

## HOUSE DUST MITES AND THEIR ALLERGENS IN DANISH MATTRESSES – RESULTS FROM A POPULATION-BASED STUDY

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**Abstract:** The purpose of this study was to identify the level of house dust mites (HDMs) and their allergens in mattresses, not selected on their owners atopic status, and to find associated factors. Dust was collected from 68 mattresses. The recruitment was population-based and conducted during the screening phase of a HDM intervention study. The visited persons declared to have had a “cold” bedroom the previous winter. HDMs were counted and dust was analysed by ELISA for Der 1 (= Der f 1+ Der p 1+ Der m 1). Multiple regression analysis was carried out to find housing conditions associated with high HDM levels. Type of housing, mattress age and self-assessed winter bedroom-temperature explained 47% of Der 1. Median concentrations were 3.77 µg Der 1/g and 1 HDM/0.1 g dust. Both immunochemically and microscopically *Dermatophagoides farinae* was dominant; *D. pteronyssinus* less frequent but important; and *D. microceras* insignificant. In 62% of these suburban homes the mattress dust exceeded 2 µg Der 1/g; and measurement of both Der f 1 and Der p 1 was necessary and sufficient to evaluate HDM allergen exposure. The association with a high HDM level was highest and most consistent for one-family houses.

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### INTRODUCTION

House dust mite (HDM) occurrence varies between geographic areas. In Denmark, a few studies on the house dust mite (HDM) allergen concentration [26, 27] or mite density [4,13] have been made among the general population. Results from such studies are more indicative for the overall HDM exposure than results from analyses based on samples from the homes of allergic patients [14,15]. Usually, lower HDM levels are seen in samples originating from homes in the general population than from HDM allergic patients [20, 30]. Currently, the majority of HDM studies have measured the major HDM allergens: Der p 1, Der f 1 and perhaps Der m 1. Microscopy is sometimes used either as the only method, or in rare cases in combination with HDM allergen analyses. Knowledge of

factors casually related to mite growth is important when planning effective intervention strategies. Some housing characteristics have been shown to be associated with the HDM level, but only a part of the HDM level can be explained by these [10, 12, 28, 34].

The aims of the present study were to identify factors that might favour high levels of *Dermatophagoides farinae*, *D. pteronyssinus* and *D. microceras*, as well as their major allergens Der f 1, Der p 1 and Der m 1. This was achieved by determination of mites and allergens in mattress dust and comparing this to a number of housing parameters.

### MATERIAL AND METHODS

**Inclusion of homes.** The present work reports cross-sectional data from the screening and inclusion phase of a

HDM intervention study. An invitation to participate was sent to 635 randomly selected 18-70 year old Danish citizens in a suburb of Copenhagen (Gladsaxe) with a broad diversity in housing standards and social conditions. Those who wanted to participate and fulfilled the inclusion criteria (see below) were visited (N = 68) from May–September 1999. The inclusion criteria were: a bedroom they considered to have been kept cold during the previous winter; and having lived in the same house and used the same mattress for at least 1 year. The cold bedrooms were chosen since the participants should be candidates for an intervention study focussing on increasing the temperature in cold bedrooms. The time-schedule for the study was chosen and recruitment for the intervention was completed before the heating season 1999–2000.

**Dust sampling.** Dust was collected for 2 min/m<sup>2</sup> by KES with a dust-sampling device containing a cellulose filter (ALK-Abelló, Hørsholm, Denmark) [11] connected to a HEPA-filter vacuum cleaner (Nilfisk-Advance UZ964 (650 W), Åmål, Sweden). The sample was taken from the entire upper surface of the mattress of the 68 persons recruited. Dust samples were kept cool (up to 8 hours) and then stored at -20°C until extraction.

**Processing of dust samples.** After being thawed at room temperature, 0.1 g dust was dispersed in 5 ml 80% lactic acid stained with lignin pink, in an 8.5-cm circular Petri dish, providing a 1-2 mm layer of lactic acid and dust [21]. The rest of the sample (including the filter paper) was extracted with 0.125 M ammonium hydrogen carbonate + 0.1% sodium azide [28, 35] added 0.1 M of the protease-inhibitor  $\epsilon$ -amino caproic acid. The extraction was carried out in a 20-ml polypropylene syringe by slow rotating for 2 hours. The dust:buffer w/v ratio was 1:5 (+0.5 ml buffer to wet the filter), except for dust samples < 0.5 g and >2.9 g where 3 respectively 15 ml buffer was used. The ooze was squeezed out of the syringe, leaving the filter and part of the coarse particles behind, and centrifuged for 20 min at 1223 g. The supernatant was filtered using a 0.8  $\mu$ m acetate filter and stored at -20°C until analysis.

The concentrations of Der p 1, Der f 1 and Der m 1 in the supernatant were assessed in duplicate by ELISA at ALK-Abelló, Hørsholm, Denmark [23]. The detection limit was 2 ng/ml for Der p 1 and Der f 1 and 4 ng/ml for Der m 1, while the detection limits per gram varied due to variation in the dilutions. When the concentration was below the detection limit, this value was used for calculations. *Dermatophagoides* group 1 allergens (Der 1) was calculated as the sum of Der p 1, Der f 1 and Der m 1. Results are expressed as  $\mu$ g allergen/gram of mattress dust.

After incubation for at least 1 day, the mites in the lactic acid preparation were counted by one of the investigators (TEH). A stereo microscope with illumination from below and a magnification  $\times$  25 (Olympus type SZ 4045 TR) was used. For the identification of mite species and their stages, the specimens were collected from the lactic acid with a

needle, placed on a slide in a drop of Hoyer's medium, and examined by normal phase-contrast microscopy by  $\times$  100 enlargement or more (Olympus type BX 40) [17, 29].

**Questionnaires.** In the invitation and questionnaire, given after a visit, several questions concerning housing conditions and habits were given, and incomplete answers were followed up by an interview. The following questions were asked: type of housing; number of children, adults and rooms; where the person slept; and during previous winter (day and night): self-assessed temperature in the bedroom and in the remainder of the dwelling, and if bedroom door or window was usually open, half-open or closed; area of bedroom and dwelling; number of persons sleeping in the bedroom; pets in the bedroom; geographical orientation of the bedroom window(s); whether the bedroom was evaluated as easy to clean; type of floor; use of heavy underblanket and thin washable underblanket; single or double-sized mattress; frequency of vacuum cleaning of the mattress and bedroom floor; frequency of washing the bedroom floor, duvet, pillow and thin washable underblanket; bedclothes change rate; age of house, mattress, duvet, pillow and thin washable and heavy underblanket; smell of mould or visible mould/water spots in the bedroom or in the remainder of the dwelling; type of window(s) in the bedroom; condensation on bedroom windows on winter mornings; daily duration of open bedroom windows and/or door during winter; drying clothes indoors; and extra ventilation measures when cooking and after baths.

**Statistical analysis.** In backward multiple regression analysis the concentrations of Der 1, Der f 1, Der p 1, Der m 1, *Dermatophagoides*, *D. farinae* and *D. pteronyssinus* were tested against 6 independent variables. These were self-assessed bedroom-temperature during previous winter; geographical orientation of the bedroom window(s); and factors suggested in other studies to be associated with HDM (allergen) concentrations as type of floor [2, 16], type of housing [6], size of household [9] and age of mattress [10, 12, 25, 28]. In the calculation time-weighted self-assessed bedroom-temperature ( $2/3 \times$  self-assessed day temperature +  $1/3 \times$  self-assessed night-temperature) and mattress age (log years) was used. Multiple linear regression analysis was used for Der 1  $\mu$ g/g (log) and Der f 1  $\mu$ g/g (log). Multiple logistic regression was used for concentration of Der p 1 and Der m 1 ( $</\geq 2 \mu$ g/g); and for density of *Dermatophagoides* spp., *D. farinae* and *D. pteronyssinus* ( $</\geq 1$  mites/0.1 g).

In univariate regression analyses (using the same transformation and discrimination limits as in the multiple regression), there were screened for associations between HDM (allergen)s concentration and the remaining information about housing conditions and habits from the questionnaires including persons per unit area; plus gender, age, mattress area and quantity of dust. If a correlation was found, it was tested in a multiple linear/logistic regression analyses with those of the above-mentioned variables, which in this study was shown to influence on HDM.

**Table 1.** Backward multiple regression between house dust mites and their allergens; and housing conditions and habits (n=68).

	Coefficient				Odds Ratio						
	Der 1	Der f 1	$\geq/ < 2 \mu\text{g Der p 1/g}$	$\pm/ - \text{Dermatophagoides}$	$\pm/ - \text{D. farinae}$	$\pm/ - \text{D. pteronyssinus}$					
Undetached one-family house vs. apartment	13.6 (4.0-45.9)**	18.9 (5.0-71.6)**	3.2 (0.5-18.7)	3.0 (0.8-10.7)	4.3 (1.1-17.2)*	1.5 (0.3-7.7)					
Detached one-family house vs. apartment	30.2 (9.6-95.5)**	28.8 (8.2-101.4)**	6.5 (1.3-33.9)*	11.5 (2.8-46.5)**	14.9 (3.6-61.4)**	2.5 (0.6-11.1)					
Time weighed bedroom temperature	1.2 (1.0-1.4)*	1.2 (1.1-1.4)*	–	–	–	–					
Mattress age (log)	5.9 (1.7-20.5)*	10.1 (2.6-39.3)*	–	–	–	–					
Rsqr	0.47	0.46									

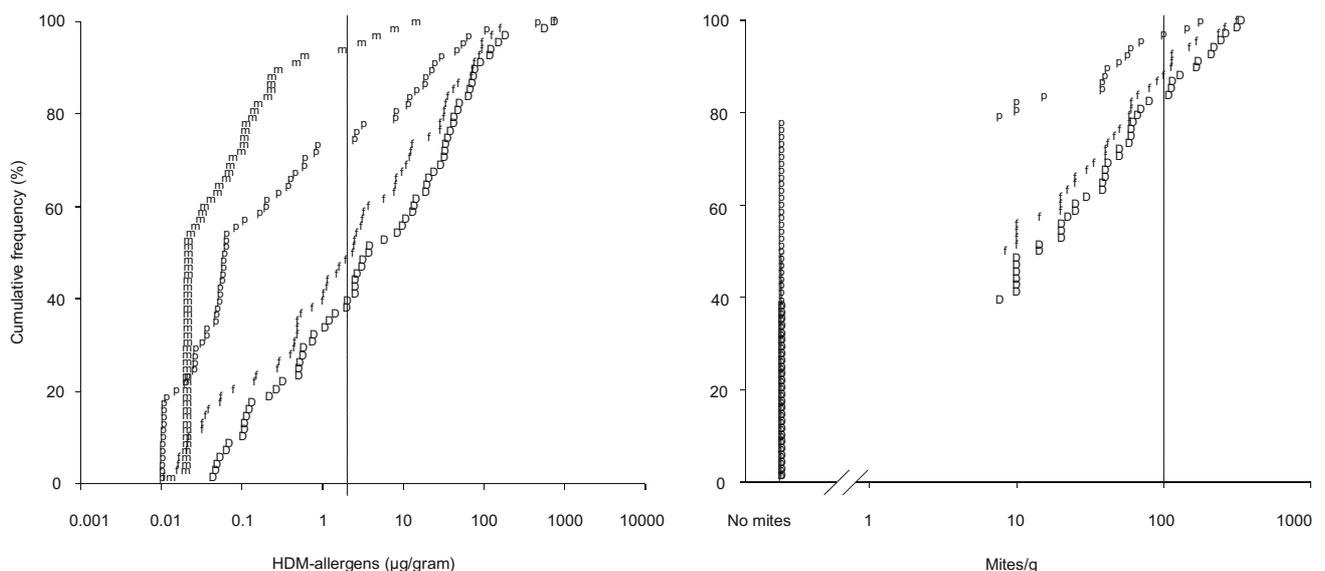
Backward multiple linear regression was carried out with  $\log(\text{Der1})\mu\text{g/g}$  and  $(\text{Der f 1})\mu\text{g/g}$ . Data given for Der 1 and Der f 1 are coefficients after back-transformation. Backward multiple logistic regression was carried out with *Dermatophagoides*, *D. farinae* or *D. pteronyssinus* ( $</>1$  mite/0.1g dust) or Der p 1 ( $</>2 \mu\text{g/g}$ ); housing type was forced to remain in the logistic regression. 95% confidence interval are given in ( ).  $P < 0.05$  are marked with \* and  $P < 0.001$  with \*\*. Besides variables listed in the table, geographical orientation of the bedroom-window(s), household and type of floor was included in the linear and logistic regression analysis, but had no influence; time-weighted bedroom-temperature and mattress age had no influence in the logistic regression analysis.

We wanted to evaluate the agreement of the measurement of HDM allergen concentration and HDM density in expressing the HDM load, and since in many of the preparations for mite identification no mites were found, this was best treated as categorical data and Kappa was calculated [1].

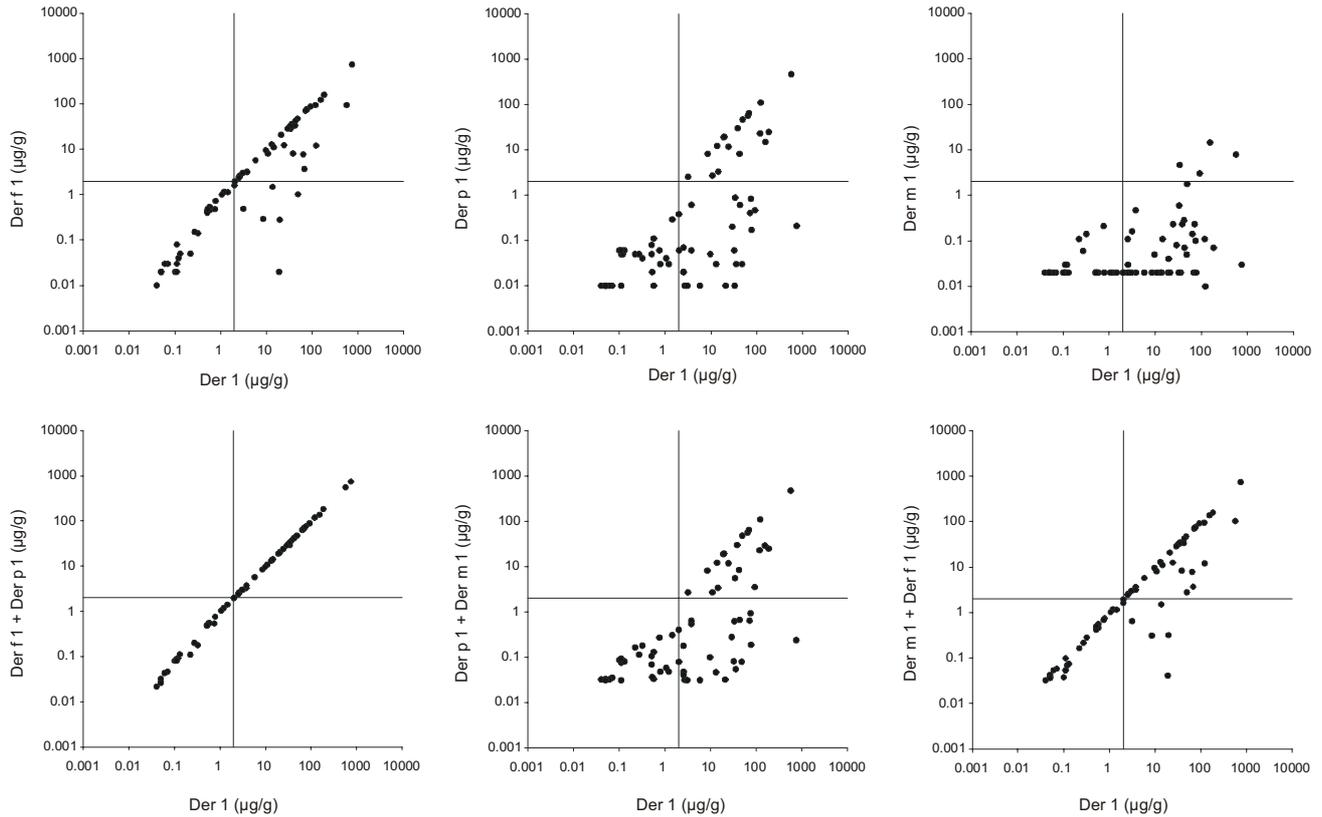
The statistical programmes of SigmaStat 2.03 (SPSS Inc., Chicago, USA), R 1.1.1 (free software ([www.r-project.org](http://www.r-project.org))) [18] and Quattro Pro 8 (Corel, Ottawa, Canada) were used.

## RESULTS

**The screening questionnaire.** Of 635 persons, 423 (67%) responded to our invitation. Of these, 64% reported that they kept their bedroom cold during winter, but only 68 persons fulfilled all inclusion criteria and were willing to participate. The median time-weighted self-assessed bedroom-temperature referring to the previous winter was for the visited group (N = 68) 17°C (range 7–20) as opposed to 18°C (3–27) for the total group responding to our invitation (N = 423). Minor differences were reported between the visited and the



**Figure 1.** Cumulative frequency of house dust mite allergen and house dust mite concentration in dust samples from 68 mattresses. A) Der 1 (D), Der f 1 (f), Der p 1 (p) and Der m 1 (m) shown as  $\mu\text{g/gram}$  mattress dust. Line corresponds to  $2 \mu\text{g/g}$ . B) *Dermatophagoides* (D), *D. farinae* (f) and *D. pteronyssinus* (p) shown as mites/gram mattress dust. Line corresponds to 100 mites/g.



**Figure 2.** Correlation between Der 1 and: Der f 1 (A), Der p 1 (B), Der m 1 (C), Der f 1 + Der p 1 (D), Der p 1 + Der m 1 (E), Der m 1 + Der f 1 (F) in dust-samples from 68 mattresses. Line corresponds to 2 µg/g.

remainder of the responders concerning habits in opening and closing doors and windows, but not concerning: age; sex; number of rooms; self-assessed temperature in the remainder of the dwelling the previous winter; and type of housing.

**HDM allergens and HDM in dust samples from 68 mattresses.** Figure 1 shows the cumulative frequency of the concentration of the allergens Der 1, Der f 1, Der p 1 and Der m 1 (medians in µg/g: 3.77, 2.41, 0.06 and < detection limit), as well as the density of *Dermatophagoides* spp., *D. pteronyssinus* and *D. farinae*. In 62% of the samples Der 1 was  $\geq 2$  µg/g. Only the concentrations of Der 1 and Der f 1 were normally distributed after log transformation. Median HDM density was 1 mite/0.1 g dust with *D. farinae* being the most prevalent mite in the microscopy. *Dermatophagoides* spp. were detected in 40 samples; of these, *D. farinae* and *D. pteronyssinus* were found together in 10, *D. farinae* alone in 25, and *D. pteronyssinus* alone in 5 samples. The species *D. microceras* was not found.

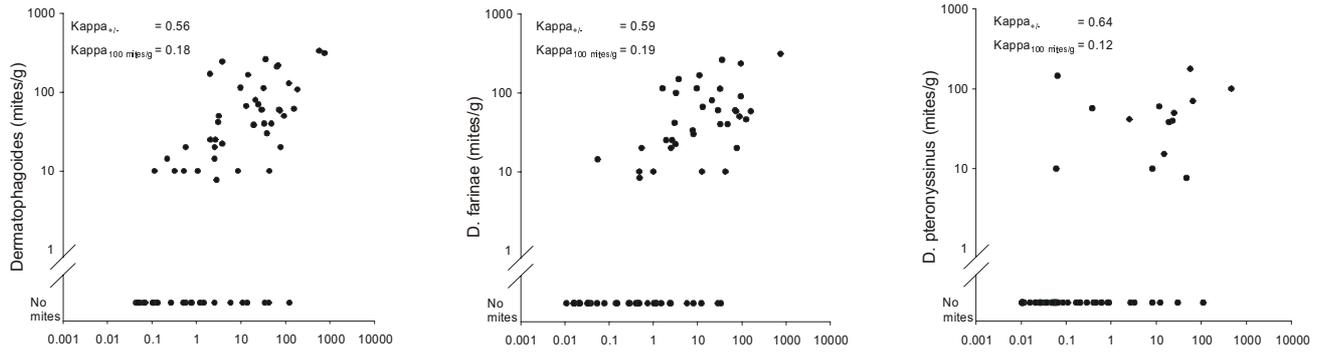
Figure 2 illustrates the correlation between the concentration of the HDM group 1 allergens. It appears that the contribution from Der m 1 is small and the concentration of Der f 1 and Der p 1 accounts for 99.8% of the Der 1 concentration.

Figure 3 shows the concentration in the same samples of the HDM and their allergens. The agreement is moderate to good for  $\leq 2$  µg HDM allergens/g versus  $\leq 1$  HDM/0.1

g dust; but poor for  $\leq 2$  µg HDM allergen/g versus  $\leq 100$  HDMs/g.

**Backward multiple regression with 6 selected independent variables.** In the backward multiple linear/logistic regression analysis (Tab. 1), detached and undetached one-family houses compared to apartments, were positively associated to all the dependent variables except *D. pteronyssinus*. Self-assessed bedroom-temperature during previous winter and mattress age were positively correlated to concentrations of Der 1 and Der f 1. No significant correlations were found to the orientation of the bedroom, household or type of floor for any of the dependent variables. The regression analyses was not valid for  $\leq 2$  µg allergen/g Der m 1, due to few samples with  $\geq 2$  µg Der m 1/g. Type of housing, mattress age and presumed bedroom-temperature during previous winter could explain 47% of the Der 1 concentration.

**Univariate regression.** In the univariate linear/logistic regression analyses the HDM (allergen)s were found to be positive correlations to: number of rooms; bedroom door or window usually closed at night during winter; bedroom evaluated as easy to clean; heavy underblanket, duvet and pillow older than median; and area of the dwelling. Negative correlations were found to storey number; bedclothes change rate; ventilation opening in bedroom more than 8-hour daily; and drying clothes indoor. However, when type



**Figure 3.** Concentration of: Der 1 allergen and *Dermatophagoides* mites (A); Der f 1 allergen and *D. farinae* mites (B); and Der p 1 allergen and *D. pteronyssinus* mites (C) in the same dust-samples. Cut-off values used for calculation of Kappa were  $\leq$  2  $\mu$ g allergen/g dust; and in the microscopy  $\leq$  1 mites/0.1 g dust ( $Kappa_{+/-}$ ) or  $\leq$  100 mites/g dust ( $Kappa_{100 \text{ mites/g}}$ ).

of housing, mattress age and self-assessed bedroom-temperature during the previous winter were included in the regression analyses the correlation only stayed significant, positive for both: Der 1 (log  $\mu$ g/g) and bedroom window usually closed at night during winter; and for Der p 1 ( $\leq$  2  $\mu$ g/g) and bedroom evaluated as easy to clean.

No significant correlation appeared between the different HDM allergen concentrations or HDM densities and the remainder of the tested variables.

## DISCUSSION

Our results may not reflect the HDM exposure for adults in Denmark, since in our study group everyone reported that they keep their bedrooms cold during the winter; they might therefore only be representative for the 2/3 of the randomly selected group who did the same. In addition, patients with allergic symptoms or in risk groups might be more willing to participate and thereby be over-represented in the study. However, with respect to the screening questionnaire no major differences were seen between the visited and the rest of the responders. Furthermore, the Der 1 concentration found in our study is in the same range as in previous studies in the general population in the Copenhagen area [26]. Similarly, the HDM density in Danish non-allergic persons' mattresses [4] was equal to the findings in our study.

In a multivariate-model, the type of housing, mattress age and self-assessed bedroom-temperature in the winter could explain 47% of the Der 1 concentration. The positive association between HDM and one-family houses (compared to apartments) was the most consistent and in concordance with other studies [7]. Often, the type of housing is linked to special housing conditions. A highly significant negative association was found between HDM concentration and drying clothes indoors, but this was a confounder with living in an apartment; so when tested with type of housing the association became a non-significant positive correlation. The storey of the building has been shown to be correlated to the occurrence of HDM, with the lower storey having most HDM [12, 25, 28]. The same association was found in univariate analyses, but it

disappeared in multivariate regression analyses when the type of house was included. Type of house should always be considered when looking for associated factors to HDM exposure and when advising patients.

As in other studies, the mattress age was positive associated with the HDM level in mattress dust [10, 12, 25, 34], therefore the focus on replacement or encasing of old mattresses seems well founded. The carpet on the floor [10, 34] as well as size of household [34] have been shown to be positively associated to HDM allergen concentrations in dwellings, and removal of the carpet is included in many intervention recommendations [32]. This association has only been confirmed in floor samples, and no association was found with our mattress samples.

An increase in temperature under constant absolute humidity will result in a lower relative humidity, and therefore a negative association between temperature and HDM concentration was expected, as seen in other studies [7, 10, 12], but not found. One reason might be that the self-assessed mean winter temperature was less reliable than the spot measurements used in other studies. We chose not to make objective measurements, since it was concluded that a self-assessed winter temperature was probably rather connected to indoor climatic influence on HDM exposure, than a spot measurement during the summertime. The summertime was chosen to make a proper time-schedule for the following intervention study. Geographical orientation of the bedroom window(s) was expected to be a determinant for differences in wind, light and heat exposure influencing life conditions of mites in the bedroom. But we found no association to geographical orientation, neither for HDM allergen concentrations nor HDM density, perhaps because most of the houses were centrally heated buildings of brick or concrete.

*D. farinae* was the dominating mite in both microscopy and allergen analyses. This finding is in agreement with a cross-sectional allergen study from Copenhagen [27]. *D. pteronyssinus* has been shown to be the most frequently occurring mite in mattress dust coming from a mixed group of allergic and non-allergic, as well as randomly selected persons [13, 24]; however, these records refer to materials collected almost 30 years ago, and a drift of species

composition might have happened. The absence or a negligible contribution of *D. microceras* to the HDM load has also been found in most other Danish studies [13, 19, 24, 27]. Only in one Danish study was Der m 1 the dominating Der 1 allergen [26]. *D. microceras* and *D. farinae* are closely related in morphology and allergen composition, and insufficient specificity of the allergen analysis in this early study might explain the discrepancy. In Denmark, testing for Der 1 includes analyses of Der p 1, Der f 1 and Der m 1, but testing with Der p 1 and Der f 1 seems both sufficient and necessary.

The proposed thresholds for HDM sensitisation of 2 µg Der 1/g or 100 HDMs/g [31] was mainly based on studies either measuring allergens [3, 5, 22, 33] or counting mites [20], but in one study, 1.5 µg/g Der p 1 was roughly found to correspond to 100 HDMs/g [8]. Fewer mites were found in samples with similar amounts of allergens, but moderate to good agreement was found between  $</\geq 2$  µg HDM allergen/g and  $</\geq 1$  HDM/0.1 g dust. A distinct conversion factor between acarological counting and allergen measurement is probably not to be found, and in the evaluation of HDM risk factors, etc., references must be to concentration of mites or allergens, and not to calculated conversions between these 2 kinds of observations.

We found measurement of HDM allergens to be more sensitive than acarological analyses, as seen previously [36]. Acarological analyses might be useful when going into more biological investigations, such as intervention studies, where rapid changes in the HDM population may be expected. In contrast, allergen analyses seem to be a more sensitive tool when testing for associated factors, and should be preferred in this type of studies. Also, the allergen analyses better illustrate the allergen exposure and seem more relevant to the HDM allergic patient. Resources available might be decisive; and whereas HDM allergen measurements are mainly expensive in material and equipment, the acarologic analyses are demanding in manpower and expertise.

In conclusion, we found that the type of housing, mattress age and self-assessed bedroom-temperature in the winter could explain 47% of the Der 1 concentration. The association to high HDM level was highest and most consistent to one-family houses. In 62% of the samples, Der 1 exceeded 2 µg/g. Both immunochemically and microscopically *Dermatophagoides farinae* was dominant; *D. pteronyssinus* less frequent, but important; and *D. microceras* insignificant. Since Der f 1 and Der p 1 accounted for 99.8% of the Der 1, the measuring of Der m 1 may be saved.

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