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## How relevant are house dust mite–fungal interactions in laboratory culture to the natural dust system?

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### ABSTRACT

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Both house dust and house dust mites *Dermatophagoides pteronyssinus* contained a wider range of fungi than laboratory mite cultures. In total, nine species of fungi were isolated from *D. pteronyssinus* in house dust, and these included three xerophilic species (*Eurotium amstelodami*, *Aspergillus penicillioides* and *Wallemia sebi*) commonly found in laboratory cultures of *D. pteronyssinus*. It is concluded that mites do interact with a similar range of fungi in natural dust and in laboratory culture, but that the diversity of fungal species in the laboratory is reduced and the density of individual fungal species in culture exceeds that of house dust. In a second experiment, dust samples were incubated at room temperature with 75% relative humidity. The diversity of fungi invariably declined from up to 13 genera to the few species recorded in laboratory culture. This suggests that the dominance of xerophilic fungi in laboratory mite rearings is mediated primarily by low relative humidity, and the exclusion of air-borne spores.

### INTRODUCTION

Relative humidity is the single most important abiotic factor influencing the reproduction, survival and population increase of pyroglyphid house dust mites (Dobson, 1979; Colloff, 1987a,b; Arlian, 1989; Fain et al., 1990). This factor alone may account for both the geographical distribution and seasonal abundance of house dust mites in temperate climates. Generally, mites are most numerous in houses of low-lying, coastal situations in warm, humid cli-

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mates (e.g. Rijckaert et al., 1981), and their abundance is correlated with indoor humidity; for example, Hart and Whitehead (1990) found that beds in rooms with humidities above 64% relative humidity contained significantly more mites than did those in rooms with lower humidities. Populations of pyroglyphid mites are greatest during the season of highest humidity (Korsgaard, 1983), and dryer parts of houses may be colonised by house dust mites only during the most humid months of the year (Van Bronswijk, 1973).

These observations are consistent with the generality that the abundance of invertebrate detritivores are primarily determined by abiotic factors in arid environments (Menge and Sutherland, 1987). As Crawford (1988) has stated, "...while the nutritional quality of materials ingested by most desert detritivores should have some bearing on their survival, in many instances, the non-nutritional quality of their habitats may be of more immediate significance". Particularly in temperate climates, competition and predation are not important factors in the population dynamics of house dust mites. Species of *Cheyletus* (the most important predatory mite found in house dust) are seldom found at densities likely to have a significant impact on pyroglyphid mite populations; probably because the optimum humidity for *Cheyletus* is 90% (Van Bronswijk et al., 1971).

Despite the accepted importance of abiotic factors in regulation of house dust mite numbers, there is a persistent strand of house dust mite study suggesting that interactions with dust fungi are an important element in the ecology of the Pyroglyphidae. Virtually all studies of the mite-fungus association have been conducted under laboratory conditions, often using mites maintained in long-established cultures. The conclusions of such studies have often been conflicting. For example, Van Bronswijk and Sinha (1973) reported that population increase of *D. pteronyssinus* was enhanced by pre-incubation of the diet with the fungus *Eurotium amstelodami*. They proposed that this fungus rendered the diet more suitable for mites by predigestion, reducing the lipid content of the substratum by assimilation of fatty acids, but the data of Douglas and Hart (1989) are not consistent with this hypothesis. Van de Lustgraaf (1978) and de Saint Georges-Grèdelet (1984) concluded that another fungus, *Aspergillus penicillioides* can either enhance and depress the growth of house dust mite populations depending on various, ill-defined factors. The fungus may represent a source of vitamins (Van Bronswijk and Sinha, 1973) and sterols (de Saint Georges-Grèdelet, 1987b) for the mites, and the antagonistic effects of *A. penicillioides* have been attributed to adverse changes in the physical nature of the substratum resulting from dense fungal colonisation (de Saint Georges-Grèdelet, 1987a).

The relevance of these laboratory studies to the relationship between mites and fungi is uncertain, because laboratory cultures differ from natural house dust systems in several ways:

- (1) Temperature and humidity are maintained at the optimum for growth

and reproduction of mites in the laboratory, i.e. 75% relative humidity and 26°C, respectively.

(2) Long-term cultures of house dust mites may be acclimated to laboratory conditions and therefore may differ physiologically from wild mite populations (Colloff, 1987a,b).

(3) Laboratory mite cultures are dominated by a single species of fungus (Hart and Douglas, 1991), the identity of which may determine the nature of the mite-fungus association.

(4) Laboratory diets are generally more dense and nutritious than house dust. For example, Van Bronswijk and Sinha (1973) and de Saint Georges-Grèdelet (1984) used diets composed of human skin scales, yeast powder and dried *Daphnia*; Van de Lustgraaf (1978) fed mites on a mixture of wheat-germ, yeast powder, dried fish meal, casein and dried *Daphnia*; and Douglas and Hart (1989) used a diet of dried yeast and dried liver powder supplemented with acetone-washed beard shavings. The physical structure and nutritional value of the substratum influence the growth and survival of house dust mites (de Saint Georges-Grèdelet, 1987a), and the response of mites to fungi under such conditions may be different from that in the natural environment.

In this study, two approaches to assess the relevance of laboratory-based investigations of the mite-fungus association to natural dust are considered. First, the incidence of fungi in mites in natural dust is examined. Second, the effect of temperature and humidity on the abundance of house dust fungi is investigated by incubation of natural dust in laboratory conditions.

## MATERIALS AND METHODS

### *Fungi isolated from Dermatophagoides pteronyssinus in house dust*

The data reported here derive from a detailed survey of dust collected in January and June 1989, from 20 houses in Oxfordshire, UK. Each of 206 dust samples was obtained by vacuuming a 0.5-m<sup>2</sup> area for 1 min. Samples were collected from mattresses, bedroom carpets, living room furniture and floors and childrens' soft toys and examined for living *D. pteronyssinus*. More than ten live adult mites were recovered from only five samples and these samples are considered here.

Each mite was surface-sterilised and pierced onto malt extract agar (containing 3% (w/v) malt extract (Oxoid L39), 0.5% (w/v) mycological peptone, (Oxoid L40) and 2% (w/v) agar No. 1 (Oxoid L11)) supplemented with 0–50% (w/v) sucrose (Douglas and Hart, 1989). As controls for surface contamination, surface-sterilised mites were placed on the agar and removed after 2 min without piercing. Plates were incubated at 25°C in darkness for up to 3 weeks. Fungi were subcultured onto recommended media (see Samson and Van Reenen-Hoekstra, 1988) and identified (Raper and Fennell,

1965; Pitt, 1980). The only fungus isolated from sterilised controls was *Penicillium chrysogenum* (2/35 mites).

*Fungi and Dermatophagoides pteronyssinus in house dust maintained in laboratory conditions for up to 10 weeks*

The study was conducted on 20 dust samples collected in June 1989. Each sample was kept in a Petri dish in the laboratory at room temperature (19–22°C) and 75% relative humidity (maintained in sealed plastic lunch boxes, with saturated sodium chloride solution). A layer of glue was applied to the rim of each dish to prevent the escape of mites.

Fungi were sampled by standard dilution plating techniques at 2-weekly intervals. Fifty mg of dust from each sample was suspended in 10 ml sterile, distilled water, vortexed for 1 min and 100 µl of suspension was spread over agar plates (prepared as above) containing 0–50% sucrose. Fungi were incubated, subcultured and identified as above. The relative abundance of each fungus was estimated by a colony count. Control plates spread with sterile, distilled water were included in all experiments.

After 10 weeks, samples were inspected for living *D. pteronyssinus* and the fungi carried by these mites were determined as above.

## RESULTS

In each of the five dust samples bearing more than ten live *D. pteronyssinus*, 66–73% of mites contained fungi that grew on agar. In all, nine species of fungi were isolated from *D. pteronyssinus* freshly removed from dust (see Table 1a). With the exception of one mite in sample LRS 1.3, each mite contained only one species of fungus.

The frequency with which fungi were isolated from *D. pteronyssinus* did not reflect their abundance in dust. *Penicillium aurantiogriseum* for example, was very abundant in dust during January, but was never encountered in mites. Likewise, in June, *Cladosporium* was found in almost every sample of dust, but was not recovered from mites found in fresh dust. *Aspergillus penicillioides*, *Eurotium repens* and *Wallemia sebi* were isolated from mites from dust samples in which these fungi were not encountered.

On collection, all 20 samples contained fungi. A total of 13 genera including species of *Absidia*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Monilia*, *Mucor*, *Penicillium*, *Rhizopus*, *Scopulariopsis* and *Wallemia* were identified. The estimated number of colonies g<sup>-1</sup> dust for the most common genera and species encountered in each sample is shown in Table 2a. Incubation under laboratory conditions resulted in a dramatic change in fungal composition over 10 weeks (see Table 2b). Species of *Absidia*, *Aureobasidium*, *Fusarium*, *Geotrichum*, *Monilia* and *Mucor* were lost within 4 weeks, and, after 10 weeks, only five fungi were found: *Eurotium*

TABLE 1

Fungi isolated from *Dermatophagoides pteronyssinus*

Dust sample	No. mites		No. mites with fungal species <sup>a</sup>									
	total	with fungi	E.a	A.p	E.r	P.a	P.b	P.c	P.ci	P.e	W.s	Cl
(a) collected from fresh dust												
(January 1989)												
LRC 23.3	22	15	.	.	.	.	.	12	2	.	1	
LRC 1.2	15	11	.	2	2	.	.	4	.	1	2	
BM 34.14	16	10	.	1	.	1	3	2	.	.	3	
(June 1989)												
LRS 23.3	33	22	1	.	.	3	8	5	.	3	2	
LRS 1.3	15	10 <sup>b</sup>	1	4	.	.	3	.	.	.	3	
(b) after 10 weeks incubation												
BM 23.34	60	21	.	3	.	.	.	.	.	.	18	.
LRC 23.3	93	56	.	8	.	.	.	.	.	.	47	1
BM 2.34	37	16	.	2	.	.	11	.	.	.	3	.
BF 2.33/4	20	10	.	.	.	.	6	.	.	.	4	.
BM 23.33	15	6	.	.	.	.	.	.	.	.	6	.

<sup>a</sup> E.a = *Eurotium amstelodami*, A.p = *Aspergillus penicillioides*, E.r = *E. repens*, P.a = *Penicillium aurantiogriseum*, P.b = *P. brevicompactum*, P.c = *P. chrysogenum*, P.ci = *P. citrinum*, P.e = *P. expansum*, W.s = *Wallemia sebi*, Cl = *Cladosporium* sp.

<sup>b</sup> One mite in this sample contained both *Aspergillus penicillioides* and *Wallemia sebi*.

*amstelodami*, *Aspergillus penicillioides*, *Cladosporium* sp., *Penicillium brevicompactum* and *Wallemia sebi*. *Aspergillus penicillioides* was not found in any freshly-collected dust sample, but, after 10 weeks, it was isolated from five samples. Likewise, *W. sebi*, recovered from only two samples before incubation was isolated from nine samples after 10 weeks. Very high densities of *W. sebi* were found in two samples; BM 23.34 and BF 23.35. This fungus was not detected in sample BF 23.35 at the time of collection and was the only fungus 10 weeks later. After 10 weeks, two samples did not contain fungi, and these samples also lacked mites.

Live mites were recovered from only one sample at the time of collection, but five samples contained living *D. pteronyssinus* at 10 weeks. These populations were presumably derived from eggs and mites previously undetected. All five dust samples in which mites were found also contained fungi, but no association between the abundance of *D. pteronyssinus* and the presence or absence of any fungal species was evident. Fungi were recovered from 35–60% of mites. Only four species of fungi were found in the mites; *A. penicillioides*, *Cladosporium* sp., *P. brevicompactum* and *W. sebi* (see Table 1b). With the exception of *Cladosporium*, recovered from a single mite, all these species were also recovered from mites found in fresh dust (see Table 1a).

TABLE 2

Abundance of fungi isolated from dust samples collected in June 1989

Dust sample	$10^{-3} \times$ mean no. colonies $g^{-1}$ dust												
	Genera of fungi <sup>a</sup>												
	Ab	Al	As	Au	Cl	Fu	Ge	Mo	Mu	Pe	Rh	Sc	Wa
(a) fresh dust													
BM 23.34	.	.	2.0	.	17.5	.	.	.	.	10.5	.	.	6.0
LRS 23.3 <sup>c</sup>	.	3.0	3.0	.	19.5	.	.	.	0.5	19.5	.	.	.
LRC 23.3	.	1.0	2.0	.	22.5	3.0	.	1.0	0.5	.	0.5	.	.
BM 23.33	.	0.5	2.5	.	9.0	.	.	.	.	23.0	.	.	.
BF 23.33	.	.	2.0	.	11.5	1.0	.	.	.	.	.	.	.
BF 23.33/4	.	1.0	2.0	0.5	68.0	1.0	.	.	.	.	.	.	.
BF 23.35	.	0.5	4.5	.	37.5	1.5	.	0.5	.	4.5	.	.	.
LRC 2.3	.	1.5	.	.	56.0	.	.	.	0.5	2.0	.	.	.
LRS 2.3	.	.	.	.	2.0	.	.	.	.	6.5	.	2.0	.
BM 2.33	.	.	2.0	.	1.0	.	.	.	.	1.0	0.5	.	.
BM 2.34	.	1.0	0.5	0.5	9.5	0.5	.	.	.	5.0	.	1.0	.
BM 2.35	.	.	1.0	.	1.0	.	.	.	.	1.0	.	.	.
ST 2.34/5	.	.	6.5	.	2.0	.	.	.	.	.	.	2.0	.
BF 2.34/5	0.5	1.0	3.0	.	9.0	0.5	.	.	.	4.5	.	0.5	.
LRS 1.3	0.5	0.5	12.0	.	25.0	.	.	1.0	.	9.5	.	0.5	.
LRC 1.3	0.5	0.5	0.5	.	13.5	.	0.5	.	.	11.5	.	1.5	1.0
BM 1.35	.	1.5	1.5	.	11.0	.	.	.	.	5.0	.	.	.
BF 1.35	.	0.5	.	.	8.0	.	.	.	.	3.5	.	.	.
BF 1.33	.	.	.	.	1.5	2.0	.	.	.	8.0	0.5	1.0	.
ST 1.33	.	.	1.0	.	1.0	.	.	.	.	1.0	0.5	.	.
(b) After 10 weeks incubation at 75% humidity and room temperature													
BM 23.34 <sup>c</sup>	.	.	2.0	.	.	.	.	.	.	.	.	.	102.0
LRS 23.3	.	.	3.5	.	.	.	.	.	.	3.5	.	.	2.0
LRC 23.3 <sup>c</sup>	.	.	6.0	.	1.0	.	.	.	.	3.5	.	.	10.0
BM 23.33 <sup>c</sup>	.	.	1.5	.	.	.	.	.	.	11.0	.	.	2.0
BF 23.33	.	.	0.5	.	.	.	.	.	.	.	.	.	.
BF 23.33/4 <sup>c</sup>	.	.	3.0	.	.	.	.	.	.	13.5	.	.	.
BF 23.35	.	.	.	.	.	.	.	.	.	.	.	.	63.5
LRC 2.3	.	.	.	.	.	.	.	.	.	1.5	.	.	.
LRS 2.3	.	.	.	.	.	.	.	.	.	3.0	.	.	.
BM 2.33	.	.	6.0	.	.	.	.	.	.	9.5	.	.	6.0
BM 2.34 <sup>c</sup>	.	.	.	.	.	.	.	.	.	1.5	.	.	.
BM 2.35	.	.	1.0	.	1.0	.	.	.	.	1.0	.	.	.
ST 2.34/5	.	.	11.5	.	2.0	.	.	.	.	.	.	1.0	.
BF 2.34/5	.	.	8.5	.	.	.	.	.	.	4.0	.	.	0.5
LRS 1.3	.	0.5	.	.	.	.	.	.	.	1.0	.	.	.
LRC 1.3	.	.	0.5	.	.	.	.	.	.	0.5	.	.	.
BM 1.35	.	.	.	.	.	.	.	.	.	0.5	.	.	0.5
BF 1.35	.	.	.	.	.	.	.	.	.	.	.	.	.
BF 1.33	.	.	.	.	.	.	.	.	.	.	.	.	2.0
ST 1.33	.	.	.	.	.	.	.	.	.	.	.	.	.

<sup>a</sup> Genera: Ab=*Absidia*, Al=*Alternaria*, As=*Aspergillus*, Au=*Aureobasidium*, Cl=*Cladosporium*, Fu=*Fusarium*, Ge=*Geotrichum*, Mo=*Monilia*, Mu=*Mucor*, Pe=*Penicillium*, Rh=*Rhizopus*, Sc=*Scopulariopsis*, Wa=*Wallemia*.

Species of fungi <sup>b</sup>										
E.a	A.p	E.r	P.a	P.b	P.c	P.ci	P.co	P.e	P.g	P.j
2.0	.	.	.	6.5	.	2.0	.	.	.	.
3.0	.	.	.	8.5	1.0	.	2.0	5.0	.	.
1.0	.	1.0	.	.	.	.	.	.	.	.
2.5	.	.	.	20.0	.	.	.	1.0	.	.
2.0	.	.	.	.	.	.	.	.	.	.
2.0	.	.	.	.	.	.	.	.	.	.
4.5	.	.	.	.	.	.	.	.	.	.
.	.	.	.	2.0	.	.	.	.	.	.
.	.	.	.	5.0	0.5	.	.	1.0	.	.
2.0	.	.	.	.	.	.	.	.	.	.
0.5	.	.	.	5.5	.	.	.	.	.	.
1.0	.	.	.	1.0	.	.	.	.	.	.
6.5	.	.	.	.	.	.	.	.	.	.
3.0	.	.	.	4.5	.	.	.	.	.	.
12.0	.	.	1.0	8.5	.	.	.	.	.	.
0.5	.	.	5.0	6.5	.	.	.	.	.	.
1.5	.	.	.	5.0	.	.	.	.	.	.
.	.	.	.	2.0	.	.	.	0.5	0.5	0.5
.	.	.	.	2.5	4.0	1.0	.	.	0.5	.
1.0	.	.	.	.	.	.	1.0	.	.	.
1.5	0.5	.	.	.	.	.	.	.	.	.
1.5	2.0	.	.	3.5	.	.	.	.	.	.
3.0	3.0	.	.	3.5	.	.	.	.	.	.
1.5	.	.	.	11.0	.	.	.	.	.	.
0.5	.	.	.	.	.	.	.	.	.	.
3.0	.	.	.	13.5	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	1.5	.	.	.	.	.	.
.	.	.	.	3.0	.	.	.	.	.	.
1.5	4.5	.	.	9.5	.	.	.	.	.	.
.	.	.	.	1.5	.	.	.	.	.	.
1.0	.	.	.	1.0	.	.	.	.	.	.
11.5	.	.	.	.	.	.	.	.	.	.
7.0	1.5	.	.	4.0	.	.	.	.	.	.
.	.	.	.	1.0	.	.	.	.	.	.
0.5	.	.	.	0.5	.	.	.	.	.	.
.	.	.	.	0.5	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.

<sup>b</sup> Species: E.a=*Eurotium amstelodami*, A.p=*Aspergillus penicillioides*, E.r=*Eurotium repens*, P.a=*Penicillium aurantiogriseum*, P.b=*Penicillium brevicompactum*, P.c=*P.chrysogenum*, P.ci=*P. citrinum*, P.co=*P. corylophilum*, P.e=*P. expansum*, P.g=*P. glabrum*, P.j=*P. janthinellum*. The only species of *Wallemia* encountered was *W. sebi*.

<sup>c</sup> Samples in which more than ten live *Dermatophagoides pteronyssinus* were found.

Also like natural dust, *W. sebi* was isolated from a proportion of the *D. pteronyssinus* in every sample, even when it was not detected in the dust from which the mites were removed.

## DISCUSSION

### *Fungi isolated from Dermatophagoides pteronyssinus*

Douglas and Hart (1989) found intact fungal spores in all parts of the gut, but not elsewhere in the body of *Dermatophagoides pteronyssinus*. Fungi do not appear to be retained selectively by *D. pteronyssinus* and are passed through the gut with the ingesta. Thus, there is no evidence to suggest that fungi are indigenous gut inhabitants of *D. pteronyssinus*.

The range of viable fungi in *D. pteronyssinus* provides an index of the feeding habit of these mites, although other ingested fungi may be killed during feeding. In particular, ingested fungal mycelium may be undetectable because it is disrupted during grazing (as in Collembola; see Hanlon and Anderson, 1979) or during transit through the gut (as in woodlice; see Coughtrey et al., 1980; Hames and Hopkin, 1989). The physical conditions in the gut of house dust mites have not been investigated, but, as in other keratin-digesting arthropods (tineid moths and mallophaga; see Waterhouse, 1952, 1953), the redox potential of the midgut is probably very low, representing a very hostile environment to obligate aerobes.

In this study, nine species of fungi were isolated from mites collected from fresh dust and only four species from cultured dust. As previously documented by Oh et al. (1986), these data suggest that a greater diversity of fungi may be isolated from mites collected from fresh dust than from laboratory-cultured mites. In previous studies on long term cultures of mites, very few fungi have been isolated. For example, Van de Lustgraaf (1978) found species of *Penicillium* and *Aspergillus penicillioides* in the majority of mites and reported that this fungus was more frequently associated with mites from 14-month rearings than with those from 7-month-old cultures. Hart and Douglas (1991) isolated only *A. penicillioides* and *W. sebi* from *D. pteronyssinus* and found that one fungus was dominant in mites from a single culture.

### *Fungi in dust and substratum of laboratory culture*

Many fungi isolated from fresh house dust did not persist through the 10 weeks incubation at 75% RH and room temperature. Relative humidity is the factor limiting growth of fungi in house dust. For example, Davies (1960) found that several fungi, such as *Mucor* and *Absidia*, grew rapidly in dust at very high humidities (93.7 and 97.8% relative humidity) but not at the lower humidities common to the house dust environment, and that at 75% relative humidity and 25°C, the slow-growing xerophilic members of the Aspergillaceae were dominant. Consequently, these xerophilic fungi are the only per-



manent fungal components of the house dust community under normal conditions (Van Bronswijk, 1981). However, hydrophilic and mesophilic species are routinely recovered from dust because they are usually abundant in outside air and sediment into house dust (Gravesen, 1978). As a result, mites in fresh dust are exposed to a far greater variety of fungi than laboratory mites maintained in culture vessels with lids.

Of the fungi which persisted during incubation at 75% relative humidity, only *A. penicillioides* and *W. sebi* increased in abundance. In particular, the density of *W. sebi* in several dust samples was greatly increased by laboratory incubation from  $< 10$  colonies  $g^{-1}$  to more than 100 colonies  $g^{-1}$ . The density of fungi in laboratory mite rearings can be considerably higher; for example,  $7.2 \times 10^5$  colonies of *A. penicillioides*  $g^{-1}$  substratum are routinely encountered in the *D. pteronyssinus* culture Holl/Cab (Douglas and Hart, 1989), and the *D. pteronyssinus* culture D53 contains  $8.5 \times 10^6$  colonies of *W. sebi*  $g^{-1}$  (D. Hay, unpublished data). At these very high densities, the fungi may have negative effect on mites (Van de Lustgraaf, 1978; de Saint Georges-Griedel, 1984; Hay et al. (in prep.)), arising from adverse modification of the physical nature of the substratum, resource competition and the accumulation of mycotoxins.

The house dust mite-fungal relationship in laboratory culture is relevant to the natural dust system to the extent that a similar range of fungi are involved. However, the fungi are at a considerably higher density in laboratory rearings and therefore the relationship between house dust mites and fungi is more likely to be antagonistic under these conditions than in natural dust.

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