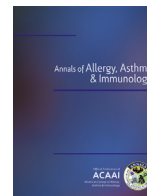




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Different sensitization to storage mites depending on the co-exposure to house dust mites

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ABSTRACT

Background: Co-sensitization to house dust mites (HDMs) and storage mites (SMs) is very frequent, although the clinical relevance is not well established.

Objective: To describe the pattern of sensitization and immunologic characterization of patients with positive skin prick test reactions to HDMs and SMs in 4 areas of Spain, selected according to high exposure to HDMs and variable exposure to SMs.

Methods: One hundred sixty-nine individuals with positive skin prick test reactions to HDMs and SMs were included. Specific IgE levels to different mite species and to Der p 1, Der p 2, and Der p 10 were determined. Immunoblots to *Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentiae* extracts were carried out, and allergograms were obtained. Results of different areas were compared.

Results: A high rate of polysensitization to SMs was observed, although 12% of participants did not have specific IgE to any SM species. Sensitization to *Dermatophagoides* species, Der p 2, and *L. destructor* were predominant, although significant differences were observed among areas depending on the grade of exposure to SMs. In areas with high exposure, the SM allergogram showed greater recognition of group 2 allergen.

Conclusion: Sensitization patterns to SMs in patients sensitized to HDMs and SMs differ depending on the exposure to SMs. Sensitization, mainly to *L. destructor*, seems to exist in areas with high exposure, possibly with group 2 allergens mainly involved. However, in areas with low SM populations, sensitizations observed by skin prick testing appear to be related to HDM exposure.

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Introduction

Mites are a major source of sensitization worldwide, especially in regions where humidity and temperature favor their development. Currently, house dust mites (HDMs) belonging to the Pyroglyphidae family are the most abundant mites in indoor habitats and thus the main indoor source of allergens. However, other mite species belonging mainly to the Glycyphagidae and Acaridae families, commonly known as storage mites (SMs), were the most cited in dwellings during the 19th and most of the 20th centuries.¹ It is

hypothesized that changes in domestic environments were responsible for this modification in prevalence. Currently, SM sensitization is related mainly to rural occupational exposure,² because they are common inhabitants in grain and foodstuff storage. However, SMs also have been identified in humid areas and in urban dwellings,³ and their allergenic capacity is increasingly the object of study.

Numerous studies have been carried out to determine the cross-reactivity between HDMs and SMs or among the different SM species.⁴ Some have shown very limited cross-reactivity,⁵ although with a certain degree of IgE inhibition.⁶ Variations may be related to actual exposure to SMs. Thus, in areas with high exposure to these 2 groups of mites, SMs may be the primary sensitizing agent.⁷

All these aspects make interpretation of skin prick test (SPT) and specific IgE (sIgE) results difficult. The use of component-resolved

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diagnosis has been proposed to improve the diagnosis of mite allergy,⁸ but it is limited by the number of purified allergens available. In addition, little information is available about the characterization of patients co-sensitized to HDMs and SMs.

The objective of the present study was to describe the pattern of sensitization and the immunologic characterization of a group of patients with positive SPT reactions to HDMs and SMs in 4 areas of Spain, selected according to high exposure to HDMs and to variable exposure to SMs, and to correlate the sensitization between the 2 groups of mites.

Methods

Areas Selected

The 4 Spanish areas of Lugo (northwest [NW]), Santander (north [N]), Barcelona (northeast [NE]), and Cartagena (southeast [SE]) were selected according to high exposure to HDMs and variable exposure to SMs. The climatic characteristics of these areas are presented in Table 1. Lugo (NW) and Santander (N) have an Atlantic climate, whereas Barcelona (NE) and Cartagena (SE) have a Mediterranean climate. Exposure to domestic mites in the 4 cities was obtained from previously published data^{9–11} (Table 1). The 2 areas located on the Atlantic coast had very high exposure to *Dermatophagoides pteronyssinus*, low exposure to *Dermatophagoides farinae*, and high exposure to SMs. The other 2 areas were on the Mediterranean coast and presented high exposure to the 2 *Dermatophagoides* species and a low presence of SMs (limited to *Glycyphagus domesticus* and *Tyrophagus putrescentiae*).

Study Design and Patient Population

The study included 169 patients who attended the allergy departments of the clinical groups (Hospital Xeral de Calde, Lugo; Hospital de Valdecilla, Santander; Hospital Clinic and Fundació Sant Pere Claver, Barcelona; and Hospital Santa María del Rosell, Cartagena). The study was approved by ethics committee of the Hospital Clinic in Barcelona (number 2009/5389). The inclusion criteria were patients with allergic symptoms and positive SPT reactions to any HDM (*D pteronyssinus*, *D farinae*, or *Euroglyphus maynei*) and any SM belonging to the Glycyphagidae (*Lepidoglyphus destructor*, *G domesticus*), Chortoglyphidae (*Chortoglyphus arcuatus*), or Acaridae (*T putrescentiae*, *Acarus siro*) family. SPT was carried out with biologically standardized, commercially available HDM and SM

extracts at 100 and 30 of histamine equivalent prick [HEP] per milliliter of biological potency, respectively (10 HEP/mL produces a wheal similar to 10 mg/mL of histamine; Laboratorios LETI, Madrid, Spain). The protein profile and immunoblot of all extracts used to manufacture the pricks are presented in Figure S1 (available online). Wheal size was measured by PC Draft software connected to a Wacom Graphic tablet and a Graphire 2 pencil (Wacom Company Ltd, Tokyo, Japan). Results were expressed in square millimeters. Wheals larger than or equal to 7 mm² were considered positive reactions.

After providing written consent, patients completed a questionnaire about personal data (sex, age, etc), allergic symptoms (type, seasonality, and allergy history), and home environment (place, kind, height, conditions, and pets). Then, patients gave oral consent to take a serum sample.

Mite Extracts

Extracts from *D pteronyssinus*, *L destructor*, *C arcuatus*, and *T putrescentiae* were manufactured using standardized internal protocols (Laboratorios LETI). Briefly, extracts were prepared with semi-purified mite bodies ($\geq 80\%$ mite bodies) in 0.01 mol/L of phosphate buffered saline (1:20 weight/volume) and centrifuged, and the supernatant was collected, dialyzed, filtered, and freeze-dried. The extracts were standardized to in-house reference preparations for each species to evaluate protein content (Lowry-Biuret method), biological potency (by enzyme-linked immunosorbent assay competition), protein profile (sodium dodecylsulfate polyacrylamide gel electrophoresis), and allergenic profile (immunoblot).

In addition, major allergen content (Der p 1 and Der p 2) was determined for *D pteronyssinus* (enzyme-linked immunosorbent assay detection kits; Indoor Biotechnologies, Charlottesville, Virginia). Main allergens of *D pteronyssinus* (Der p 1, Der p 3, Der p 8, and Der p 10), *L destructor* (Lep d 2), and *T putrescentiae* (Tyr p 2) were identified by mass spectrometry (Plataforma Proteómica, Parc Científic, Barcelona, Spain). Briefly, proteins were in-gel digested with trypsin (Promega Corporation, Madison, Wisconsin) in the automatic Investigator ProGest robot of Genomic Solutions (Digilab, Marlborough, Massachusetts). Peptides were identified by liquid chromatography and tandem mass spectrometry in a NanoAcquity (Waters, Milford, Massachusetts) high-performance liquid chromatograph coupled to an Orbitrap Velos mass spectrometer (Thermo Scientific, Kanagawa, Japan). Raw data were

Table 1
Climatic characteristics and exposition to domestic mites in 4 Spanish areas^a

	Lugo (NW)	Santander (N)	Barcelona (NE)	Cartagena (SE)
Climatic characteristics				
Altitude (m.a.s.l.)	445	5	4	4
Annual mean temperature (°C)	11.5	14.5	15.5	17.1
Mean maximum temperature (°C)	16.8	18.1	20.0	22.0
Mean minimum temperature (°C)	6.3	10.2	11.1	12.3
Mean annual rainfall (mm)	1,084	1,246	640	339
Mean relative humidity (%)	78	75	72	71
Prevalence of domestic mites (% mattresses with mites)				
Samples analyzed, n	84	57	148	20
House dust mites				
<i>Dermatophagoides pteronyssinus</i>	98.8%	96.5%	49.2%	90.0%
<i>Dermatophagoides farinae</i>	4.8%	21.1%	65.1%	80.0%
<i>Euroglyphus maynei</i>	41.0%	24.6%	4.0%	0%
Storage mites				
<i>Lepidoglyphus destructor</i>	34.9%	21.1%	0%	0%
<i>Glycyphagus domesticus</i>	7.2%	5.3%	6.3%	0%
<i>Chortoglyphus arcuatus</i>	38.6%	22.8%	0.8%	0%
<i>Tyrophagus putrescentiae</i>	15.7%	3.5%	7.1%	5.0%
<i>Acarus siro</i>	3.6%	1.8%	0%	0%

Abbreviations: N, northern Spain; NE, northeastern Spain; NW, northwestern Spain; SE, southeastern Spain; m.a.s.l, meters above sea level.

^aSources: Agencia Estatal de Meteorología (www.aemet.es), the Agricultural Geographical Information System, Iraola and Fernández-Caldas,⁹ Pagán et al,¹⁰ and Boquete et al.¹¹

collected with Thermo Xcalibur 2.2. The database search was performed by the Mascot search engine using Thermo Proteome Discover 1.3.0.339 against a Uniprot database. In addition, immunoblot with specific monoclonal antibodies (Indoor Biotechnologies) was used for Der p 2 identification.

Specific IgE Determination

Specific IgE to *D pteronyssinus*, *D farinae*, *E maynei*, *L destructor*, *G domesticus*, *C arcuatus*, *T putrescentiae*, *A siro*, and the allergen components nDer p 1, rDer p 2, and rDer p 10 were quantified with the commercially available ImmunoCAP system (Thermo Scientific, Uppsala, Sweden), except for *C arcuatus*. This extract was biotinylated with a biotin labeling kit (Roche Diagnostics, Indianapolis, Indiana) and incubated with streptavidin ImmunoCAP. Levels higher than 0.35 kU/L were considered positive, with an upper limit of 100 kU/L.

Immunoblots

The allergenic profile was determined by western blot using 40 µg of protein from *D pteronyssinus*, *L destructor*, and *T putrescentiae* or 0.6 µg from Der p 1 (Indoor Biotechnologies) in the solid phase under reducing conditions. Proteins were separated according to their molecular weight by sodium dodecylsulfate polyacrylamide gel electrophoresis. Proteins were electronically transferred into a polyvinylidene difluoride membrane (Immobilon-P, Millipore, Billerica, Massachusetts). Afterward, they were incubated with individual serum diluted 1:2. Western blots were developed by a chemiluminescent reaction using the ImmunoStar HRP Chemiluminescent kit (BioRad, Hercules, California).

Images were analyzed using Image Quant TL 2005 (Pharmacia Biotech, Uppsala, Sweden).

Statistical Analysis

Statistical analysis of the data was performed with SigmaStat 3.5 (San Jose, California). Data description, Fischer exact test, and χ^2 test were used to analyze relations between sensitizations, Spearman rank-order correlation was used to compare wheal size or sIgE between species, and analysis of variance (Mann-Whitney and Kruskal-Wallis tests with the Dunn test to compare groups) and the Wilcoxon signed rank test were used for intragroup comparison.

Results

Patient Population and Clinical Characteristics

The personal characteristics of patients are listed in Table 2. There were substantial variations among areas, especially in the percentage of participants living in a rural habitat (localities with <2,000 inhabitants), ranging from 58% in the NW to only 2.2% in the NE.

Concerning clinical characteristics, the incidence of asthma with and without rhinitis was higher in the NW (72%), followed by the SE (63%), N (49%), and NE (36%). However, the incidence of rhinitis and rhinoconjunctivitis was similar in all areas (96–86%). For seasonality of symptoms, most participants (91.1%) reported perennial

symptoms (minimal in the NE at 82.2%; maximal in the NW at 100%).

Skin Prick Tests

The percentage of sensitization to the different species is presented in Table 3, and Figure 1 presents a boxplot showing the wheal sizes.

The SPT results showed a high rate of polysensitization to the different SM species, with more than 97% of participants in the NW sensitized to the different SMs. In the N area, the percentage was lower (72%) but higher than in the 2 Mediterranean areas (61–63%).

The relation between the age of the participants and the number of species to which they were sensitized was evaluated. Patients' ages were stratified into 5 classes: 0 to 20, 20 to 30, 30 to 40, and older than 50 years. The evaluation showed that the number of sensitizations did not increase significantly ($P > .05$) with age.

Statistically significant differences were observed among areas. The wheal size induced by *D pteronyssinus*, *D farinae*, and all SMs was significantly larger in the NW than in the other areas ($P < .05$). In addition, *L destructor* in the N area induced a significantly larger wheal than in the NE, and *E maynei* induced significantly larger wheals in the NW than in the NE ($P < .05$).

Analysis within the areas showed that *D pteronyssinus* induced significantly larger wheals than the other mite species ($P < .001$), with the exception of *L destructor* ($P = .391$) in the NW. In this Atlantic area, this latter species produced significantly larger wheals ($P < .05$) than the other mites (*D farinae* included). In contrast, in the Mediterranean areas, *D farinae* and *E maynei* induced significantly larger wheals than all other SMs ($P < .001$).

In all 4 areas, there was a significant correlation among the 3 HDM species ($r > 0.570$, $P < .001$). For the SMs, there was a significant correlation among all species ($P < .05$) in the NW, N, and NE, with the highest correlation coefficients ($r > 0.6$) between mites belonging to the same phylogenetic group (Glycyphagidae or Acaridae). In the SE, no significant correlation ($P > .05$) was observed between *L destructor* and Acaridae mites ($r = 0.213$) or between *G domesticus* and *A siro* ($r = 0.193$).

The correlation between *Dermatophagoides* species and SMs in the Mediterranean areas was significant ($P < .05$) only for *A siro* and *C arcuatus*, whereas in the Atlantic areas, *L destructor* and *T putrescentiae* significantly correlated with *D farinae* (NW and N) and *D pteronyssinus* (NW only; $r > 0.5$, $P < .001$), and *A siro* was significantly related to the 2 *Dermatophagoides* species in the NW ($r = 0.4$, $P < .001$).

Specific IgE to Mites

Specific IgE to mites was determined in all participants (169). In 7 (4.1%), no sIgE to any mite species was detected so they were excluded from further analysis. Table 4 lists the median levels of sIgE and the percentages of positive serum levels in the remaining participants (NW 43; N 45; NE 43; SE 31). In each area, significantly higher levels of sIgE were found to *D pteronyssinus* than to the other species. Similarly, the second highest value of sIgE, for *D farinae*, was significantly higher than for the other species, with the exception of *L destructor*, in the NW.

Table 2
Characteristics of participants included in the study

	All	Lugo (NW)	Santander (N)	Barcelona (NE)	Cartagena (SE)
Participants, n	169	43	48	45	33
Women, %	57.1	46.5	56.3	68.2	57.6
Age (y), mean $\pm$ SD	30.7 $\pm$ 13.1	31.7 $\pm$ 14.9	26.7 $\pm$ 10.5	31.4 $\pm$ 11.4	34.3 $\pm$ 15.2
Rural habitat, %	27.2	58.1	27.1	2.2	9.1

Abbreviations: N, northern Spain; NE, northeastern Spain; NW, northwestern Spain; SE, southeastern Spain.

Table 3Percentage of patients with positive skin prick test reaction ($>7 \text{ mm}^2$) to mite species and median wheal size in the 4 areas studied

	NW		N		NE		SE	
	%	mm^2	%	mm^2	%	mm^2	%	mm^2
<i>Dermatophagoides pteronyssinus</i>	100	115	100	78.5	100	81	97.0	65
<i>Dermatophagoides farinae</i>	97.7	88	91.7	58.5	93.3	58	97.0	50
<i>Euroglyphus maynei</i>	95.3	80	91.7	49.5	95.6	47	100	51
<i>Lepidoglyphus destructor</i>	97.7	107	87.5	60.5	84.4	24	87.9	29
<i>Glycyphagus domesticus</i>	95.3	78	72.9	33	64.4	17	51.5	9
<i>Tyrophagus putrescentiae</i>	97.7	61	75.0	22	55.0	7	51.5	0
<i>Acarus siro</i>	95.3	55	89.6	28	84.4	18	72.7	21
<i>Chortoglyphus arcuatus</i>	100	59	83.3	27	75.6	21	78.8	25

Abbreviations: N, northern Spain; NE, northeastern Spain; NW, northwestern Spain; SE, southeastern Spain.

The sIgE values for each mite and area are shown in Figure 2. No significant differences among areas were observed for *Dermatophagoides* species or individual allergens. However, sIgE levels to SMs were significantly higher in the NW than in the other areas ($P < .001$).

Most participants with positive sIgE to *D pteronyssinus* had sIgE to Der p 1 and Der p 2 (NW 74.4%; N 70.5%; NE 74.4%; SE 83.9%). No participants with sensitization to only Der p 1 were found in the SE. In the other Mediterranean area (NE), 14% were sensitized to Der p 1 alone, whereas in the Atlantic zones, the rates were lower (NW 4.7%; N 9.1%). Percentages of exclusive sensitization to Der p 2 were similar in all areas (9.1–9.7%) excepting the NE (4.7%). The percentage of patients sensitized to *D pteronyssinus* but negative to Der p 1 and Der p 2 was higher in the Atlantic zones (NW 9.3%; N 11.4%) than in the Mediterranean zones (NE 7%; SE 6.5%).

Only 5 patients showed positivity to Der p 10: 1 reported a seafood allergy and another had seafood and cockroach sensitization, whereas the other 3 did not report an allergy to insects or foods.

Worth noting was the presence of 19 participants (11.7%) who, despite positive SPT reactions to SMs, only presented sIgE to HDMs. This occurred more frequently in the N, NE, and SE (14–16%) than in the NW, where only 1 patient had no sIgE to SMs. Analysis of these participants without sIgE to SMs showed that in the N area they had significantly lower levels of sIgE to Der p 2 ($P = .034$) and lower rates of positive SPT reactions to all SM species ($P < .05$) compared with those with sIgE to SMs. In the Mediterranean areas, they had

lower levels ($P < .05$) of sIgE to *D pteronyssinus*, *D farinae*, Der p 1 (only in NE), and Der p 2 and a smaller wheal only to *T putrescentiae* ($P < .05$). In contrast, only 1 patient (from the N) presented sIgE exclusively to SMs.

A significant correlation ($r > 0.8$, $P < .001$) was observed between sIgE to species belonging to the same group (HDMs or SMs) in the 4 areas. In the Mediterranean areas, a significant correlation ($r > 0.5$, $P < .001$) between HDMs and SMs was found, but in the Atlantic areas, the correlation coefficient ($r < 0.05$) and significance level ($P < .05$) were lower or did not exist (HDMs and Glycyphagidae in the N; $r = 0.2$, $P > .05$). Similar results were obtained in the correlation with sIgE to individual *D pteronyssinus* allergens. In the Mediterranean areas, significant ($P < .05$) and positive ($r > 0.4$) correlations were found between all species and Der p 1 and Der p 2, but in the Atlantic areas, a positive correlation was found for Der p 1 and all SM species in the NW ($r < 0.2$) and to *L destructor* in the N ($r = 0.243$) but without statistically significant differences ($P > .05$). Der p 10 was not significantly correlated with sIgE to any mite species, except for *A siro* in the NW ($r = 0.339$, $P = .026$). Interestingly, in the N and NE, the correlation coefficients between Der p 10 and sIgE to *A siro* ($r = 0.259$ and 0.296) were higher than those obtained with the other mite species ($r < 0.2$).

Immunoblotting

Dermatophagoides pteronyssinus

The *D pteronyssinus* extract presented a protein content of 0.32 mg/mg. Der p 1 and Der p 3 were identified by mass spectrometry in the same band at 30.6 kDa, whereas Der p 8 and Der p 10 were detected in 1 band at 29.0 kDa. Der p 2 was identified with monoclonal antibody anti-Der p 2 at 16.1 kDa. The major allergen content was $15.1 \mu\text{g}$ of Der p 1 and $11.5 \mu\text{g}$ of Der p 2 per milligram.

Immunoblot analysis was performed on the 161 sera with positive sIgE to *D pteronyssinus*. No bands were detected in 6 sera from the NW (14%), 5 from the N (11.4%), 5 from the NE (11.6%), and 6 from the SE (19.4%). The mean sIgE to *D pteronyssinus* in these 22 sera was significantly lower ($P < .01$) than the mean value for sera that recognized any allergen (6.0 vs 37.2 kU/L).

The allergograms (Fig 3A) showed a similar pattern of recognition, with 2 allergens being the most recognized in all zones. The most frequently recognized band was at 14.5 kDa by 75.8% of total sera (NW and NE 79.1%; N 68.2%). The molecular weight of this band corresponds to group 2 allergens. There was a significant relation between sIgE to Der p 2 and the recognition band ($P < .001$ by χ^2 test), and there was a significant difference in mean sIgE to Der p 2 between the sera that recognized this allergen and those that did not (25.7 vs 2.7 kU/L). The second most commonly recognized band (34.8%) was at 27.2 kDa (maximal in the NW at 41.9%; minimal in the SE at 25.8%). This allergen could correspond to Der p 1, because there was a significant relation between sIgE to Der p 1 and the presence of this band ($P < .001$), and sIgE levels in the sera that detected this band were significantly higher

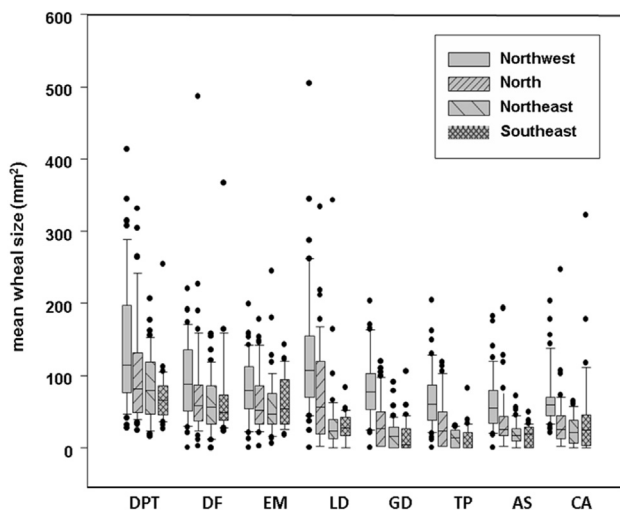


Figure 1. Boxplot of wheal sizes. AS, *Acarus siro*; CA, *Chortoglyphus arcuatus*; DF, *Dermatophagoides farinae*; DPT, *Dermatophagoides pteronyssinus*; EM, *Euroglyphus maynei*; GD, *Glycyphagus domesticus*; LD, *Lepidoglyphus destructor*; TP, *Tyrophagus putrescentiae*.

Table 4

Percentage of patients with specific IgE levels higher than 0.35 kU/L and median specific IgE levels

	NW		N		NE		SE	
	%	kU/L	%	kU/L	%	kU/L	%	kU/L
<i>Dermatophagoides pteronyssinus</i>	100	25.8 ^a	97.8	17.7 ^a	100	26.1 ^a	100	32.3 ^a
<i>Dermatophagoides farinae</i>	100	17.3 ^b	93.3	8.7 ^b	95.3	24.0 ^b	100	20.7 ^b
<i>Euroglyphus maynei</i>	81.4	2.3 ^c	68.9	0.9 ^d	88.4	3.6 ^c	87.1	2.4 ^c
<i>Lepidoglyphus destructor</i>	97.7	13.5 ^b	75.6	1.2 ^c	65.1	0.8 ^d	77.4	1.1 ^d
<i>Glycyphagus domesticus</i>	93.0	8.8 ^d	66.7	0.7 ^d	67.4	0.8 ^e	64.5	0.6 ^e
<i>Tyrophagus putrescentiae</i>	95.3	9.2 ^c	66.7	0.9 ^d	69.8	0.8 ^d	71.0	1.0 ^e
<i>Acarus siro</i>	97.7	4.6 ^e	64.4	0.5 ^e	69.8	0.9 ^d	80.6	1.1 ^d
<i>Chortoglyphus arcuatus</i>	93.0	3.8 ^e	60.0	0.4 ^e	60.5	0.7 ^d	64.5	0.7 ^e
Der p 1	79.1	6.5	77.8	4.6	88.4	7.6	83.9	7.0
Der p 2	83.7	13.3	77.8	6.8	79.1	8.2	93.5	7.7
Der p 10	4.7	0.0	2.2	0.0	4.7	0.0	0.0	—

Abbreviations: N, northern Spain; NE, northeastern Spain; NW, northwestern Spain; SE, southeastern Spain.

In each area, specific IgE values with different superscript letters are statistically significant different.

(29.9 vs 7.3 kU/L; $P < .001$). To confirm this finding, some positive sera were incubated with nDer p 1. The band obtained presented the same molecular weight as the band in the complete extracts.

Lepidoglyphus destructor

The *L destructor* extract used for the immunoblot presented a protein content of 0.32 mg/mg. Lep d 2 was identified by mass spectrometry at 19.4 kDa.

The *L destructor* immunoblot was analyzed in 122 sera from patients with positive SPT reactions and sIgE levels to *L destructor* (NW 42; N 32; NE 27; SE 21), and 14.7% of them did not recognize any allergen. Differences in sIgE levels between these 2 groups were significant only in the N ($P < .001$) and the SE ($P = .048$).

The allergograms (Fig 3B) showed clear differences among areas. Two allergens of approximately 14 to 15 kDa were the most frequently observed, especially in the Atlantic regions (approximately 70%). In contrast, a decrease in the recognition of these 2 allergens and an increase in the recognition of high-molecular-weight allergens were observed in the Mediterranean areas.

Tyrophagus putrescentiae

The *T putrescentiae* extract used for the immunoblot presented a protein content of 0.47 mg per milligram of lyophilized extract. Tyr p 2 was identified by mass spectrometry in 2 different bands, namely 15.42 and 17.48 kDa.

The immunoblot was performed on 92 sera from participants with SPT and sIgE reactions to this species (NW 40; N 29; NE 11;

SE 12). A substantial number did not recognize any allergen, but with differences depending on the zone (minimal in the NW at 7.5%; maximal in the SE at 58.3%). The allergograms (Fig 3C) showed that the allergens recognized by more than 50% of the sera in the Atlantic zones had low molecular weight (14–16 kDa), although there was another predominant allergen at 24.5 kDa in the NW. In the Mediterranean zones, there were no clear dominant allergens, although allergens at 37 and 66 kDa had more than 20% of recognition in the SE.

Discussion

Previous studies have focused on SM-sensitization prevalence in patients sensitized to *Dermatophagoides* species,^{12–14} but little information is available about patients with positive SPT reactions to HDM and SM.

The present objective was to describe the sensitization profile of patients sensitized to HDMs and SMs, diagnosed by SPT, in 4 areas of Spain. Selection of the zones was done according to results of a large survey of domestic mites in mattresses in Spain.⁹ That study showed that in the areas chosen for the present study, exposure to HDM is high, but there are variations in relation to SM exposure. In the NW and N, there is a high prevalence of SMs related to high humidity and rural habitat, especially *L destructor* and *C arcuatus*, whereas in the NE and SE, the abundance of SMs is low, limited to *T putrescentiae*. Although the real level of mites living in patients' homes was not determined and no allergen exposure results were obtained, the data of prevalence of mites^{9–11} in these areas are, in the authors' opinion, robust enough to support the hypothesis that these participants were actually exposed to similar percentages of the different mite species. Moreover, a large number of patients was used in the study to be representative of the population, and the different selected areas correspond to different mite exposition patterns of the coastal areas of Spain.

The results of the SPT showed a very high rate of polysensitization to the 5 SM species in the Atlantic areas and a notably high rate in the Mediterranean areas. A common finding in the 4 zones was the preeminence of *D pteronyssinus*, followed by *D farinae*, *E maynei*, and *L destructor*. The sIgE determination showed an interesting finding: the existence of a relatively large percentage (12%) of participants without sIgE to SM. In the Atlantic area, these patients had significantly smaller wheals to all SM species, but in the Mediterranean zones, they had significantly fewer positive SPT reactions only to *T putrescentiae*. This fact is remarkable because *T putrescentiae* is the most common of the scarce SMs in this area,⁹ suggesting a different sensitization to this species.

The analysis of sIgE confirmed the dominance of HDMs in all areas, although *L destructor* could be considered an important

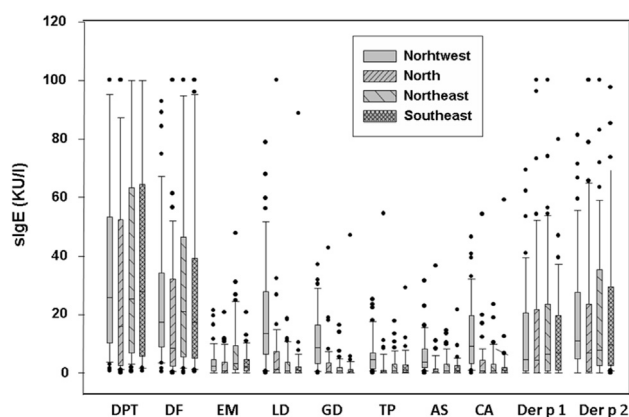


Figure 2. Boxplot of specific IgE levels. AS, *Acarus siro*; CA, *Chortoglyphus arcuatus*; DF, *Dermatophagoides farinae*; DPT, *Dermatophagoides pteronyssinus*; EM, *Euroglyphus maynei*; GD, *Glycyphagus domesticus*; LD, *Lepidoglyphus destructor*; TP, *Tyrophagus putrescentiae*.

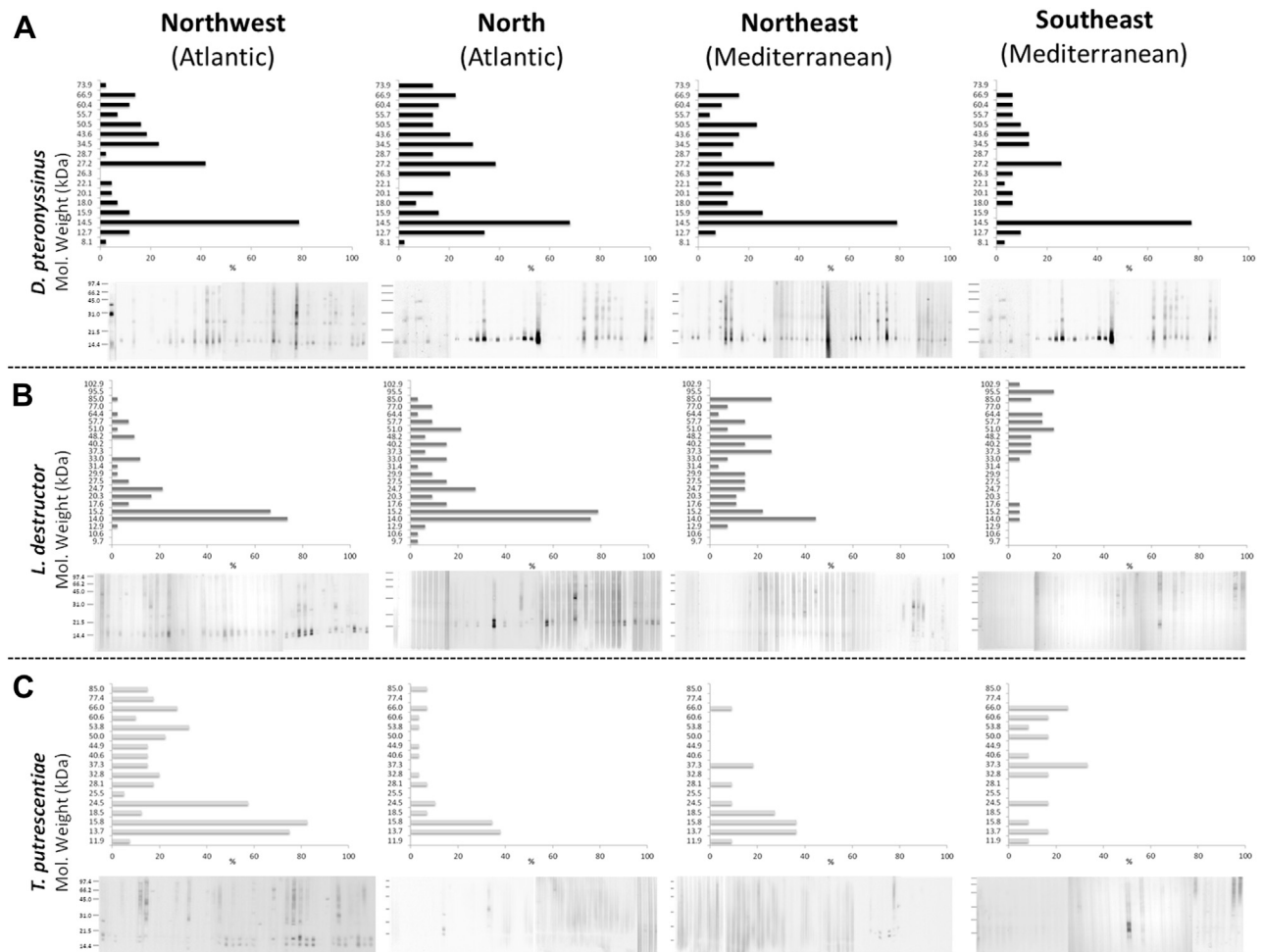


Figure 3. Allergograms and individual immunoblots. Percentage of recognition of bands of *Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentiae* in the 4 geographic areas.

sensitizing species in Spanish Atlantic areas, confirming the findings of previous studies,¹⁵ consistent with the abundance of this species in the zone.¹¹ However, the considerable prevalence of sensitization in the SE observed previously¹⁶ and in the present study is not related to the real mite exposure to *L. destructor*.¹⁰

Regarding other mite species, it is noteworthy that the high exposure to *C. arcuatus* in the NW did not correspond to similar importance in sensitization in this specific group of patients (HDM⁺ and SM⁺), this being different from the results previously obtained in the general allergic population in the same area.¹⁷

Regarding sIgE to *D. pteronyssinus* allergens, in areas with high exposure to SMs (the NW and N), mean levels of sIgE and percentages of serum with sIgE to Der p 2 were clearly higher than those to Der p 1. Furthermore, a larger percentage of patients exclusively sensitized to Der p 2 than to Der p 1 was observed. This fact has been observed previously in different regions of Spain⁸ and particularly in the NW area,¹⁸ although not specifically in patients sensitized to HDMs and SMs. However, in areas with the exclusive presence of HDMs (especially the NE), there were more sera with positive sIgE to Der p 1 than to Der p 2 and a larger percentage of patients sensitized to Der p 1 but not to Der p 2. Interestingly, a similar result has been reported in Marseille, which is close to the NE area.¹⁹ This fact could be related to the lack of exposition to SM species, because group 2 allergens are more abundant in SMs, being the mite sensitization dominated by group 1 HDM allergens.

The low prevalence of sensitization to mite tropomyosin Der p 10 also has been observed in patients with mite allergy in Spain⁸ and Europe.²⁰ Der p 10 sensitization has been associated with seafood allergy, so the latter could be the sensitizing tropomyosin in some cases.²¹

Immunoblots were performed using the most prevalent species of each family (*D. pteronyssinus*, *L. destructor*, and *T. putrescentiae*) in the solid phase and incubating with serum from patients with confirmed sensitization to each allergen (positive SPT and sIgE findings). Although the presence of relevant allergens in the extracts was confirmed by mass spectrometry (from sodium dodecylsulfate gel bands), the identity of the allergens detected on the allergograms by immunoblot cannot be confirmed because of variability in the techniques. However, the identities of Der p 1 and Der p 2 were confirmed by immunoblot (using nDer p 1 and Der p 2 specific monoclonal antibodies, respectively). Furthermore, there was a good correspondence between sensitization to Der p 2 and recognition of the corresponding band.

The allergen recognition pattern for SMs showed differences among zones. In the Atlantic areas with high exposure to SMs, *L. destructor* and *T. putrescentiae* had a distinctive pattern with a very high recognition of allergens coincident with group 2. This group of allergens is considered the major group in all domestic mite species, and it has been cited as responsible for cross-reactivity between taxonomically related species. Thus, HDM group 2 allergens

share an 86% to 89% homology, although homology is lower than 45% between HDMs and SMs and among different SMs, with the exception of *L destructor* and *G domesticus* (79%).²² It also should be considered that some other allergens, such as group 5, described in HDMs and SMs, present a similar molecular weight.

In the authors' opinion, exposure to SM is crucial to explain all these data. According to the present results, an SPT reaction to all SM species in patients with a positive SPT reaction to *Dermatophagoides* species could be considered unnecessary, but the strategy may differ depending on the zone. In areas with high exposure to SMs, a real sensitization to these species seems to exist, mainly involving group 2 allergens, especially to the most abundant species, *L destructor*, which can be considered the representative SM and should be included in the diagnosis.

However, in areas with low exposure to SMs, sensitizations observed by SPT are not relevant and appear to be related to HDM exposure. Similar results were obtained in different areas where patients with positive SPT reactions to certain SM species are often found with no exposure to this species. This is the case of *Blomia tropicalis* in the United Kingdom²³ or in the Spanish Mediterranean area.¹⁶ However, the exposure data and the fact that *T putrescentiae* is the only species that produces a significantly smaller wheal when there is no sIgE to SM suggest that this species could be included in the diagnosis.

Additional inhibition studies will be necessary to discern cross-reactivity from real sensitization in areas with high SM exposition.

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Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2014.10.005>.

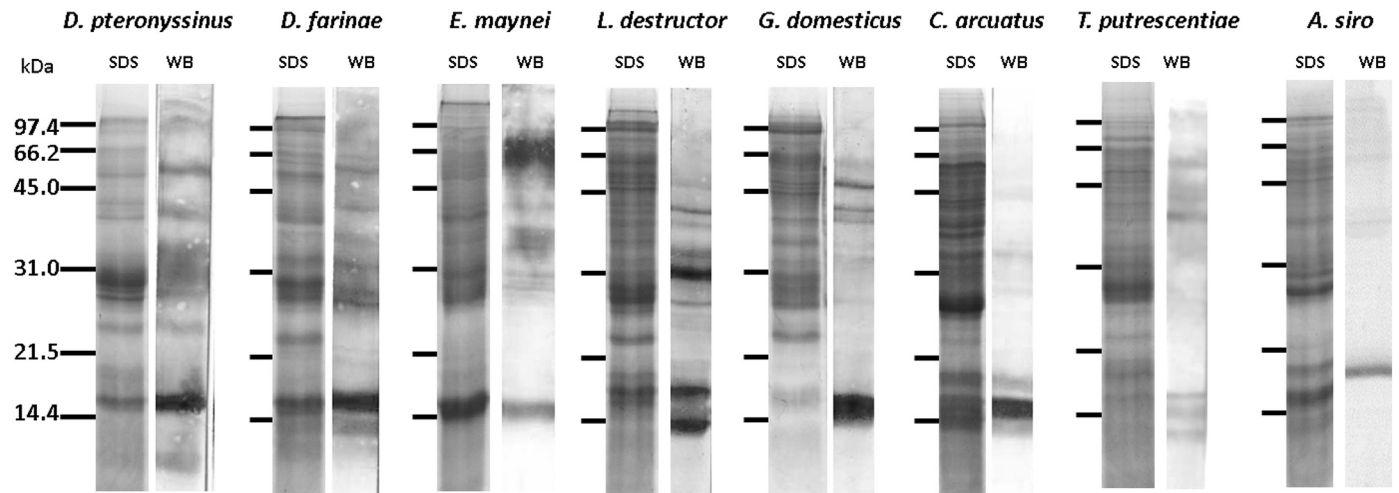


Figure S1. Protein profile (sodium dodecylsulfate [SDS]) and immunoblot (western blot [WB]) of all mite extracts used to manufacture the pricks.