

Antigenic Interrelationships Among Mite Allergens (*Blomia* and *Dermatophagoides* spp)

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Mite Allergens and Asthma

Dust mites of the class Arachnida are found in dwellings world-wide. In 1964, Voorhorst et al. discovered that mites of the genus *Dermatophagoides* (family Pyroglyphidae) are the major source of allergen in house dust (1). In vitro culture of *Dermatophagoides* spp and the subsequent development of mite extracts for skin testing have resulted in the recognition that sensitization to *Dermatophagoides* spp is a major risk factor for asthma (2). Immunochemical studies have identified and sequenced 10 groups of allergens from *Dermatophagoides* spp to date (3) and monoclonal antibodies (MAbs) have been produced to several of these, enabling accurate quantification of mite allergen exposure by enzyme-linked immunosorbant assay (ELISA) (4,5). Many epidemiological studies have used these techniques to measure sensitization and exposure to *Dermatophagoides* spp, resulting in the proposal of threshold levels of mite allergen exposure that carry a risk for sensitization and for exacerbations of asthma (2).

Other pyroglyphid mites, (i.e., *Euroglyphus maynei*) are less well-studied, as they grow poorly in culture, and standardized extracts for skin testing are not readily available, nor are MAbs for quantification of allergen exposure. The prevalence of such mites can only be mea-

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Table 1
Taxonomy of the genus *Blomia* (6,7)

Species	Description
<i>Blomia freemani</i>	Hughes, 1948
<i>Blomia khaliovae</i>	Zachvatkin, 1949
<i>Blomia kulagini</i> ^a	Zachvatkin, 1936
<i>Blomia thori</i>	Zachvatkin, 1936
<i>Blomia tjibodas</i> ^a	Oudesman, 1910
<i>Blomia tropicalis</i> ^a	Bronswijk, 1974

^aFound in house dust.

sured by mite counting; consequently their allergenicity and role in the etiology of asthma is not well-defined.

Evidence supporting the role of pyroglyphid mites in the etiology of asthma has been reviewed elsewhere (2). We propose to review the role of storage mites in occupational and nonoccupational asthma, and use the mites *Blomia tropicalis* and *Dermatophagoides pteronyssinus* to illustrate the techniques utilized to explore the antigenic relationships between mite species. These have important implications for allergy diagnosis and therapeutics and also for epidemiological studies on the role of mite allergens in the etiology of asthma.

Phylogenetic Relationships of Mite Species

Mites form part of the class Arachnida and as such are phylogenetically more closely linked to spiders than to insects. They belong to the astigmata group of the subclass Acari. The group astigmata comprises 69 families of mites. Those found in house dust include the Pyroglyphidae (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Euroglyphus maynei*), which have adapted to live in human houses and feed from human skin scales (house dust mites); Glycyphagidae (*Glycyphagus domesticus*, *Glycyphagus destructor*, *Lepidoglyphus destructor*, and *Blomia tropicalis*); Acaridae (*Acarus siro*, *Tyrophagus putrescentiae*); and Chortoglyphidae (*Chortoglyphus* spp), which have all adapted to feed on stored grains and grain products (storage mites). Of the six species assigned to the genus *Blomia* (Table 1), only three have been found in house dust (6,7); of these, *Blomia tropicalis* has been studied in most detail.

Barn Allergy

Storage mites of the families Glycyphagidae and Acaridae feed on grain and flour and are found in stables, barns, and mills. In 1925, Storm van Leeuwen recognized that the frequent and severe asthma suffered by Dutch farmers was as a result of allergy to storage mites (8). In 1979,

Cuthbert et al. coined the term "Barn Allergy" to describe the asthma that they found in farmworkers who were sensitized to storage mites and occupationally exposed to mites (principally *T. putrescentiae*) found in barns on the Orkney Islands, UK (9). In that study, storage mites were also identified in some damp homes. Since then, many other studies have confirmed storage mites as an occupational cause of sensitization and asthma using skin test, RAST, and bronchial challenge studies (10–13). The term "domestic mites" is now used to describe pyroglyphid and nonpyroglyphid mite species that are found in homes and that induce IgE antibody responses (2). In tropical and subtropical areas, storage mites, especially *B. tropicalis*, have also been found in homes and often form a significant part of the acarofauna.

Storage Mites as a Cause of "Nonoccupational" Asthma

The role of storage mites in causing asthma in the home is now being studied. However, even in homes with high levels of storage mites, pyroglyphid mites tend to coexist and be numerically dominant. As a result, patients exposed to mites at home are often sensitized to more than one mite species. It is unclear whether this occurs because of recognition by IgE of crossreactive allergens between the different mite species or because of sensitization to species-specific allergens. This has important implications for diagnosis and therapeutics in populations exposed to multiple mite species.

Domestic Exposure to *B. tropicalis*

B. tropicalis has been identified in house dust samples from several parts of the world, especially Latin America, where *B. tropicalis* is often the most prevalent mite in house dust along with *D. pteronyssinus*. The presence of *B. tropicalis* is detected either by mite counting, using light microscopy, or, more recently, by RAST inhibition, using pooled sera from *B. tropicalis* allergic patients or by fluorescent immunosorbent tests (14). In 1972, van Bronswijk described *B. tropicalis* in Surinam, Indonesia, Phillipines, Taiwan, Burma, and India (6). This species has also been identified in homes in Japan (15), Nigeria (16), Spain (17), Barbados (18), Hong Kong (19), and other countries. *B. tropicalis* was first identified in Colombia in 1977 (20), and recent studies in Cartagena identified *B. tropicalis* in 96% of floor and mattress samples. Studies also found that it accounted for 40.1% of total mites, whereas *D. pteronyssinus* was found in 90% of floor and mattress samples and accounted for 35.7% of total mites (14). *B. tropicalis* is the most numerous nonpyroglyphid mite in homes in Caracas, Venezuela (21) and is an abundant species in Costa Rica (22) and Guatemala (23). Studies in Sao Paulo, Brazil found *B. tropicalis* in 100% of homes sampled, comprising nearly 80% of the

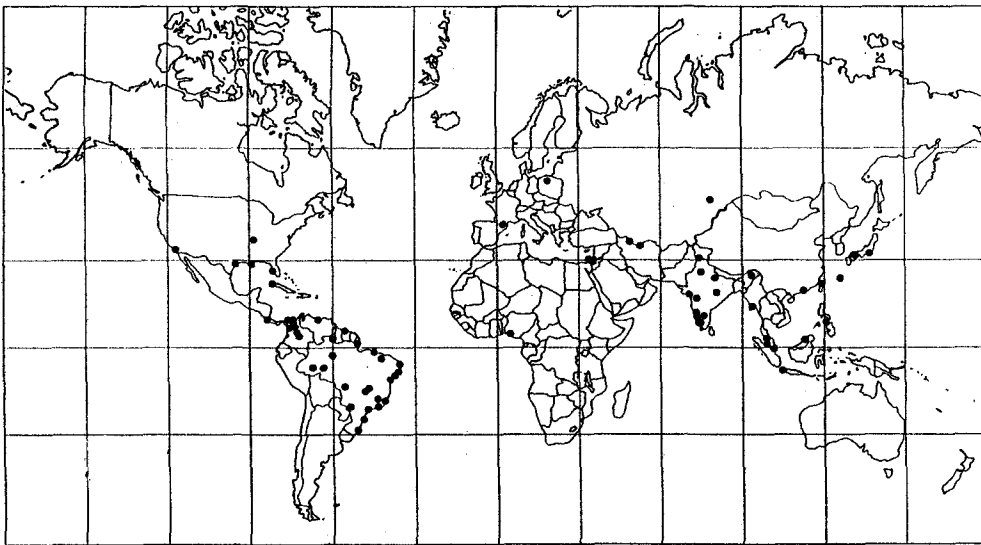


Fig. 1. Distribution of *Blomia tropicalis*.

total mites recovered (24). In Florida, *B. tropicalis* was present in 30% of samples from homes of mite-sensitized patients, although it was rarely the numerically dominant mite (25). With *D. pteronyssinus*, *D. farinae*, and *E. maynei*, *B. tropicalis* is one of the four most important domestic mites in the United States. It has also been identified in New Orleans, LA and San Diego, CA (26). Clearly this mite is prevalent in homes in many areas of the world, especially South America (Fig. 1). Domestic dwellings in the United Kingdom and Europe do not contain *B. tropicalis*, with the exception of a few areas in Spain (e.g., Tenerife).

Clinical Studies of *Blomia tropicalis*

In Sao Paulo, Brazil, skin test studies have suggested that the proportion of asthmatic and/or rhinitic adults with a positive skin test to *B. tropicalis* is similar to that for *D. pteronyssinus* (27). In asthmatic children there, a raised serum specific IgE level to *B. tropicalis* was found in 55–80% of cases and to *D. pteronyssinus* in 95% of cases; the levels to *D. pteronyssinus* tend to be higher (28,29). In Cartagena, Colombia, 86% of subjects with a positive skin prick test to *Dermatophagoides* spp had a positive RAST to *B. tropicalis* (30). A study of the prevalence of sensitization to six mite species among asthmatic adults and children in five Latin American countries found that sensitization to *B. tropicalis* ranged from 46% in Mexico City to 94% in Sao Paulo (31).

These studies suggest that in areas where *B. tropicalis* forms part of the acarofauna, a significant proportion of mite allergic asthmatics have

a positive skin prick test to *B. tropicalis*. This may be owing to the production of specific IgE antibodies to *B. tropicalis* allergens or due to IgE antibodies that are directed against allergens of *Dermatophagoides* spp. These may crossreact with *B. tropicalis* allergens because of their structural homology.

A study of the prevalence of skin test reactivity to *B. tropicalis*, *D. pteronyssinus*, and *D. farinae* among a population of 167 patients in Tampa, FL, presenting for evaluation of upper and lower respiratory complaints, found that 67% had a positive reaction to at least one mite, and 38% to *B. tropicalis* (32). Of these, 34% were positive to all three mites, 2% were positive to both *B. tropicalis* and *D. pteronyssinus*, and only 2% of the population studied had a positive reaction to *B. tropicalis* alone, a rate similar to that for *D. farinae* alone and *D. pteronyssinus* alone (2 and 4%, respectively). This study identified a small population sensitized to *B. tropicalis* alone, suggesting that *B. tropicalis* has some species-specific allergens.

Allergenic Relationships Between *B. tropicalis* and *Dermatophagoides* spp

Crossed-radioimmuno-electrophoresis and immunoblotting studies have shown that *B. tropicalis* contains 27 antigens, 7 of which are allergens. Heterologous crossed immunoelectrophoresis (CIE) studies identified two crossreactive allergens between *B. tropicalis* and *D. pteronyssinus* and two crossreactive allergens between *D. farinae* and *B. tropicalis* (33–37), the binding of which was weak in comparison to the binding in homologous CIE studies. This suggests limited structural homology and few crossreactive epitopes between the allergens. These results suggest that *B. tropicalis* produces multiple major allergens and that a minority of these allergens are able to crossreact with human IgE produced to other domestic mites (i.e., that most *B. tropicalis* allergens are species-specific).

Arruda et al. have performed immunoabsorption studies using sera from Brazilian children with a known high RAST to *B. tropicalis* and to *D. pteronyssinus* (28). By absorbing the sera with whole body extract of *D. pteronyssinus*, they were able to remove only 36% of the IgE to *B. tropicalis* as measured by RAST. Similarly, only 32% of the IgE to *D. pteronyssinus* was removed by absorbing the sera with extract of *B. tropicalis*. These results suggest that approx 70% of the IgE to both *D. pteronyssinus* and to *B. tropicalis* is made to species-specific allergens. MAb-based assays were used to measure Der p 1, Der f 1, and Der p 2 in *B. tropicalis* extract (both mite body and whole-culture), and found levels of <50 ng/mL (28), suggesting that allergens other than those of Group 1 and 2 contain the crossreactive epitopes.

Identification and Cloning of *B. tropicalis* Allergens

In vivo studies of crossreactivity have been carried out in the United Kingdom, where *B. tropicalis* does not form part of the acarofauna (38). In a population of asthmatics and rhinitics with known sensitization to *D. pteronyssinus* but previously unexposed to *B. tropicalis*, 60% showed a positive reaction on intradermal skin testing with *B. tropicalis* extract. A *B. tropicalis* allergen has recently been identified using cDNA cloning techniques and produced in recombinant form in *E. coli*. This allergen shows 40% amino acid sequence homology with Der p 5 and has been designated Blo t 5, in accordance with WHO/IUIS allergen nomenclature (39). Among patients from Brazil who were both sensitized and exposed to *B. tropicalis*, 70% gave a positive skin test reaction to rBlo t 5, showing that this recombinant protein has strong reactivity with IgE antibody in vivo (40). However when we skin-tested the UK patients who were sensitized, but not exposed, they all failed to react to intradermal skin testing with rBlo t 5 at concentrations of 5 µg/mL. Forty percent had a positive reaction on intradermal testing with rDer p 5 (38). By antigen binding RIA, serum IgE antibody to Der p 5 (41) and to Blo t 5 was detected in 21 and 0% of cases, respectively (42). Serum IgE antibodies to Blo t 5 were detected in 20% of sera from patients from Poole, UK and from Charlottesville, Virginia (unexposed populations); however, the levels of IgE ab measured were very much lower than those found in the serum of patients from Brazil, Puerto Rico, and Florida, where 45% of patients react to Blo t 5 (43). Our results suggest that Blo t 5 is a major allergen in areas where patients are exposed to *B. tropicalis*. Although there is 40% sequence homology with Der p 5, patients exposed to *D. pteronyssinus*, but not to *B. tropicalis*, generally do not make IgE antibody to Blo t 5. This suggests that other allergens are responsible for the crossreactivity seen when patients are skin tested with *D. pteronyssinus* or *Blomia* extracts. Several other *B. tropicalis* allergens have recently been identified by immunochemical or cDNA cloning techniques (Table 2) (40,44–46). Most of these are small proteins (10–20 kDa), and one cDNA clone (bt 8) is a troponin. Expression of these *Blomia* allergens in *Dermatophagoides* spp has not yet been investigated. Using PCR and nucleic acid hybridization techniques, it will be possible to establish which of the *Blomia* allergens are expressed by *D. pteronyssinus* and to determine the degree of structural homology between them by DNA sequencing. Another approach would be to use sera from *D. pteronyssinus* sensitized patients to screen a *B. tropicalis* cDNA expression library and pick out antigenically (IgE) crossreactive allergens. We have attempted this approach using sera from mite-sensitive patients living in Manchester, UK. However, although these patients showed crossreactivity to skin testing, their

Table 2
Immunochemical Characteristics of *B. tropicalis* Allergens

Allergen	Prevalence of IgE Ab	Function	Mol wt	Sequence
Blo t 5 (37)	45–69%	Unknown	14 kDa	cDNA
bt 8 (37)	50%	Troponin-C	18 kDa	cDNA
"14" kDa (44)	50%	Unknown	14 kDa	Protein
Bt 11a (41)	50%	Unknown	16.3 kDa	cDNA
Bt1 (41)	47%	Unknown	11–13 kDa	cDNA
Bt 6 (41)	10%	Fatty acid	14.8 kDa	cDNA
Bt-M (42)	47%	binding protein unknown, 78% homology to Blo t 5 C-terminus	8.4 kDa	cDNA

serum IgE antibody to *B. tropicalis* by RAST was low, and we were unable to obtain any positive clones.

Unlike *D. pteronyssinus*, commercial extracts of *B. tropicalis* are not available. Our results with Blo t 5 suggest that by using cloned allergens, it will be possible to develop suitable diagnostic reagents for demonstrating IgE responses to *Blomia*. Thus, using a cocktail of Blo t 5, bt 8 and other recombinant allergens for skin testing would circumvent the need for using whole mite extract. The diagnostic potential of recombinant allergens is currently being investigated.

Conclusions

Asthma can occur as a result of allergy to both house dust mites and to storage mites. Many people worldwide are exposed to more than one mite species in the domestic environment. The most common domestic mites are *D. pteronyssinus*, *D. farinae*, *E. maynei*, and *B. tropicalis*; other storage mites are found in homes much less frequently, even in tropical climates. Because of the ready availability of allergen extracts for skin testing and immunotherapy, patients with respiratory symptoms are generally evaluated for sensitization to *D. pteronyssinus* and *D. farinae* only. The evidence reviewed here suggests, however, that, although there are crossreactive allergens between many mite species, most species have specific allergens that can be an important cause of IgE responses. Therefore, the role of *B. tropicalis* and *E. maynei* in the etiology of asthma merits further investigation. Identification of species-specific and crossreactive allergens and production of recombinant allergens would be useful tools in allergy practice. By skin-testing patients, physicians would then be able to evaluate whether patients recognize crossreactive allergens or species-specific allergens and whether or not there is a need for immunotherapy to more than one mite species.

Acknowledgments

This work was supported in part by National Institute of Health Grants AI 34607 and AI 32557. The authors would like to thank Dr. Matthew Colloff for supplying Fig. 1 and Table 1.

References

1. Voorhorst, R. F., Spieksma, Th. M., Varekamp, H., Leupen, M. J., and Lyklema, A. W. (1967), *J. Allergy* **39**, 325–330.
2. Platts-Mills, T. A. E., Thomas, W. R., Aalberse, R. C., Vervloet, D., and Chapman, M. D. (1992), *J. Allergy Clin. Immunol.* **89**, 1046–1060.
3. Thomas, W. R. (1993), *Clin. Exp. Allergy* **23**, 350–353.
4. Luczynska, C. M., Arruda, L. K., Platts-Mills, T. A. E., Miller, J. D., Lopez, M., and Chapman, M. D. (1989), *J. Immunol. Methods* **118**, 227–235.
5. Yaseuda, H., Mita, H., Yui, Y., and Shida, T. (1990), *Int. Arch. Allergy Immunol.* **90**, 182–189.
6. Bronswijk, J. E. M. H., van de Cock, A. W. A. M., and Oshima, S. (1973), *Acarologia* **15**, 477–489.
7. Bronswijk, J. E. M. H., van de Cock, A. W. A. M., and Oshima, S. (1973), *Acarologia* **15**, 490–505.
8. Storm van Leeuwen, W. (1925), *Allergic Diseases: Diagnosis and Treatment of Bronchial Asthma, Hay Fever and Other Allergic Diseases*, JB Lippincott, Philadelphia.
9. Cuthbert, O. D., Brostoff, J., Wraith, D. G., and Brighton, W. D. (1979), *Clin. Allergy* **9**, 229–236.
10. Ingram, C. G., Jeffrey, I. G., Symington, I. S., and Cuthbert, O. D. (1979) *Lancet* **ii**, 1330–1332.
11. van Hage-Hamsten, M., Johansson, S. G. O., Hoglund, S., Tull, P., Wiren, A., and Zetterstrom, O. (1985), *Clin. Allergy* **15**, 555–564.
12. Terho, E. O., Husman, K., Vohlonen, I., Rautalahti, M., and Tukiainen, H. (1985), *Allergy* **40**, 23–26.
13. Blainey, A. D., Topping, M. D., Ollier, S., and Davies, R. J. (1989), *J. Allergy Clin. Immunol.* **84**, 296–303.
14. Fernandez-Caldas, E., Puerta, L., Mercado, D., Lockey, R. F., and Caraballo, L. R. (1993), *Clin. Exp. Allergy* **23**, 292–297.
15. Miyamoto, T., Oshima, S., Mizuno, K., Sasa, M., and Ishizaki, T. (1969), *J. Allergy* **44**, 228.
16. Somorin, A. O., Hunponu-Wusu, O. O., Mumcuoglu, Y., and Heiner, D. C. (1978), *Irish J. Med. Sci.* **147**, 26.
17. Fernandez-Caldas, E. (1986), Ph. D. Thesis, Universidad de la Laguna, Tenerife, Canary Isles, Spain.
18. Pearson, R. S. and Cunningham, A. M. (1973), *Clin. Allergy* **3**, 299–306.
19. Gabriel, M., Cunningham, A. M., Allan, W. G. L., Pickering, C. A. C., and Wraith, D. G. (1982), *Clin. Allergy* **12**, 157–171.
20. Charlet, L. D., Mulla, M. S., and Sanchez-Medina, M. (1977), *Intl. J. Acar.* **3**, 55–64.
21. Hurtado, I. and Parini, M. (1987), *Ann. Allergy* **59**, 128–130.
22. Vargas, M. V. and Mairena, H. A. (1991), *Int. J. Acarol.* **17**, 141–144.
23. Garcia-Ibanez, R., Fernandez-Caldas, E., Garcia-Romas, E., Arango, L., and Lockey, R. F. (1991), *J. Allergy Clin. Immunol.* **87**, 293 (abstract).
24. Rosa, A. E. and Flechtmann, C. H. W. (1979), *Intl. J. Acar.* **5**, 195–198.
25. Fernandez-Caldas, E., Fox, R. W., Bucholtz, G., Trudeau, W. L., Ledford, D. K., and Lockey, R. F. (1990), *Allergy Proc.* **11**, 263–267.

26. Arlian, L., Bernstein, D., Bernstein, I. L., Friedman, S., Grant, A., Lieberman, P., Lopez, M., Metzger, J., Platts-Mills, T. A. E., Schatz, M., Spector, S., Wasserman, S. I., and Zeiger, R. S. (1992), *J. Allergy Clin. Immunol.* **90**, 292–300.
27. Ambrozio, L. C., Baggio, D., Mori, J. C., Fernandes, M. F., Kase, M. T., and Mello, J. F. (1989), *Cad. Immunol-alergol. Pediatr.* **4**, 24–30.
28. Arruda, L. K., Rizzo, M. C., Chapman, M. D., Fernandez-Caldas, E., Baggio, D., Platts-Mills, T. A. E., and Naspitz, C. K. (1991), *Clin. Exp. Allergy* **21**, 433–439.
29. Rizzo, C. R., Arruda, L. K., Chapman, M. D., Fernandez-Caldas, E., Baggio, D., Platts-Mills, T. A. E., and Naspitz, C. K. (1993), *Ann. Allergy* **71**, 152–158.
30. Llerena, L. P., Fernandez-Caldas, E., Gracia, L. R. C., and Lockey, R. F. (1991), *J. Allergy Clin. Immunol.* **88**, 943–950.
31. Fernandez-Caldas, E., Baena-Cagnani, C. E., Lopez, M., Patino, C., Neffen, H. E., Sanchez-Medina, M., Caraballo, L. R., Lopez, J. H., Malka, S., Naspitz, C., and Lockey, R. F. (1993), *J. Invest. Allergol. Clin. Immunol.* **3**, 245–249.
32. Stanaland, B. E., Fernandez-Caldas, E., Jacinto, C. M., Trudeau, W. L., and Lockey, R. F. (1994), *J. Allergy Clin. Immunol.* **94**, 452–457.
33. Caraballo, L., Puerta, L., Martinez, B., and Moreno, L. (1993), *Clin. Exp. Allergy* **24**, 1056–1060.
34. Arlian, L. G., Vyszynski-Moher, D. L., and Fernandez-Caldas, E. (1993), *J. Allergy Clin. Immunol.* **91**, 1042–1050.
35. Arlian, L. G., Bernstein, I. L., Geis, D. P., Vyszynski-Moher, D. L., and Gallagher, J. S. (1987), *J. Allergy Clin. Immunol.* **79**, 457–466.
36. Arlian, L. G., Bernstein, I. L., Vyszynski-Moher, D. L., and Gallagher, J. S. (1987), *J. Med. Entomol.* **24**, 252–259.
37. Arlian, L. G., Bernstein, I. L., Vyszynski-Moher, D. L., and Gallagher, J. S. (1987), *J. Allergy Clin. Immunol.* **79**, 467–477.
38. Smith, A., Green, R., Custovic, A., Woodcock, A., Arruda, K., and Chapman, M. D. (1996), *J. Allergy Clin. Immunol.* **97**, 420 (abstract).
39. Arruda, L. K., Fernandez-Caldas, E., Naspitz, C. K., Montealegre, F., Vailes, L. D., and Chapman, M. D. (1995), *Int. Arch. Allergy Immunol.* **107**, 456–457.
40. Arruda, L. K., Vailes, L. D., Platts-Mills, T. A. E., Fernandez-Caldas, E., Montealegre, F., Lin, K-L., Chua, K-Y., Rizzo, M. C., Naspitz, C. K., and Chapman, M. D. (1997), *Am. J. Respir. Crit. Care Med.* **155**, 343–350.
41. Lin, K-L., Hsieh, K-H., Thomas, W. R., Chiang, B-L., and Chua, K-Y. (1995), *J. Allergy Clin. Immunol.* **94**, 989–996.
42. Smith, A., Green, R., Custovic, A., Woodcock, A., Arruda, L. K., and Chapman, M. D. (1996), Cross Reactivity Between IgE Ab to *Blomia* and *Dermatophagoides* spp—*In Vivo* and *In Vitro* Techniques For Group 5 Allergens. Proceedings of BSACI, Manchester, UK, p. 34.
43. Vailes, L. D., Rizzo, M. C., Naspitz, C. K., Montealegre F., Fernandez-Caldas, E., Platts-Mills, T. A. E., Chapman M. D., and Arruda, L. K. (1996), *J. Allergy Clin. Immunol.* **97**, 213 (abstract).
44. Montealegre, F. Personal communication.
45. Caraballo, L., Puerta, L., Jimenez, S., Martinez, B., Avjioglu, A., and Marsh, D. (1996), *J. Allergy Clin. Immunol.* **97**, 420 (abstract).
46. Caraballo, L., Avjioglu, A., Marrugo, J., Puerta, L., and Marsh, D. (1996), *J. Allergy Clin. Immunol.* **98**, 573–579.