ . In contrast, more than nine measurements of total culturable fungi will be needed to maintain a bias of less than 20%.

### Residential Characteristics Associated with Allergen Concentrations

Relative humidity, which was  $48 \pm 14\%$  RH (mean  $\pm$  SD) over a 1-year period of study, was inversely associated with allergens expressed as loading (per  $m^2$ ). The results of the present study are consistent with other studies that indicate that dust collection efficiency in carpets is more likely to increase at moderate RH levels (30–60% RH) than at extremely low

or high RH.<sup>(44,45)</sup> In this moderate range, neither electrostatic charge in carpets is strong nor is dust hygroscopic. Dust collection efficiency on a floor is determined as a ratio of dust weight recovered to dust weight deposited on the floor.

Even though the actual amount of dust deposited on floors is not known, the dust sampling scheme in the present study where dust was collected from at least three nonadjacent 1  $ft^2$  areas of the same sample locations on every home visit may have minimized the variability of dust loading in different sites. Therefore, total dust weight collected from the floors in this study can be used as a proxy for dust collection efficiency. Since allergen loading for the most part depended on the mass of dust collected from the floor, RH was more closely associated with allergen loading than with allergen concentrations (per g dust) in the present study.

Race/ethnicity was associated with cat and cockroach allergen concentrations. Nine of 19 English-speaking households had a cat and had higher cat allergen and lower cockroach concentrations compared with Spanish- and Somali-speaking households. After adjusting for the effect of language, households with family income  $< \$20,000$  had higher cockroach allergen than those  $\geq \$20,000$ . Other studies have had similar results, indicating that low family income and race/ethnicity are associated with high cockroach allergen and low dust mite and cat allergen concentrations in house dust samples.<sup>(1–3,18)</sup>

### Strengths and Limitations

The major strength of this study is that dust samples and indoor climate were taken up to six times over a 1-year period from a diverse population of 47 households. It

was possible to examine the seasonal variation of allergens and to characterize the residential characteristics associated with higher or lower allergen concentrations. The effect of primary household language and other sociodemographic characteristics on allergen concentrations in house dust over a 1-year period was estimated. The large number of repeated measurements for each allergen made it possible to study the within- and between-home variability to an extent not seen in previous studies and enabled the authors to draw conclusions and make recommendations regarding the number of repeated measurements needed in epidemiologic studies to limit the amount of bias to some acceptable level.

More than 70% of the dust samples showed levels of dust mite and cockroach allergen levels below detectable limits. While dust mite allergens may vary by geographic location and season,<sup>(17,19,22–27,46–49)</sup> the large fraction of nondetects may explain why the authors did not observe high, season-adjusted within-home correlations for Der f 1 and Der p 1 observed in other studies.<sup>(23,30,32)</sup> The authors imputed missing values with half the MQL, thereby reducing between- and within-home variability. They performed a sensitivity analysis using 100% MQL, 10% MQL, 1% MQL and a combination of 100% MQL and 1% MQL for longitudinal analyses. There was no substantial bias due to the imputation method on the effects of residential characteristics on allergen concentrations over time. However, this does not preclude each of these assumptions leading to similar reductions in estimated variances; therefore, results for dust mite and cockroach allergens must be treated with care.

## CONCLUSIONS

This study presents results of seasonal variability of dust mite, cat, and cockroach allergens, and fungal concentrations and identified residential characteristics associated with these agents in Minneapolis homes. The authors estimated the magnitude of within- and between-home variability in the levels of these agents. Total culturable fungi showed a significant seasonal effect that outweighed the effect of the residential characteristics and larger within-home variance compared to between-home variance.

While dust mite allergens similarly showed significant seasonal variation, a large fraction of these measurements were below limits of detection. Observed cockroach allergen concentrations were higher in Spanish- and Somali-speaking households than in English-speaking households, while English-speaking households had significantly higher cat allergen concentrations compared with the other language groups. There was no seasonal variation of cat and cockroach allergens and within-home variance of these agents was smaller than between-home variance.

An analysis of between- and within-home variability indicates that greater than nine repeated measurements of total culturable fungi are required for robustly estimating long-term exposure. A similar analysis indicates that a single measurement of cat allergen is sufficient for the same purpose.

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