

Allergenicity of the mite *Hemisarcoptes cooremani*

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Background: A researcher experienced allergic symptoms while working with the astigmatid mite *Hemisarcoptes cooremani* cultured on scale insects. This mite is a predator of scale insects that often parasitize many perennial vascular plants in orchards, gardens, and ornamental nurseries worldwide; therefore, orchard and ornamental nursery workers and gardeners may be exposed to this mite.

Objective: We investigated the possible allergenicity of *H. cooremani* and the cross-reactivity between it and other allergy-causing astigmatid mites.

Methods: Serum from a subject who experienced allergic symptoms while working with *H. cooremani* was analyzed for IgE and IgG to proteins in an extract of this mite and of other astigmatid mites known to cause allergic reactions. The serum was used to probe proteins fractionated by SDS-PAGE or precipitated by CIE using rabbit antiserum. In addition, the subject's serum was used to directly precipitate proteins in extracts of *H. cooremani* and other mite species.

Results: SDS-PAGE and immunoblotting of proteins in an *H. cooremani* extract showed the reference serum contained IgE directed at 16-kD and 19-kD proteins. Crossed radioimmuno-electrophoresis reaction showed that the subject's serum contained antibody that precipitated a protein in an *H. cooremani* extract and that IgE bound to this protein. The proteins in an extract of *H. cooremani* did not precipitate when reacted with rabbit antisera against the dust mites *D. farinae*, *D. pteronyssinus*, and *E. maynei*, or the storage mites *B. tropicalis*, *L. destructor*, and *T. putrescentiae*. This indicated there was no cross-reactivity between *H. cooremani* and these mites.

Conclusion: These results indicated that an extract of the mite *H. cooremani* contained at least two prominent IgE binding proteins that were not present in the other astigmatid mites. Thus, *H. cooremani* is the source of unique allergenic proteins and allergy to this mite may develop in orchard and ornamental nursery workers and gardeners.

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INTRODUCTION

Many species of mites in the group Astigmata elicit IgE-mediated allergic reactions.¹ Examples include the storage mites (*Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, and *Blomia tropicalis*) and house dust mites (*Dermatophagoides farinae*, *D. pteronyssi-*

nus, and *Euroglyphus maynei*).²⁻¹⁴ Astigmatid mites of the genus *Hemisarcoptes* are commonly found on many species of woody vascular plants.¹⁵ These mites are cosmopolitan and occur on all continents of the world, possibly with the exception of the under-sampled continent of South America. There are several species of *Hemisarcoptes* in the United States and Canada with *H. cooremani* being the dominant species in the southern tier of states. These mites are predators of scale insects that often parasitize many perennial vascular plants in orchards and yards worldwide; therefore, orchard and ornamental nursery workers and gardeners may be exposed to *H. coore-*

mani. It came to our attention that a researcher experienced allergic symptoms (rhinitis and watery eyes) while working with *H. cooremani* cultured on scale insects. Since other astigmatid mites are known to be highly allergenic, the purpose of this study was to investigate the possible allergenicity of *H. cooremani* and the cross-reactivity between it and other astigmatid mites.

METHODS

Patient profile

Serum was obtained (under a protocol approved by the Wright State University Human Subjects Committee) from a 50-year-old female researcher who had worked with *Hemisarcoptes cooremani* cultures for 13 years and reported experiencing allergic symptoms (rhinitis and watery eyes) when handling these mites. She did not work with house dust or storage mites. The subject had previously produced a 4+ skin reaction to *D. farinae* and had been undergoing immunotherapy for 4 years. Serum from this subject was commercially tested by ImmunoCAP and was class 1/0 to *D. farinae* but negative to *D. pteronyssinus* and *E. maynei*. Total IgE was 66 U/mL.

Antigen extracts

Aqueous extracts (1:10, wt/vol) were prepared from whole mite bodies of *H. cooremani*, *D. farinae*, *D. pteronyssinus*, *E. maynei*, *B. tropicalis*, *T. putrescentiae*, and *L. destructor* as previously described.¹¹ *Dermatophagoides farinae*, *D. pteronyssinus*, *E. maynei*, *B. tropicalis*, *T. putrescentiae*, and *L. destructor* were harvested from laboratory cultures. *Hemisarcoptes cooremani* for these studies were individually removed from scale insects using a needle while viewing under a stereomicroscope; therefore, very limited numbers of *H. cooremani* were avail-

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able for this study because this mite cannot be mass produced like the other species. Protein concentrations were determined by the method of Bradford.¹⁶

Antisera

Antisera to *D. farinae*, *D. pteronyssinus*, *E. maynei*, *B. tropicalis*, *T. putrescentiae*, and *L. destructor* were produced in rabbits as previously reported.^{4,7,14,17,18}

SDS-PAGE/Immunoblotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were carried out on 12% gels under non-reducing conditions as previously described.¹¹ Except for *H. cooremani* each lane was loaded with 10 µg of mite protein. Because of the limited quantity of *H. cooremani* available, only 1.2 µg of protein was loaded in this lane. Separated proteins from each mite species were electrophoretically transferred to a PVDF membrane that was blocked and incubated in 4 mL serum diluted in 50 mL of buffer. IgE-binding allergens were then visualized with ¹²⁵I-labeled anti-human-IgE (2 million cpm in 40 ml buffer) and au-

toradiography. IgG binding on this same blot was detected by using peroxidase-labeled goat anti-human-IgG (Fisher Scientific, Pittsburgh, PA) at 1:2000 in buffer. The blot was developed for 5 minutes by using the 4-chloronaphthol/3,3'-diaminobenzidine/H₂O₂ chromogenic substrate of Young.¹⁹ Following IgE (autoradiography) and IgG (colorimetric) determinations, the blot was probed with the mouse monoclonal antibody 1D8 (1:500; obtained from Dr. Martin D. Chapman, University of Virginia, Charlottesville) to identify group 2 allergens. This monoclonal antibody recognizes an epitope present on the group 2 allergens Der p 2, Der f 2 and Eur m 2 of *D. pteronyssinus*, *D. farinae*, and *E. maynei*, respectively.^{11,20} Murine antibody binding was detected using alkaline phosphatase-labeled goat anti-mouse-IgG with BCIP/NBT chromogen (5-bromo,4-chloro,3-indolylphosphate/nitroblue tetrazolium).

CIE/CRIE

To determine the cross-reactivity of *H. cooremani* with *D. farinae*, *D. pteronyssinus*, *E. maynei*, *B. tropicalis*, *L. destructor*, and *T. putrescentiae*, sev-

eral sets of homologous and heterologous crossed immunoelectrophoresis (CIE) reactions were performed using procedures identical to those reported in previous studies.^{4,7,10,11,14}

In one set of homologous CIE reactions, 10 µL of mite extract was electrophoresed in the first dimension at 200 V for 30 minutes to separate the proteins. The separated proteins were then electrophoresed into a second dimension anodal gel containing 200 µL of the corresponding rabbit antiserum in 3 mL of 1% agarose. The cathodal gel contained 40 µL of antisera in 1 mL of agarose. Crossed radioimmuno-electrophoresis (CRIE) was then performed using serum from the subject (0.5 mL serum/20 mL buffer) to determine whether this serum contained any IgE directed at determinants on cross-reacting antigens. Autoradiography was conducted using 1 million cpm ¹²⁵I-labeled anti-human-IgE per tray.

Using heterologous CIE, *H. cooremani* extract was reacted with rabbit antiserum built to the other mite species to determine if *H. cooremani* was cross-reactive with *D. farinae*, *D. pteronyssinus*, *L. destructor*, *T. putrescentiae*, *E. maynei*, and *B. tropicalis*. Antigen-antibody complexes on the heterologous gels were visualized by Coomassie blue staining.

In a third experiment, extracts of the various mite species were electrophoresed in the second dimension into 300 µL of the allergic subject's serum in 3 mL of 1% agarose in the anodal gel and 50 µL of her serum in 1 mL of 1% agarose in the cathodal gel. Autoradiography was then performed as above.

RESULTS

Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis revealed that extracts of each of the seven astigmatid mites contained a large number of soluble proteins. When serum from the *H. cooremani*-sensitive individual was used to probe an immunoblot of the 7-mite species, it contained circulating IgE that bound to bands of 16 and 19 kD in an *H. cooremani* extract (Fig 1). IgE binding was also observed to a 16-kD band in an

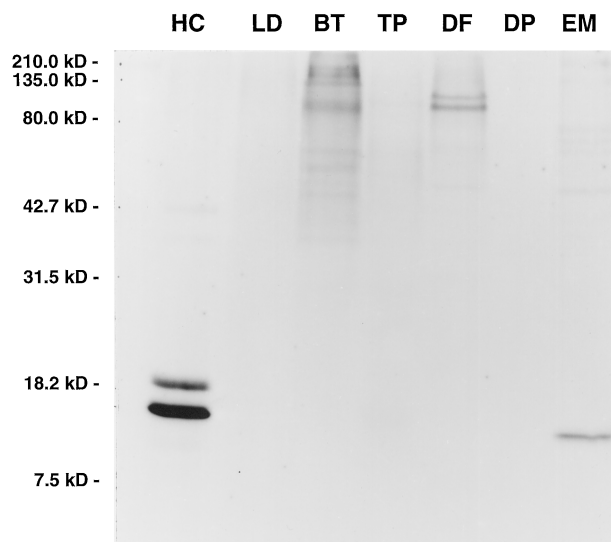


Figure 1. Immunoblot of extracts of seven astigmatid mites probed with the serum of the *Hemisarcoptes cooremani*-sensitive subject. Binding of circulating IgE was detected autoradiographically. Molecular weight markers were run in the first lane and are indicated at the left and mite species are indicated at the top. HC, *Hemisarcoptes cooremani*; LD, *Lepidoglyphus destructor*; BT, *Blomia tropicalis*; TP, *Tyrophagus putrescentiae*; DF, *Dermatophagoides farinae*; DP, *Dermatophagoides pteronyssinus*; and EM, *Euroglyphus maynei*.

extract from the house dust mite *E. maynei*. Weaker IgE binding to some higher molecular weight components of *H. cooremani*, *B. tropicalis*, *D. farinae*, *E. maynei*, and *T. putrescentiae* was also observed.

The reference serum showed weak IgG binding directed at two to three proteins of *H. cooremani* and *D. pteronyssinus* and strong IgG binding to multiple proteins of *B. tropicalis*, *T. putrescentiae*, *E. maynei*, and *D. farinae* (Fig 2). The high IgG titer to *D. farinae* was expected due to the *D. farinae* immunotherapy.

The mouse monoclonal antibody 1D8 (that recognizes an epitope present on the allergens Der p 2, Der f 2, and Eur m 2^{11,20}) bound to a low molecular weight protein present in these mite species but not to any protein of the other mite species on our blot (Fig 2). The serum from the study subject did not contain IgE that bound to the same proteins that bound the 1D8 monoclonal antibody.

Homologous and heterologous CIE analyses confirmed the SDS-PAGE results. Homologous CIE reactions of *D. farinae*, *D. pteronyssinus*, *E. maynei*, *T. putrescentiae*, *B. tropicalis*, and *L. destructor* each with its corresponding rabbit antiserum resulted in the typical large number of protein precipitin peaks. After incubation of each of these gel plates in the serum of the reference subject and ¹²⁵I-labeled anti-IgE, autoradiographs revealed no proteins in extracts of *D. farinae*, *D. pteronyssinus*, *E. maynei*, *L. destructor*, and *T. putrescentiae* that bound IgE from this subject's serum. One protein of *B. tropicalis* exhibited weak IgE binding that was visible only after 15 days of autoradiography which indicated that the subject had some circulating IgE antibody to one protein of this mite.

Heterologous CIE reactions of *H. cooremani* extract with rabbit antiserum to *D. farinae*, *D. pteronyssinus*, *E. maynei*, *L. destructor*, *B. tropicalis*, and *T. putrescentiae* resulted in no visible antigen-antibody precipitin peaks following Coomassie blue staining of the gels. This indicated there was no

cross-reactivity between *H. cooremani* and these other mite species.

In another set of CIE experiments, *H. cooremani*, *D. farinae*, *D. pteronyssinus*, *E. maynei*, *L. destructor*, *B. tropicalis*, and *T. putrescentiae* extracts were reacted directly in the second dimension with the serum from the reference subject and then immunodetection for IgE binding was performed. Following autoradiography one antigen-antibody precipitin peak of *H. cooremani* exhibited IgE binding. Coomassie blue staining of the gels revealed two precipitin peaks for *L. destructor* and one for *T. putrescentiae*.

DISCUSSION

The results of this study indicate that an extract of the mite *Hemisarcoptes cooremani* contained at least two prominent IgE binding proteins of 16-kD and 19-kD molecular weight. The 16-kD band of *H. cooremani* matched a similar molecular weight protein in an extract of *E. maynei* which was separated in a parallel SDS-PAGE lane under identical conditions. Whether or not there is any sequence homology between the *E. maynei* and *H. cooremani* IgE-binding proteins

was not determined so it is not known whether these are cross-reacting epitopes on proteins of similar molecular weight. Clearly, since no protein in the extracts of the other mite species showed IgE binding to either a 16-kD or 19-kD protein, it can be assumed that these were allergens (epitopes) unique to *H. cooremani*. The 16-kD or 19-kD allergens of *H. cooremani* were clearly not related to the similar molecular weight group 2 allergens of *D. pteronyssinus*, *D. farinae*, and *E. maynei* since these proteins of *H. cooremani* did not bind the monoclonal antibody 1D8 while proteins in the dust mite extracts did. In parallel lanes of the same gel *D. farinae*, *D. pteronyssinus*, and *E. maynei* had proteins in the same molecular weight region that did bind this monoclonal.

The existence of *H. cooremani* allergens was further confirmed by the results of the homologous and heterologous CIE and CRIE experiments in which *H. cooremani* extract was reacted directly with the subject's serum or with polyclonal antisera built to the other mite species. Immunodetection using the subject's serum showed IgE binding after *H. cooremani* extract was

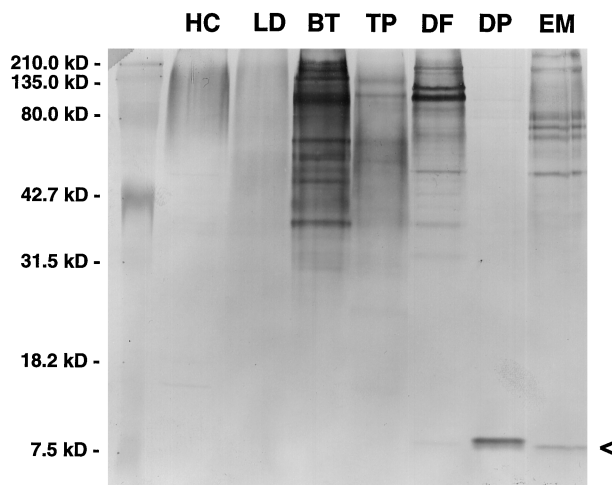


Figure 2. Immunoblot of extracts of seven astigmatid mites probed with the serum of the *Hemisarcoptes cooremani*-sensitive subject. Binding of circulating IgG was detected colorimetrically. The blot was then probed with the murine monoclonal antibody 1D8 and developed colorimetrically using a different color substrate. Bands detected with 1D8 are indicated (<). Molecular weight markers were run in the first lane and are indicated at the left and mite species are indicated at the top. HC, *Hemisarcoptes cooremani*; LD, *Lepidoglyphus destructor*; BT, *Blomia tropicalis*; TP, *Tyrophagus putrescentiae*; DF, *Dermatophagoides farinae*; DP, *Dermatophagoides pteronyssinus*; and EM, *Euroglyphus maynei*.

directly reacted with serum from the subject. Clearly, the subject had sufficient circulating antibody to precipitate a mite protein and some of this antibody was IgE. Homologous CIE reactions of the dust and storage mites resulted in numerous antigen-antibody precipitin peaks but the reference individual had serum IgE directed only at *B. tropicalis*. None of the numerous antigen-antibody complexes of the other mite species bound IgE in the reference serum. Likewise, heterologous CIE reaction of *H. cooremani* extract reacted with rabbit antisera to each of the other mite species resulted in no precipitin peaks suggesting no cross-reactivity.

Taken together, these immune assays indicate that *H. cooremani* is the source of allergenic proteins. We realize this conclusion is based on the analysis and case history of only one subject; however, the circumstances that identified this individual were unusual and the scope of the work was limited by the availability of only a few mites to make an extract. If larger numbers of this mite can be obtained the studies should be expanded to a larger population of individuals whose occupation brings them into contact with scale insects and this predaceous mite. Allergy to *H. cooremani* may develop in orchard and ornamental nursery workers and those who garden. The presence of circulating IgE to proteins of this mite and a history of symptoms by an individual who worked with this mite raises this possibility.

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REFERENCES

- Arlian LG. House-dust-mite allergens: a review. *Exp Appl Acarol* 1991;10:167–186.
- Johansson E, Johansson SGO, van Hage-Hamsten M. Allergenic characterization of *Acarus siro* and *Tyrophagus putrescentiae* and their cross-reactivity with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1994;24:743–751.
- van Hage-Hamsten M, Johansson SGO, Hogland S, et al. Storage mite allergy is common in a farming population. *Clin Allergy* 1985;15:555–564.
- Arlian LG, Geis DP, Vyszynski-Moher DL, et al. Antigenic and allergenic properties of the storage mite *Tyrophagus putrescentiae*. *J Allergy Clin Immunol* 1984;74:166–171.
- Fernandez-Caldas E, Baena-Cagnani CE, Lopez M, et al. Cutaneous sensitivity to six mite species in asthmatic patients from five Latin American countries. 1993;3:245–249.
- Puerta L, Fernandez-Caldas E, Lockey RF, Caraballo L. Mite allergy in the tropics: sensitization to six domestic mite species in Cartagena, Colombia. *J Invest Allergy Clin Immunol* 1993;3:198–204.
- Arlian LG, Vyszynski-Moher DL, Fernandez-Caldas E. Allergenicity of the mite, *Blomia tropicalis*. *J Allergy Clin Immunol* 1993;91:1042–1050.
- Caraballo L, Puerta L, Martinez B, Moreno L. Identification of allergens from the mite *Blomia tropicalis*. *Clin Exp Allergy* 1993;24:1056–1060.
- Puerta L, Fernandez-Caldas E, Caraballo Gracia LR, Lockey RF. Sensitization to *Blomia tropicalis* and *Lepidoglyphus destructor* in *Dermatophagoides* spp.-allergic individuals. *J Allergy Clin Immunol* 1991;88:943–950.
- Arlian LG, Geis DP, Vyszynski-Moher DL, et al. Cross antigenic and allergenic properties of the house dust mite *Dermatophagoides farinae* and the storage mite *Tyrophagus putrescentiae*. *J Allergy Clin Immunol* 1984;74:172–179.
- Morgan MS, Arlian LG, Fernandez-Caldas E. Cross-allergenicity of the house dust mites *Euroglyphus maynei* and *Blomia tropicalis*. *Ann Allergy Asthma Immunol* 1996;77:386–392.
- Chapman MD, Platts-Mills T. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus* antigen P1. *J Immunol* 1980;125:587–592.
- Platts-Mills TAE, Heymann PW, Chapman MD, et al. Cross-reacting and species-specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. farinae*; development of a radioimmunoassay for antigen P1 equivalent in house dust and house dust mite extracts. *J Allergy Clin Immunol* 1986;78:398–407.
- Arlian LG, Rapp CM, Fernandez-Caldas E. Allergenicity of *Euroglyphus maynei* and its cross-reactivity with *Dermatophagoides* species. *J Allergy Clin Immunol* 1993;23:110–116.
- Houck MA. Adaptation and transition into parasitism from commensalism: a phoretic model. In: Houck MA, ed. *Mites: ecological and evolutionary analyses of life-history patterns*. New York: Chapman & Hall, 1994:252–281.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–254.
- Arlian LG, Bernstein IL, Vyszynski-Moher DL, Gallaher JS. Antigenicity and allergenicity of body and fecal extracts of the mite *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *J Med Entomol* 1987;24:252–259.
- Arlian LG, Bernstein IL, Geis DP, et al. Investigations of culture medium-free house dust mites. III. Antigens and allergens of body and fecal extract of *Dermatophagoides farinae*. *J Allergy Clin Immunol* 1987;79:457–466.
- Young PR. Enhancement of immunoblot staining using a mixed chromogenic substrate. *J Immunol Methods* 1989;121:295–296.
- Ovsyannikova IG, Vailes LD, Li Y, et al. Monoclonal antibodies to group II *Dermatophagoides* spp. allergens: murine immune response, epitope analysis, and development of a two-site ELISA. *J Allergy Clin Immunol* 1994;94:537–546.

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