

# Mould-devouring mites differ in guanine excretion from dust-eating Acari, a possible error source in mite allergen exposure studies

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## Summary

**Background and Objective** Measurement of guanine in dust proved a good assessment of mite allergen exposure.

**Methods** Exposure to mite allergens may lead to atopic afflictions. In a semi-natural test system the development of *Dermatophagoides pteronyssinus* (Trouessart) and *Glycyphagus domesticus* (De Geer), and the presence of their guanine excretion, was examined in a dust-soiled and mouldy environment. Mites were counted after heat-escape, and guanine was detected by means of capillary zone electrophoresis. For each species, 50 mites randomly taken, were inoculated on soiled test-surfaces of 10 × 10 cm. Rough wooden board, gypsum board, tufted carpet, and a self-made mattress representing wall surfaces and home-textiles, respectively, were used. Eight weeks after inoculation with mites only, the surfaces were all mould ridden, and mite and guanine measurements were taken. The Spearman rank correlation test and the Mann–Whitney *U*-test were used in statistical analysis. The confidence limit was set at 1%.

**Results** Among the various test-surfaces, no differences were found regarding total mite numbers and amount of guanine present ( $P > 0.01$ ). For the dust-eating mite *D. pteronyssinus*, total mite numbers correlated with the amount of guanine present ( $P = 0.002$ ) on all inoculated surfaces, indicating feeding on the protein-rich dust. For the mould devouring mite *G. domesticus*, however, no such correlation was found ( $P = 0.72$ ). Apparently, they mainly consumed fungal carbohydrates during this experiment.

**Conclusion** The allergological relevance of storage mites has been under discussion for the last 25 years. In humid homes, these mites will feed almost exclusively on fungi and may produce allergenic or irritating substances different from those arising on protein-rich laboratory media used in allergen extract production or present in carpets, bedding and furniture.

**Keywords:** domestic mites, guanine, mite allergens, avoidance studies

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## Introduction

Domestic mites, especially house dust mites (*Pyroglyphidae*), are common inhabitants of houses. Exposure to mite allergens may lead to atopic afflictions such as rhinitis, asthma and atopic dermatitis [1]. The significance of

exposure to house dust mite (HDM) allergens in these disorders has been well documented [2]. In the 1980s, the significance of exposure to storage mites (SM) in allergic diseases, however, was established in rural communities only, and was related to damp environments and agricultural occupations [3–5]. At present, several studies report SM allergy in combination with HDM allergy not only in rural areas, but also in urban and sub-urban surroundings [6–8].

Home textiles as well as wall surfaces may be inhabited

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by both types of mites [9,10]. Mite excretion products, guanine and allergens, are measured in the dust to deduce mite exposure, before an individual avoidance plan is made up [11]. In addition, the efficacy of mite avoidance measures can be easily assessed by the measurement of guanine [1,12].

In houses, HDM and SM share the same niches, although their feeding differs [13]. However, when storage mites (*Acaridae* and *Glycyphagidae*) occur, their growth coincides with fungal growth [10,14].

Mite development and fungal growth depend on the availability of water, and to a lesser extent on the presence of nutrients or predators in the niche. Under suitable conditions, mites and moulds prosper, and produce allergens in clinically relevant amounts [9,15].

Some studies report on cross-allergenicity between HDM and SM allergens. Other studies revealed distinct HDM and SM allergens [reviewed in 16].

Guanine measurement is an important tool in the evaluation of avoidance studies with all species of mites. Therefore, we examined the correlation between HDM and SM and their guanine excretion in a soiled and mould-ridden environment.

## Materials and methods

The domestic mites, *Dermatophagoides pteronyssinus* (Trouessart) and *Glycyphagus domesticus* (De Geer), were reared at room temperature and 55%, 75%, 85%, 88% or 90% relative humidity, under stationary conditions. Mite allergens were assessed by guanine determination. Simulated dwelling conditions were created as described for a semi-natural test system [11,17].

Organisms used were derived from our own laboratory cultures. For each species, 50 mites randomly taken were put on each test-surface. Test-surfaces used were pieces of 10×10 cm of rough wooden board, gypsum board, tufted carpet and self-made mattress, representing wall surfaces and home-textiles.

Different relative humidities (RH) were achieved by the following saturated salt-solutions: calcium nitrate (55% RH), ammonium sulphate (75% RH), potassium chloride (85% RH), sodium bicarbonate (88% RH). Beside these salt-solutions we also used water to achieve a RH of ≥ 90%. Equilibrium situations were assessed with a moisture meter (Rotronic AG, Zurich, Switzerland).

## Experimental set-up

All surfaces were soiled prior to acclimatization. Textile surfaces were soiled with 1.0 g of artificial dust and smooth surfaces with 0.5 g [18].

After soiling, the surfaces were put in a microwave at 450–550 W for 1 min in order to diminish biotic agents

present in the dust. These microwaved and soiled surfaces were acclimatized in polyethylene bags (ARE, Polypax b.v., Barneveld, the Netherlands) for 2 weeks. These bags were placed in a cupboard in a stationary environment of  $T = 20^{\circ}\text{C}$ , and  $\text{RH} = 55\%$  (mean values).

Eight surfaces were enclosed in each bag: two of each surface type. Surfaces were placed in petri-dishes, and their rims were covered with tangle trap (The Tanglefoot Company, Grand Rapids, Michigan, USA). The dishes were covered with a poplin cloth, and tied with an elastic band by hand.

After 2 weeks, the surfaces were inoculated with 50 mites each. Two blanks were used, both microwaved but with no organisms added.

Every 2 weeks, the surfaces were aerated for 10 s by gently waving of the hands above the surface. The whole experiment was repeated after 1 week.

## Analysis

Eight weeks after inoculation, after approximately two life cycles, analysis was performed. This period ensures sufficient amount of mites and a stable mite population [21]. Contaminating fungi were scored by means of a grid with a 1×1 cm matrix, as large as the test-surface. Fungal scores ranged from 0 to 100.

A tape-imprint was also taken of each surface in order to identify the contaminating fungi. These tapes were inoculated for 3 weeks on Oxoid-malt agar with water activities of 0.99 and 0.82 [10], after which mould colonies were identified.

Mite analysis was done by vacuuming each surface for 25 s. In the collected dust a rough estimation of the numbers of living mites was made. This was done by examining the dust for 1.5 minute under a dissecting microscope (10× magnification) and counting the moving mites. Following, a more accurate estimation of the number of remaining living mites was made by use of the heat-escape method [19].

Guanine was quantitatively assessed in the collected dust by means of capillary zone electrophoresis (CZE) [20,21] (10 mM Tartaric acid,  $\beta$ -Alanine, pH 3; capillary of 50 cm in length, internal diameter 50  $\mu\text{m}$ , 30 kV, detection at 254 nm). A Beckman p/ace system 2000 (Beckman Instruments, California, USA) was used. Results were expressed as guanine in mg per gram dust.

## Statistical analysis

The Spearman rank correlation test and the Mann–Whitney U-test were employed. The confidence limit was set at 1%. Statistical analysis was performed by use of SPSS-pc version 4.0.1 [22].

**Table 1.** Guanine content in mg/g dust of non-inoculated surfaces and guanine content and total mite numbers of *D. pteronyssinus* and *G. domesticus* are grouped by the relative humidity (RH). The median and range (in brackets) values are given

| Relative humidity (%) | Blank Guanine | <i>D. pteronyssinus</i> |              | <i>G. domesticus</i> |              |
|-----------------------|---------------|-------------------------|--------------|----------------------|--------------|
|                       |               | Guanine                 | Total mites  | Guanine              | Total mites  |
| 55                    | 34 (14–58)    | 31 (10–57)              | 0 (0–3)      | 28 (10–55)           | 0 (0–10)     |
| 75                    | 18 (10–90)    | 58 (12–194)             | 102 (0–1750) | 29 (10–124)          | 9 (0–1098)   |
| 85                    | 32 (10–65)    | 42 (10–83)              | 6 (0–36)     | 47 (10–138)          | 8 (0–95)     |
| 88                    | 41 (15–60)    | 40 (10–194)             | 58 (10–500)  | 27 (10–179)          | 228 (38–396) |
| 90                    | 27 (10–75)    | 44 (18–125)             | 36 (0–243)   | 81 (17–905)          | 153 (48–336) |

## Results

At the end of the experiment, all surfaces were mould ridden. The contaminating moulds belong predominantly to the species *Aspergillus repens* and *Penicillium chrysogenum*. Other mould taxa found include *Mucor racemosus*, *Paecilomyces variotii* and *Cladosporium*.

*D. pteronyssinus* was present in a range of 0–1750 per dish (median = 18). The maximum number of *D. pteronyssinus* was found on spur wood at 75% RH. *G. domesticus* mites were present in a range of 0–1098 per dish (median = 27). The maximum number of *G. domesticus* was also present on spur wood at 75% RH.

The blanks stayed free of mites but they were covered with moulds to the same extent as the surfaces inoculated with mites, indicating that the moulds originated from the dirt used for soiling. Between blanks and surfaces inhabited by *D. pteronyssinus* the amount of guanine

differed significantly ( $P = 0.0002$ ). However, no significant difference was found in guanine content between blanks and surfaces populated with *G. domesticus* ( $P = 0.02$ ).

Among the various test-surfaces, no differences were found regarding total mite numbers and guanine ( $P > 0.01$ ). Consequently, all results were grouped by relative humidity only (Table 1). The soiling of the surfaces with moulds was equal for all types of surface, except for spur wood and gypsum board inoculated with *G. domesticus*. Gypsum board surfaces inoculated with *G. domesticus* were more covered with moulds, as compared with spur wood inoculated by *G. domesticus* ( $P = 0.008$ ).

As was found previously [9,23,24], total mite numbers of both species correlated with the relative humidity. However, the amount of guanine excreted by *D. pteronyssinus* in the dust did not correlate with the relative humidity. On surfaces populated by *G. domesticus*, the amount of

**Table 2.** Spearman rank correlation (rs) matrix of the variables guanine content, total mites, guanine/mite, and relative humidity (RH) for *D. pteronyssinus* (right, top) and *G. domesticus* (left, bottom). In each cell  $n = 96$ . Significant correlations are in bold type

|                      |                              | <i>D. pteronyssinus</i>           |                                   |                                 |
|----------------------|------------------------------|-----------------------------------|-----------------------------------|---------------------------------|
|                      | Guanine content              | Total mites                       | Guanine/mite                      | Humidity (RH)                   |
| Guanine content      |                              | <b>rs = 0.32</b><br>P = 0.002     | rs = -0.08<br>P = 0.44            | rs = 0.10<br>P = 0.32           |
| Total mites          | rs = 0.19<br>P = 0.72        |                                   | <b>rs = -0.96</b><br>P < 0.000001 | <b>rs = 0.37</b><br>P = 0.0002  |
| Guanine/mite         | rs = -0.18<br>P = 0.07       | <b>rs = -0.90</b><br>P < 0.000001 |                                   | <b>rs = -0.38</b><br>P = 0.0001 |
| Humidity (RH)        | <b>rs = 0.26</b><br>P = 0.01 | <b>rs = 0.68</b><br>P < 0.000001  | <b>rs = -0.58</b><br>P < 0.000001 |                                 |
| <i>G. domesticus</i> |                              |                                   |                                   |                                 |

excreted guanine did correlate significantly with the relative humidity ( $r_s = 0.26$ ,  $n = 96$ ,  $P = 0.01$ ) (Tables 1 & 2).

For the dust-eating mite *D. pteronyssinus* total mite numbers correlated significantly with the amount of guanine present ( $r_s = 0.32$ ,  $n = 96$ ,  $P = 0.002$ ). For the mould-devouring mite *G. domesticus*, however, no such correlation was found. For both mite species total mite numbers were negatively correlated with the amount of guanine per mite (Table 2).

## Discussion

Measurement of guanine has shown to be a good assessment of mite allergen exposure. Previous studies did show a correlation between guanine and mite group I allergens and between guanine and mite numbers [25,26]. The correlation between guanine in mattress dust and mite numbers did however, disappear when mattresses were previously vacuumed [26]. In this study guanine was correlated with total mite numbers in the case of the dust-eating mite *D. pteronyssinus*. In the case of colonization by the mould-devouring species, *G. domesticus*, no such relation was found. This indicates that in mite avoidance studies, assessment of guanine in mouldy environments underestimates the exposure to storage mites. Mite allergens being digestive enzymes, feeding differences may result in differences in faecal pellet composition. A shift from nitrogenous (guanine) to more carbohydrate-rich may be expected, due to the fungal diet of storage mites. This could be an explanation for the variability found in cross-allergenicity studies of storage mites and house dust mites [reviewed in 16].

In mite avoidance studies, humidity management has proven to be successful in reducing mite numbers. For rapid results a combination with short-term procedures, such as acaricidal cleaning, is advised [11,27]. In this study the ambient relative humidity influenced the number of mites. Guanine excretion on surfaces inhabited by *D. pteronyssinus* is not related to ambient humidity, indicating no increase of protein digestion by humidity in the range of 50–90% relative humidity. On surfaces colonized by fungi-eating *G. domesticus*, humidity did increase the guanine amount in the dust, probably by the association of humidity with mould growth.

The relevance of storage mites in allergic disorders has been under discussion for the last 25 years [8,28]. Mite allergens and guanine are both metabolic products of mites. When the diet of the mites changes the composition of their metabolic products will change too. In humid homes, storage mites will feed almost exclusively on fungi and may produce allergenic or irritating substances different from those arising on protein-rich laboratory media as used in allergen extract production. This implies that for storage

mite sensitive persons, who are living in humid environments, specific storage mites assays should also be used, both in sensitization and exposure.

We conclude that the different feeding habits of *Dermaphagoides* and *Glycyphagus* may explain some error on exposure assessment by guanine determination.

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