

Allergenicity of the mite, *Blomia tropicalis*

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Background: *Blomia tropicalis* (BT) occurs in a significant percentage of homes in tropical and subtropical geographic regions of the United States and Europe and in countries in South America and Asia along with the pyroglyphid mites, *Euroglyphus maynei* (EM), *Dermatophagoides pteronyssinus*, and *D. farinae*. *Blomia* species may be major sources of allergens in house dust in addition to *Dermatophagoides* species and *E. maynei*.

Methods: Crossed immunoelectrophoresis and crossed radioimmunoelectrophoresis were used to identify the antigens and allergens of BT and to determine the cross-reactivity between BT and the house dust mites, *D. farinae*, *D. pteronyssinus*, and the stored product mite, *Tyrophagus putrescentiae*.

Results: Homologous crossed immunoelectrophoresis of BT resulted in 27 antigen-antibody complexes. Crossed radioimmunoelectrophoresis of these gels showed 21 different immunoglobulin E binding antigens when they were incubated in the sera from 14 patients with asthma whose radioallergosorbent test and skin test results were positive. Heterologous crossed immunoelectrophoresis reactions with BT and rabbit *D. farinae* body and feces, *D. pteronyssinus* body and feces, and *T. putrescentiae* body and feces antisera resulted in one to four precipitin lines. BT extract, reacted with rabbit antisera produced against the bodies of *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*, produced 3, 2, and 4 antigenic peaks, respectively; whereas crossed radioimmunoelectrophoresis of these gels with the sera of the 14 patients with asthma resulted in immunoglobulin E binding to 2, 2, and 4 peaks, respectively.

Conclusions: The results of this study indicated that BT contained multiple allergens of which most were species-specific. There was a limited amount of cross-reactivity between BT and the two common house dust mite species and the stored product mite. The amount of cross-reactivity appears to parallel the phylogenetic relatedness. (*J ALLERGY CLIN IMMUNOL* 1993;91:1042-50.)

Key words: *Blomia tropicalis*, *Dermatophagoides farinae*, *D. pteronyssinus*, *Tyrophagus putrescentiae*, antigens, allergens, phylogenetic, cross-reactivity, mites, house dust

Surveys show that the nonpyroglyphid mite, *Blomia tropicalis* (BT) occurs in a significant percentage of homes in tropical and subtropical geographic regions of the United States and Europe and in countries in South America and Asia along with the pyroglyphid mites, *Euroglyphus maynei* (EM), *Dermatophagoides pteronyssinus* (DP), and *D. farinae* (DF).¹⁻¹⁵ Most surveys report that homes with BT mites are usually coinhabited by DF, DP, or both. Sometimes EM is also present when BT is found. BT mites may constitute a significant portion of the total mite population in individual homes, and in some instances they are the most numerous mites that occur in house

Abbreviations used

CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
RAST:	Radioallergosorbent test
DF:	<i>Dermatophagoides farinae</i>
DP:	<i>Dermatophagoides pteronyssinus</i>
BT:	<i>Blomia tropicalis</i>
LD:	<i>Lepidoglyphus destructor</i>
TP:	<i>Tyrophagus putrescentiae</i>
EM:	<i>Euroglyphus maynei</i>
AS:	<i>Acarus siro</i>
GD:	<i>Glycyphagus domesticus</i>
SS:	<i>Sarcoptes scabiei</i>
IgE:	Immunoglobulin E

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dust.^{1, 2, 12, 13} Therefore in certain homes or geographic localities, *Blomia* species may be major sources of allergens in house dust in addition to *Dermatophagoides* species and EM.

BT occurs in significant numbers in some homes in the southern United States. BT was reported in 23 of 154 homes sampled in New Orleans, Galveston,

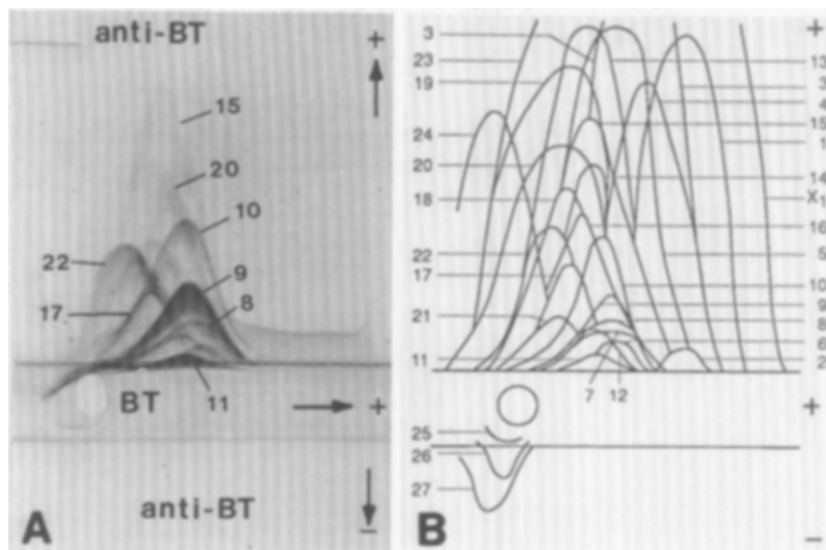


FIG. 1. BT extract reacted with rabbit anti-BT serum. **A**, Homologous CIE pattern; **B**, schematic profile.

Delray Beach, San Diego, and Memphis.¹ BT was present in 44% and 25% of the homes that were sampled in San Diego and Delray Beach respectively. BT was found in 12 of 40 homes that were sampled in Tampa, Florida, and this species was the dominant mite in the mattresses of three homes.² Seven of 32 homes that were sampled in Texas and 3 of 59 homes in Louisiana were reported to contain BT.²

In tropical climates this species is common and may be the dominant species. It has been found in dust from homes located in Spain, India, Burma, Taiwan, the Philippines, Indonesia, Venezuela, Nigeria, Uruguay, Brazil, and Columbia.³⁻¹⁶ A survey of mites in 72 homes in Brazil showed that BT occurred in all of the homes and constituted 79.5% of the total number of mites that were counted in the samples.¹² Another survey, which was conducted in São Paulo, Brazil, identified DP and BT as the most frequent mite species in house dust.¹⁵

Several studies have demonstrated positive skin tests and/or presence of circulating IgE antibodies (RAST) to BT in sera from individuals that have been exposed to BT.^{13, 14, 16} A study in São Paulo, Brazil, of a group of 123 patients with asthma and/or rhinitis demonstrated that 77% and 78% had skin tests that were positive to DP and BT, respectively.¹³ A study by Llerena et al.¹⁴ of mite-allergic individuals in Cartagena, Columbia, showed that 66% and 90% of the subjects had positive RAST results to BT and DF, respectively, with a correlation coefficient of 0.59 for sensitivity to both species. However, these investigators, using RAST inhibition assays and a pool of sera from 11 individuals who were highly allergic to DF and BT, showed only moderate cross-reactivity

between DF and BT.¹⁴ Arruda et al.¹³ observed that 13 of 20 serum samples from DF and DP allergic asthmatic children in São Paulo, Brazil, were RAST positive (>200 RAST U/ml) for BT. Immunoabsorption and RAST studies by this group showed that 64% of the IgE antibody in the sera was species-specific for BT and 36% recognized cross-reactive antigens of both BT and DP. These RAST inhibition and immunoabsorption results clearly show that among individuals who have results of skin tests or RAST that are positive to BT and *Dermatophagoides* species, BT is the source of primarily species-specific allergens and also some allergens that cross-react with DF and DP. However, with RAST inhibition and immunoabsorption it was not possible to determine whether the cross-reactivity between BT and other domestic mites and the specificity of BT involved a few or many allergens. With crossed immunoelectrophoresis (CIE) and crossed radioimmuno-electrophoresis (CRIE) techniques, the allergenicity of individual antigens and the cross-allergenicity between allergens of different mite species can be characterized. Characterization of individual allergens in BT extracts is important for: (1) the eventual characterization and standardization of BT extracts, (2) proper diagnosis of mite induced allergy, (3) choice of immunotherapy, (4) development of assays for determining BT allergen levels in dust, and (5) eventual cloning by recombinant techniques of specific allergens for use in diagnosis and immunotherapy. Therefore the purpose of this study was to evaluate the antigenic and allergenic properties of BT and its cross-reactivity with DF, DP, and *Tyrophagus putrescentiae* (TP) by means of CIE and CRIE.

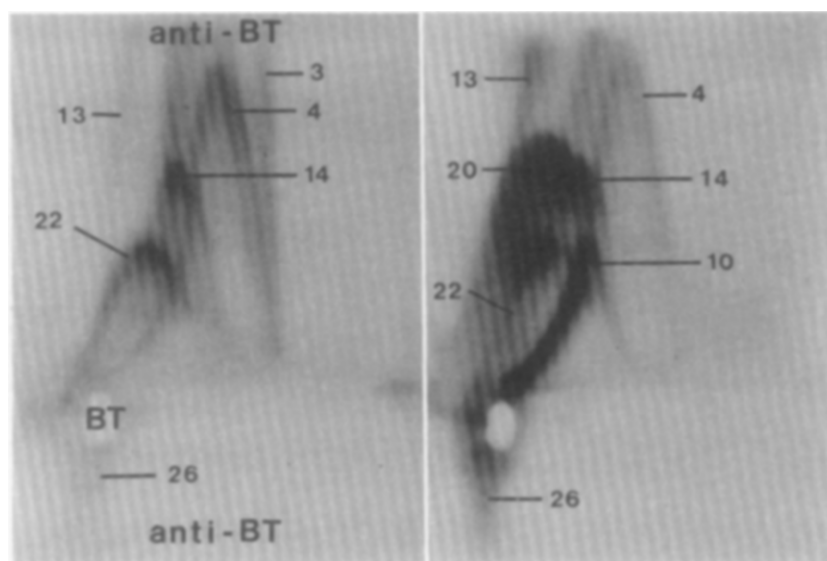


FIG. 2. Homologous CRIE IgE binding pattern of BT extract reacted with rabbit anti-BT serum. Autoradiograms show two representative sera. Corresponding CIE pattern is shown in Fig. 1, A.

MATERIAL AND METHODS

Mite material

DF, DP, and TP mites and fecal material were harvested from mature cultures as previously described.¹⁷⁻²² Briefly, mites that were free of culture medium were harvested by aspirating or brushing as the mites migrated from cultures, killed by freezing, and either dried by warming to 50° to 60° C or by placing them over Drierite (W.A. Hammond Drierite Co., Xenia, Ohio). DF and DP fecal materials were collected by sieving (106 μ m) dried spent culture material. BT was cultured on autoclaved rodent chow at 75% relative humidity. These mites were harvested with a Berlese-Tullgren funnel.²³ An extract was also made from BT spent culture after most of the food had been consumed. This material contained a high concentration of fecal material. Extract was also prepared from fecal-rich TP spent culture material after live mites were driven out with a Berlese-Tullgren funnel.

Extract preparation

Methods for extract preparations for BT, DF, DP, TP, and the culture medium for BT were described previously.¹⁷⁻²¹ Briefly, 1 gm of dried mite material was ground, defatted in anhydrous ethyl ether, extracted in 10 ml of distilled water (1:10 wt/vol resulting extract) for at least 48 hours, centrifuged, and filtered (0.2 μ m). Extracts were stored at 4° C.

Antisera

Antisera to the different antigenic extracts were prepared as previously reported.¹⁷⁻²¹ For each extract, New Zealand white rabbits were initially subcutaneously immunized with 0.5 to 3.7 mg of protein emulsified in complete Freund's adjuvant, followed by subsequent injections every 1 to 3

weeks of 0.5 to 5 mg protein in incomplete Freund's adjuvant until potent antisera were produced. Antiserum titers were periodically monitored by CIE during the immunization schedule. Antisera were frozen (-20° C) until use.

Human sera

Sera from 14 patients with asthma who had skin test (4+) and RAST (classes 3 and 4) results that were positive to BT, DF, and DP were used for this study. Eleven negative sera were used for the homologous BT CRIE gels; six negative controls were used for the cross-reactive gels.

CIE and CRIE

CIE and CRIE were performed as previously reported.¹⁷⁻²² To investigate the number of antigens present in BT, 18 μ l of extract (1:10 wt/vol) were horizontally electrophoresed in the first dimension at 200 V, at 14° C for 30 minutes. The second-dimension agarose gels, which contained corresponding antiserum (300 μ l anodic side and 60 μ l cathodic side), were electrophoresed at 50 V, at 14° C and for 15 to 16 hours. In order to identify the cross-reactivity of BT body and spent culture extracts (predominantly fecal material) with other common mite species, heterologous CIE gels were run with the same methods. Eighteen to fifty microliters (BT spent culture and body extracts) were electrophoretically run into the various DF body and feces, DP body and feces, or TP body and feces antisera (200 to 400 μ l anodic gels and 60 μ l cathodic gels). Reciprocal heterologous gels were run by electrophoresing 25 μ l of the various mite extracts into BT antiserum (300 μ l anodic side and 60 μ l cathodic side). Electrophoresed gels were washed, pressed, and stained with Coomassie Brilliant Blue R-250 (Sigma Chemical Co., St. Louis, Mo.).

CRIE was performed to determine which of the BT an-

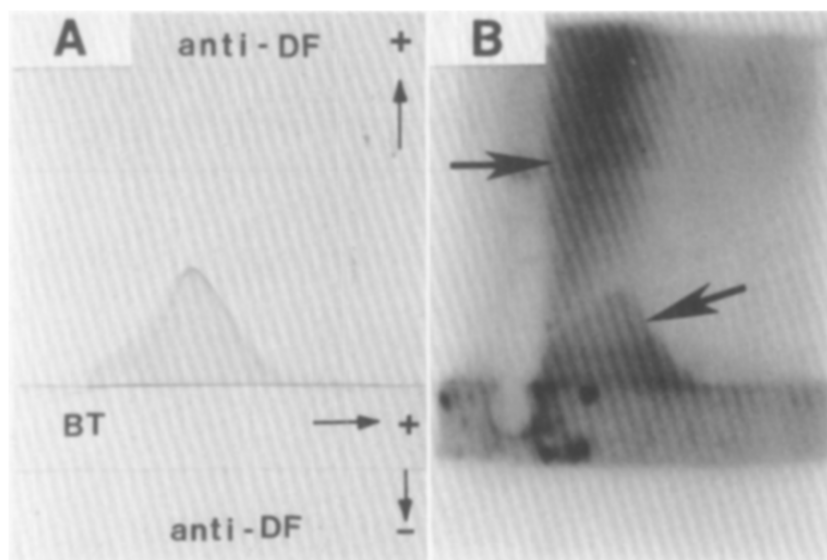


FIG. 3. Heterologous CIE and CRIE patterns. **A**, BT extract reacted with rabbit anti-DF serum. The CIE gel contained three antigen/antibody precipitates, but only one stained sufficiently to be seen in the photograph. **B**, Autoradiogram showing IgE binding to two allergens (arrows) for one representative serum sample.

tigens would bind to allergic patients' IgE. Homologous CIE gels that contained 18 μ l of extract and 300 μ l and 60 μ l of rabbit antiserum (anodal and cathodal gels, respectively) were incubated overnight in 1 ml of patient's serum per 19 ml RAST A buffer, washed three times in RAST B buffer, and then incubated overnight in iodine-125-labeled anti-human IgE (800,000 cpm per 20 ml RAST C buffer). The compositions of RAST A, B, and C buffers were as previously described.^{21, 22} Autoradiography was performed for specific periods from 3 to 21 days. To standardize and compare IgE binding both within and between serum samples, all gels were processed identically in one batch with one batch of reagents (e.g., buffers, ¹²⁵I-labeled anti-human IgE, x-ray film, etc.). The system for scoring the potency of allergens for individual serum samples was similar to that used previously²⁴ and reported by Lind and Lowenstein.²⁵ An allergen was defined as "potent," "moderate," or "weak" if the antigen produced radiostaining on the autoradiographs after 3, 7, or 14 days of exposure, respectively. Allergens that were recognized by $\geq 50\%$ of the reference sera were considered to be "major" allergens.

In addition, heterologous CRIEs were performed to determine which of the cross-reacting antigens were allergens and would bind to BT-allergic patients' IgE. Heterologous gels contained 50 μ l of BT extract and 200, 200, and 450 μ l for DF, DP, and TP anodic gels, respectively, and 30 μ l for all cathodic gels.

RESULTS

Detection of BT antigens and allergens

The CIE of BT body extract reacted with its corresponding antiserum resulted in 27 antigen-antibody precipitin complexes that stained with Coomassie bril-

liant blue (Fig. 1). One additional antigen-antibody complex (X_1) that did not stain with Coomassie brilliant blue was identified by autoradiography. Twenty-five of the antigens were electronegative and anodically migrating. CRIE studies were performed with individual serum samples from 14 patients with asthma who had RAST and skin test results that were positive to BT and 11 individuals who had skin test results that were negative (Fig. 2). Autoradiograms showed IgE binding and radiostaining to 21 different antigens (Table I) by the 14 allergic patients' sera. Sera of individual patients with asthma contained circulating IgE directed at from as few as 5 to as many as 14 different antigens (Table I). Eleven patients had circulating IgE directed at eight or more antigens. Sera of nine patients showed moderate or strong binding to at least one antigen, as judged by intensity of radiostaining. All patients had IgE binding to antigen nos. 10, 20, and 22 (Table I). Fifty-seven percent or more of the patients had IgE binding to antigen nos. 3, 5, 10, 13, 20, 22, and 26. Individuals with negative RAST and skin test results showed no IgE binding to any antigen. Five different antigens were recognized as potent allergens by at least 1 of the 14 serum samples.

It was possible that the observed IgE binding was directed at antigens that were derived from the mites' culture medium. To investigate this possibility, extract made from the mites' culture medium was reacted with anti-BT serum. No Coomassie brilliant blue-stained antigen-antibody precipitin lines were visible

TABLE I. Frequency of patients' IgE binding to BT antigens that were precipitated by rabbit anti-BT serum with CIE and CRIE

Antigen numbers	Patient numbers													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3		X		X	X	X		X		X			X	X
4			X	X	X			X	X	X				
5	X		X				X		X		X	X	X	X
6							X						X	
10	X	X	X	X	X	X	X	X	X	X	X	X	X	X
11	X		X				X	X					X	
13	X	X	X				X	X	X	X	X		X	X
14				X					X	X	X		X	
15								X						
17					X								X	
18			X											
19													X	
20	X	X	X	X	X	X	X	X	X	X	X	X	X	X
21				X								X		
22	X	X	X	X	X	X	X	X	X	X	X	X	X	X
23	X	X		X				X						X
24				X	X								X	
25										X				
26		X	X	X	X	X	X	X	X	X	X		X	X
27				X	X	X					X		X	X
X ¹		X	X											

X denotes IgE binding.

TABLE II. Number of cross-reacting antigens between various mites when extracts were reacted by two-dimensional CIE

Mite extract	Mite antiserum					
	DF _b	DF _f	DP _b	DP _f	TP _b	TP _{sc}
BT _b	3	3	2	1	4	1
BT _{sc}	1	3	1	1	2	1
DF _b	35	15	19	12	2	2
DF _f	15	20	12	13	0	0
DP _b	21	5	30+	6	2	1
DP _f	3	9+	9	13+	0	0
TP _b	2	1	0	0	20	5
TP _{sc}	1	1	ND	ND	0	18

BT_b, *Blomia tropicalis* bodies; BT_{sc}, *B. tropicalis* spent culture; DF_b, *Dermatophagoides farinae* bodies; DF_f, *D. farinae* feces; DP_b, *D. pteronyssinus* bodies; DP_f, *D. pteronyssinus* feces; TP_b, *Tyrophagus putrescentiae* bodies; TP_{sc}, *T. putrescentiae* spent culture; ND, no data.

on these CIE gel plates. These gel plates were then reacted by CRIE with sera from the 14 patients with asthma with IgE directed at BT. No radiostaining was evident, which proved that none of the antigens and allergens in BT extract originated from the mites' culture medium.

Cross-reactivity between BT and DF, DP, or TP

Heterologous CIE was performed to evaluate the potential cross-reactivity between BT and other mites found in dust. Heterologous CIE of BT body extract electrophoresed into a second-dimension gel that con-

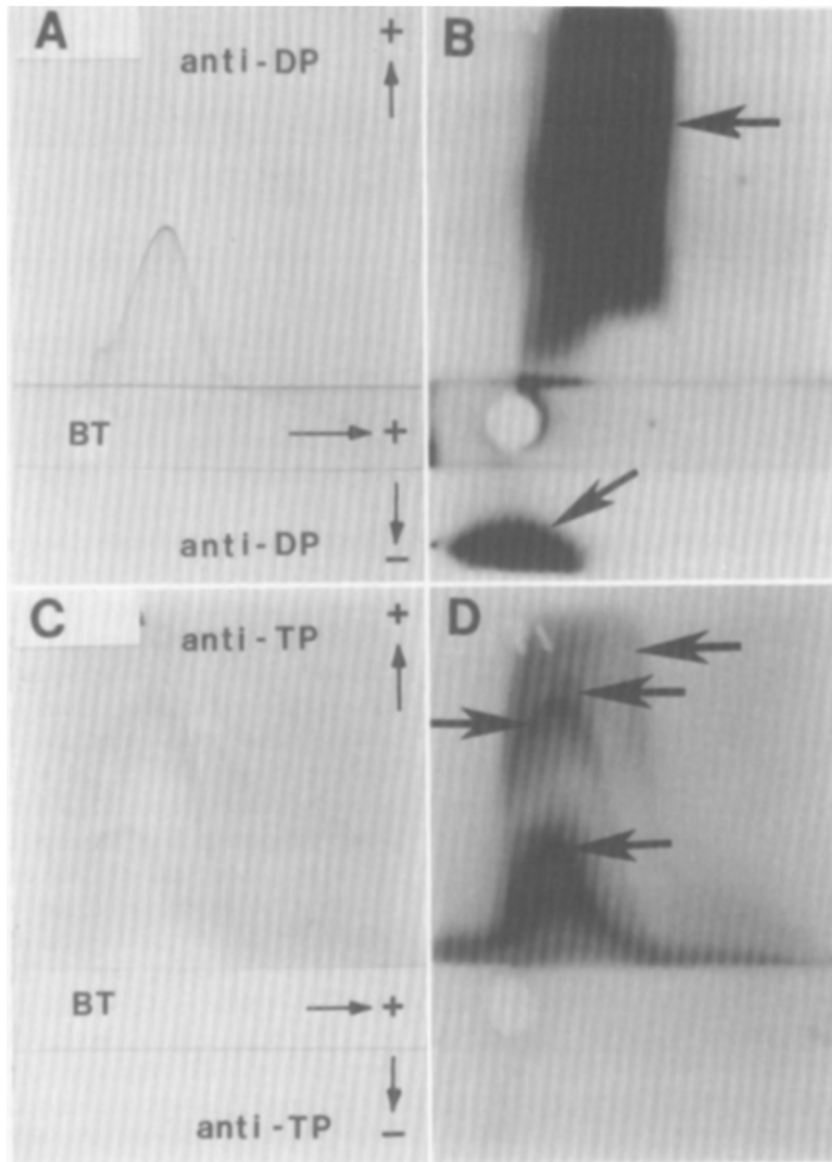


FIG. 4. Heterologous CIE and CRIE patterns. **A**, BT extract reacted with rabbit anti-DP serum; **B**, autoradiogram of **A** showing IgE binding to two cross-reacting antigens (*arrows*) by a representative serum sample; **C**, BT extract reacted with rabbit anti-TP serum; **D**, autoradiogram of **C** showing IgE binding to four cross-reacting antigens (*arrows*) by a representative serum sample.

tained antiserum produced to DF bodies resulted in three antigen-antibody precipitates (Fig. 3, *A*). One precipitate was clearly resolved and well stained with Coomassie brilliant blue; two others were faintly stained and not very visible. Two of these bound IgE when incubated in the sera of the 14 mite-sensitive patients with asthma (Fig. 3, *B*). Likewise, reaction of BT bodies with anti-DF feces serum produced three precipitin lines. BT spent culture (predominantly feces) reacted with DF bodies and feces produced one and three precipitates, respectively (Table II). BT

bodies or BT spent culture reacted with DP bodies, DP feces, TP bodies, and TP spent culture (feces rich) produced one to four precipitin lines (Fig. 4, *A* and *C*, Table II). CRIE showed that two cross-reacting antigens from reaction of BT bodies with DP body-antiserum bound IgE (Fig. 4, *B*). Therefore cross-reactivity and allergenicity between BT and DP mites was limited to two antigens. Finally, CIE reaction of BT bodies with TP bodies produced four weakly staining antigen-antibody precipitates. All four precipitates bound IgE (Fig. 4, *D*).

TABLE III. Relationship of species and families of astigmatid mites

Mite family			
Pyroglyphidae	Glycyphagidae	Acaridae	Sarcoptidae
<i>Dermatophagoides farinae</i>	<i>Lepidoglyphus destructor</i>	<i>Tyrophagus putrescentiae</i>	<i>Sarcoptes scabiei</i>
<i>D. pteronyssinus</i>	<i>Blomia tropicalis</i>	<i>Acarus siro</i>	
<i>Euroglyphus maynei</i>	<i>Glycyphagus domesticus</i>		

DISCUSSION

Our results indicate that BT is the source of at least 27 antigens that can be precipitated by a rabbit anti-serum. Twenty-one of these antigens bound IgE when incubated in the serum of patients with asthma whose RAST and skin tests were positive to BT, DF, and DP. At least 12 of these antigens were "potent" allergens, since IgE binding was evident after 2 to 3 days of autoradiography for one or more of the reference serum samples. Eighty-six percent of the 14 reference patients' serum samples had high levels of IgE directed at three to eight BT antigens, and 86% of the patients' serum samples showed high IgE levels directed at one particular antigen. Eighty-six percent of the reference sera had IgE to at least seven different allergens. Therefore our results show that BT is also the source of multiple "major" allergens.

It could be suggested that the sensitivity to BT was the result of a cross-reactivity with DF or DP, the most common mites in dust in the Tampa area where the patients lived. However, heterologous CIE and CRIE clearly showed that there was limited cross-antigenicity and cross-allergenicity between BT and *Dermatophagoides* species or TP. This was evident by both the number of cross-reacting antigens identified and the reduced amount of IgE binding by the cross-reacting BT antigens. Heterologous CIE reactions of BT with DF, DP, or TP resulted in a maximum of three, two, and four antigen-antibody precipitates, respectively; whereas homologous CIE reactions of BT, DF, DP, and TP extracts reacted with their corresponding antisera yielded 27+, 35, 30+, and 20 antigens, respectively. Autoradiography with CIE gels in which BT antigens were precipitated with anti-DF, DP, or TP antiserum showed IgE directed at only three, two, and four cross-reacting antigens, respectively. This binding of IgE to individual cross-reacting antigens was weak (on the basis of reduced radiostaining for comparable times) compared with the binding that occurred when the same patients' serum samples were reacted with homologous CIE plates in which BT extract was reacted with anti-BT serum. In contrast, autoradiography of homologous CIE of BT reacted with anti-BT serum identified 21

allergens. Likewise, autoradiography with homologous CIE reactions of DF or DP showed 15 and 16 allergens, respectively, from these species.^{17, 19} These results indicate that: (1) BT is the source of multiple potent allergens; (2) BT is the source of several "major" allergens, although sensitivity to the various BT allergens varies both between and within individuals; (3) most allergens of BT are species-specific; and (4) BT shares a few cross-reacting allergens with the common house dust mites DP and DF and the storage mite TP, but the low IgE binding to the cross-reacting antigens suggests limited structural homology and few cross-reacting epitopes for individual cross-reacting allergens. Conclusions 3 and 4 are indirectly supported by previously published RAST inhibition and immunoabsorption data, although cross-reactivity of individual antigens and allergens cannot be evaluated with these techniques. Using sera of individuals who were highly sensitive to DF and BT, Llerena et al.¹⁴ showed only moderate cross-reactivity between DF and BT. Likewise, using sera from patients with DF- and DP-allergic asthma who had RAST results that were positive to BT, Arruda et al.¹³ showed that 64% of the serum IgE was species-specific for BT and 36% recognized cross-reacting antigens of both BT and DP.

On the basis of this study and those of others, it generally appears that the number of cross-reacting antigens (allergens) and the amount of cross-reactivity between individual allergens (structural homology) of different mite species parallels the phylogenetic relatedness between the mites being compared. BT, DF, DP, and EM (the most common mites found in house dust) and TP, *Lepidoglyphus destructor* (LD), *Acarus siro* (AS), and *Glycyphagus domesticus* (GD) (stored product mites) are phylogenetically related mites belonging to the suborder astigmata (Table III).^{11, 26} On the basis of distinct differences in morphologic characteristics, behavioral attributes, and ecologic associations BT, GD, and LD belong to the family Glycyphagidae; TP and AS are in the family Acaridae, and DP, DF, and EM belong to the family Pyroglyphidae. Most glycyphagid species are strictly nidicolous, but some lineages of glycyphagid mites, in-

cluding *Blomia* species live in stored products (grains and hay) and feed primarily on fungal growth. *Dermatophagoides* species and EM mites are primarily nidicoles found in the nests of mammals and birds.²⁶ The acarids are strictly stored-product mites.^{11, 26} Direct immunologic reactions (with techniques that allow visualization of individual antigens) of different mite extracts and antisera indicate that the more closely mites are related phylogenetically, the greater the number of cross-reacting antigens. The pyroglyphid mites, DP, DF, and EM share a high number of cross-reacting antigens within the group but few cross-reacting antigens with nonpyroglyphid storage mites (Table II). Presumably, species of the same genus exhibit the highest number of cross-reacting allergens. For example, DF and DP share 20+ cross-reacting antigens and eight to nine cross-reactive allergens.¹⁸ However, EM shares 15+ cross-reacting antigens with DF and DP, which is fewer than are shared by DF and DP. EM possesses six and four allergens that cross-react with allergens of DP and DF, respectively.²⁴ Members of the same family are more antigenically similar than species of different families. For example, DF and DP exhibit 20+ cross-reacting antigens and many cross-reacting allergens,¹⁸ whereas DF and DP showed only two cross-reacting antigens and allergens with the storage mite TP.²⁰ TP is the source of 20 antigens and five allergens.²¹ Likewise, BT extracts of this study showed at most three cross-reacting antigens with DF and DP and only three cross-reacting allergens. In addition, BT showed only four cross-reacting antigens and allergens with TP. The ectoparasitic mite, *Sarcoptes scabiei* (Family Sarcoptidae) shares seven cross-reacting antigens with DF and DP.^{22, 27} Without the use of CIE and CRIE, it is difficult to determine the number of cross-reacting antigens between species. The low number of cross-reacting antigens and reduced level of IgE binding by these cross-reacting allergens that has been found between various storage mites and house dust mites with CIE and CRIE is consistent with the conclusions of previous investigations in which RAST inhibition was used. van Hage-Hamsten et al.²⁸ report a lack of cross-reactivity between DP and the storage mite, LD and no significant cross-reactivity between DP and the storage mites, LD and GD (*Glycyphagidae*), TP and AS (*Acaridae*). LD and GD are antigenically more closely related to each other than either is to AS. Using RAST inhibition, Llerena et al.¹⁴ demonstrated considerable cross-reactivity between BT and LD, both species in the family Glycyphagidae. In contrast, they found only moderate cross-reactivity between DF and BT or LD.

With the exception of group I, II, and III allergens

of *Dermatophagoides* species and EM, few studies have investigated the structural homology (amino acid sequence and epitopes) between cross-reacting allergens of different mite species. Therefore the molecular relatedness of cross-reacting allergens of different mite species is largely unknown. Group I, II, and III allergens of the related species DF, DP, and EM as expected, show a relatively high level of homology. Thomas et al.²⁹ reported that the deduced amino acid sequence of the group I allergens, *Der p* I and *Der f* I, showed 81% homology and that the group II allergens, *Der p* II and *Der f* II, showed 88% homology, which confirmed the work of Heymann et al.^{30, 31} The N-terminal amino acid sequence of group III allergens, *Der p* III and *Der f* III show 75% homology.^{31, 32} The coding regions of the genes for *Der p* I and *Eur m* I are highly conserved between the two species (89% nucleotide identity; 86% amino acid identity).³³ The relatedness of cross-reacting allergens of BT, TP, and EM will not be known until other molecular studies are completed. It is likely that the number of cross-reacting allergens and the level of homology will be much less than is present for the pyroglyphid mites DF, DP, and EM.

It is important to recognize these phylogenetic antigenic relationships and the cross-reactivity between mite species because of the potential for mites to cause allergic manifestations in specific populations of the world. Realistically, for the typical metropolitan or urban dweller, DF, DP, EM, and BT are the major mites of concern. For these populations, sensitivity to the many species of storage mites and their cross-reactivity with mites found in house dust (DF, DP, BT, and EM) are not important because exposure to storage mite species is minimal. On the other hand, for farmers and other agricultural workers who handle stored grains or hay, both storage and house dust mites may act as inhalant allergens. Thus for these populations, sensitivity to the various species and cross-reactivity between species may be of clinical significance. Therefore for agricultural workers, all species should be considered for diagnostic testing and possible immunotherapy.

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