

Diet Influences Growth Rates and Allergen and Endotoxin Contents of Cultured *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* House Dust Mites

Swetha Avula-Poola Marjorie S. Morgan Larry G. Arlian

Department of Biological Sciences, Wright State University, Dayton, Ohio, USA

Key Words

Dermatophagoides farinae • *Dermatophagoides pteronyssinus* • House dust mite • Growth rate • Allergen • Endotoxin

Abstract

Background: The house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are cultured to obtain material for the production of allergen extracts for research, diagnostic and immunotherapeutic purposes. **Methods:** We cultured mites on two different diets that supported thriving populations and determined the population growth rates, dynamics of allergen accumulation, and endotoxin concentrations in extracts made from mites harvested from the cultures. **Results:** *D. farinae* populations grew faster on a diet of rodent chow/yeast than on an egg/yeast diet but a larger peak population size was achieved on the egg/yeast diet. Diet influenced the dynamics of the production of groups 1 and 2 allergens and the group 1/2 ratios for both species. To population peak, Der f 1 was produced at a faster rate on the chow/yeast diet but greater amounts of Der f 1 were produced by mites grown on the egg/yeast diet. *D. pteronyssinus* populations grew faster and achieved greater

density on the egg/yeast diet compared to the chow/yeast diet. *D. pteronyssinus* produced more Der p 1 than Der p 2 when grown on chow/yeast while more Der p 2 than Der p 1 was produced on egg/yeast. Endotoxin concentrations in extracts made from whole cultures for both species at maximum population density were very different in the two diets. Washing the mites resulted in the loss of up to 88% of the allergen. **Conclusion:** Mite-culturing diet directly effects population growth, the dynamics of allergen accumulation, the group 1/2 allergen ratio and the endotoxin contents in extracts of cultured house dust mites.

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Introduction

The house dust mites, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are the source of many potent allergens. Extracts of these mites are heterogeneous mixtures that contain many allergens as well as other non-allergenic molecules. Notably, extracts of these mites contain significant amounts of endotoxin. Previous studies found that *D. farinae* extracts contained more endotoxin than *D. pteronyssinus* [1–3]. Mite extracts also contain

molecules that induce cytokine release by cultured dermal fibroblasts, epidermal keratinocytes, endothelial cells of the dermal microvasculature, and lung epithelial cells and thereby may influence inflammation in the skin and respiratory system [4–9]. Thus, molecules in mite extracts may influence the course of allergic reactions.

These mites are cultured in research laboratories and commercially to produce allergen extracts for research, diagnostic and immunotherapeutic purposes. The amounts of the major allergens and endotoxins in extracts has been shown to differ between different source materials used for extraction [1–3, 10–12]. Climatic conditions (temperature and relative humidity) greatly affect population growth rates in cultured mites and the allergen content and endotoxin concentration in the extracts made from these mites [3, 13–15]. It was recently found, for example, that the accumulation rates for the allergens Der p 1 and Der p 2 vary independently for mites cultured on the same diet at 20 and 25°C [3]. Likewise, the *D. pteronyssinus* material harvested from cultures grown at 20°C contained more endotoxin than when the mites were cultured at 25°C [3].

Different research laboratories and commercial producers of mite allergen extracts use different diets to grow their mites. The effect of these different diets on the allergen and endotoxin profiles of cultured mites grown on different diets has never been investigated. An understanding of how varied diets and climatic conditions affect allergen profiles and endotoxin levels is a key to developing new standardizing protocols for culturing mites, harvesting mite materials from cultures for allergen extraction, and standardizing extracts that meet the requirements of the Food and Drug Administration in the USA [16] and the European Medicines Agency [17] and the European Pharmacopoeia [18] in the European Union. The purpose of this research was to determine how diet influences the cultivation of house dust mites and the allergen and endotoxin contents of extracts produced from mites cultured on different foods.

Materials and Methods

Most of the materials and all of the methods utilized in this study have previously been described in detail [3]. A brief description is given here of the specific details of the present study.

Mite Cultures

All mites for this study were taken from thriving cultures maintained at Wright State University. Prior to the start of the study, stock cultures of *D. farinae* and *D. pteronyssinus* were pre-

pared and maintained on two different foods (diets A and B). Diet A was composed of a 9:1 (vol:vol) mixture of dried whole egg (Bio-Serv, Frenchtown, N.J., USA) and baker's yeast. Diet B was a 9:1 mixture of laboratory rodent chow (Teklad Rodent Diet 8640; Harlan Laboratories, Indianapolis, Ind., USA) and baker's yeast and was the diet used in the earlier study [3]. All stock cultures were maintained at room temperature and 75% relative humidity.

At the beginning of each experiment, 6 g of thriving culture (about 2 teaspoons) on the appropriate food was added to 30 g of diet A or 60 g of diet B (each about 1/3 cup). As in the earlier study, five test cultures were prepared in half-pint Ball® jars for each mite/food combination. All cultures were maintained at normal fluctuating room temperature (averages ranged from 19.6 to 21.8°C) in ventilated humidity chambers at 75% relative humidity. At 2-week intervals, all cultures were thoroughly mixed and several 10- to 100-mg aliquots were removed for immediate live mite counts or frozen for subsequent allergen and biochemical analysis. Cultures were terminated when visual inspection indicated that the food had been depleted.

Mite Population Growth

The number of live mites in precisely weighed 10- to 50-mg aliquots of each individual culture at each sample time was determined with the aid of a stereomicroscope. Mites that were moving, regardless of life stage (larvae, protonymphs, tritonymphs or adults), were counted as live. Mite counts for the five replicates of each mite/food/time were normalized to 100 mg, averaged, and the standard error of the mean (SEM) was determined. Population growth rates to the peak population densities for each mite/food combination were determined using an exponential model [3, 19].

Allergen Analyses

Extracts were prepared from frozen aliquots of whole culture collected from each culture at each sample time as previously described [3]. The amounts of group 1 (Der f 1 or Der p 1) and group 2 allergens were determined in these extracts using enzyme-linked immunosorbent assay (ELISA) kits obtained from Indoor Biotechnologies (Charlottesville, Va., USA) using the universal standard supplied as reference. Parallel samples of whole culture were washed as before and the resulting washed mite samples were also extracted and assayed for allergen content.

Protein and Endotoxin Determinations

Another aliquot of each culture at each sample time was extracted into endotoxin-free water as described [3]. These extracts were analyzed for protein content using the A280 protein program of a NanoDrop 1000 spectrophotometer. The Lonza QCL-1000 endotoxin test kit was used to determine the endotoxin levels in these aqueous extracts.

Results

Mite Population Growth

The live mite population densities of both *D. farinae* and *D. pteronyssinus* increased exponentially over time on both of the test diets (fig. 1).

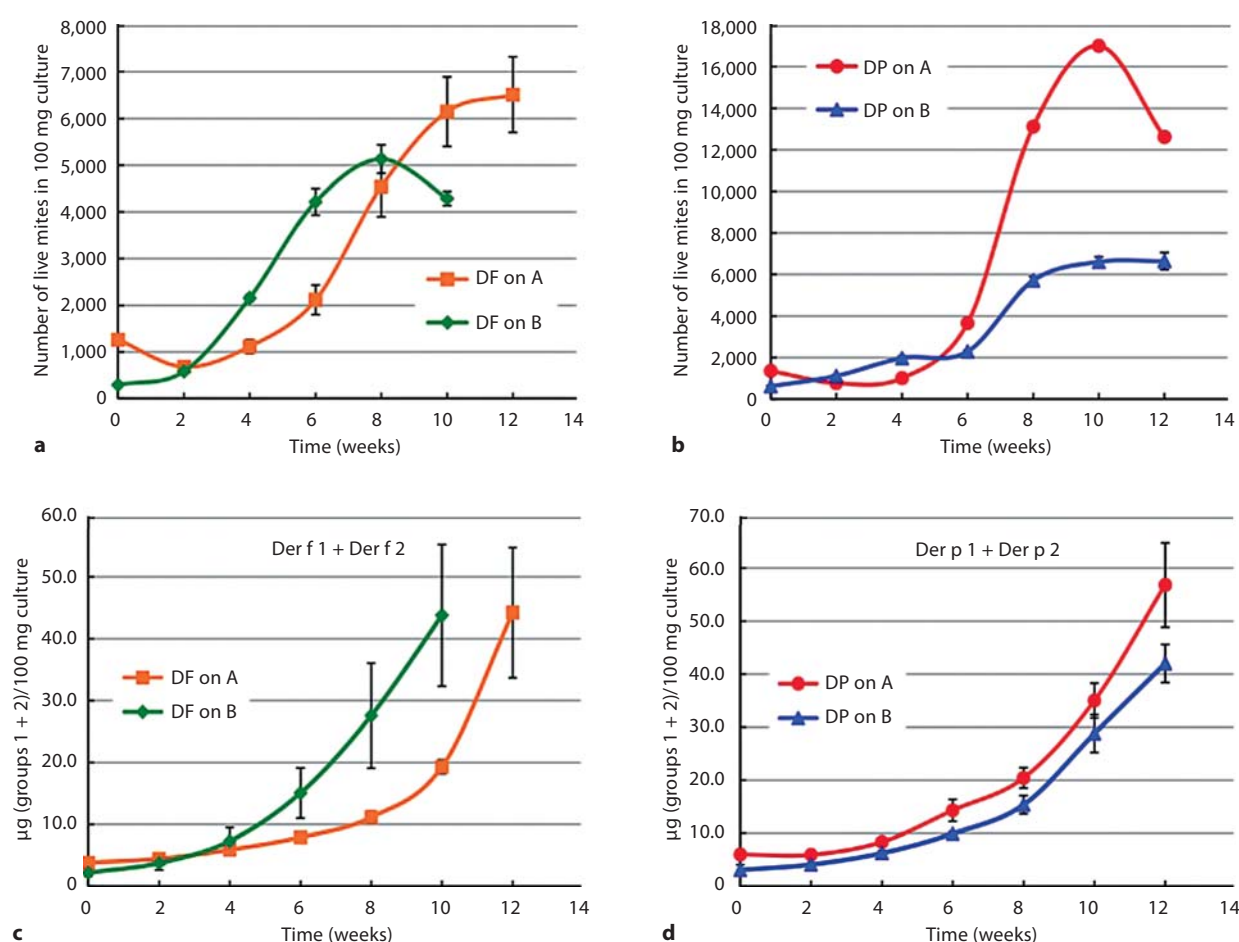


Fig. 1. Population growth (a, b) and allergen accumulation (c, d) by *D. farinae* (DF) and *D. pteronyssinus* (DP) cultured on two foods (diets A and B) at room temperature.

Cultures of *D. farinae* grown on diet A reached a maximum population density in 12 weeks while *D. farinae* grown on diet B reached a maximum density at 8 weeks (fig. 1; table 1). After these times, the food became limited on both diets. The *D. farinae* population growth rate to the peak was slower on diet A (19.1% per week) compared to diet B (38.5% per week). Although the growth rate was slower, larger populations of *D. farinae* developed on diet A compared to diet B. Diet A cultures peaked at a population density of $6,511 \pm 808$ mites per 100 mg of whole culture compared to $5,131 \pm 304$ mites in 100 mg of culture material when mites were grown on diet B.

In contrast to *D. farinae*, *D. pteronyssinus* grew faster on diet A with a population growth rate of 32.1% per week while the growth rate on diet B was 21.0% per week (fig. 1; table 1). On diet A, the *D. pteronyssinus* population growth peaked at $16,998 \pm 112$ mites per 100 mg of whole culture after 10 weeks of growth before the food was depleted and the population declined. This mite density was 2.6 times greater than the peak population size achieved at 12 weeks of culturing on diet B.

Therefore, diet A was the best diet for growing *D. pteronyssinus* while diet B resulted in the fastest growth of *D. farinae*. On these diets with the fastest growth, peak population density was reached in 8 weeks for *D. farinae* and

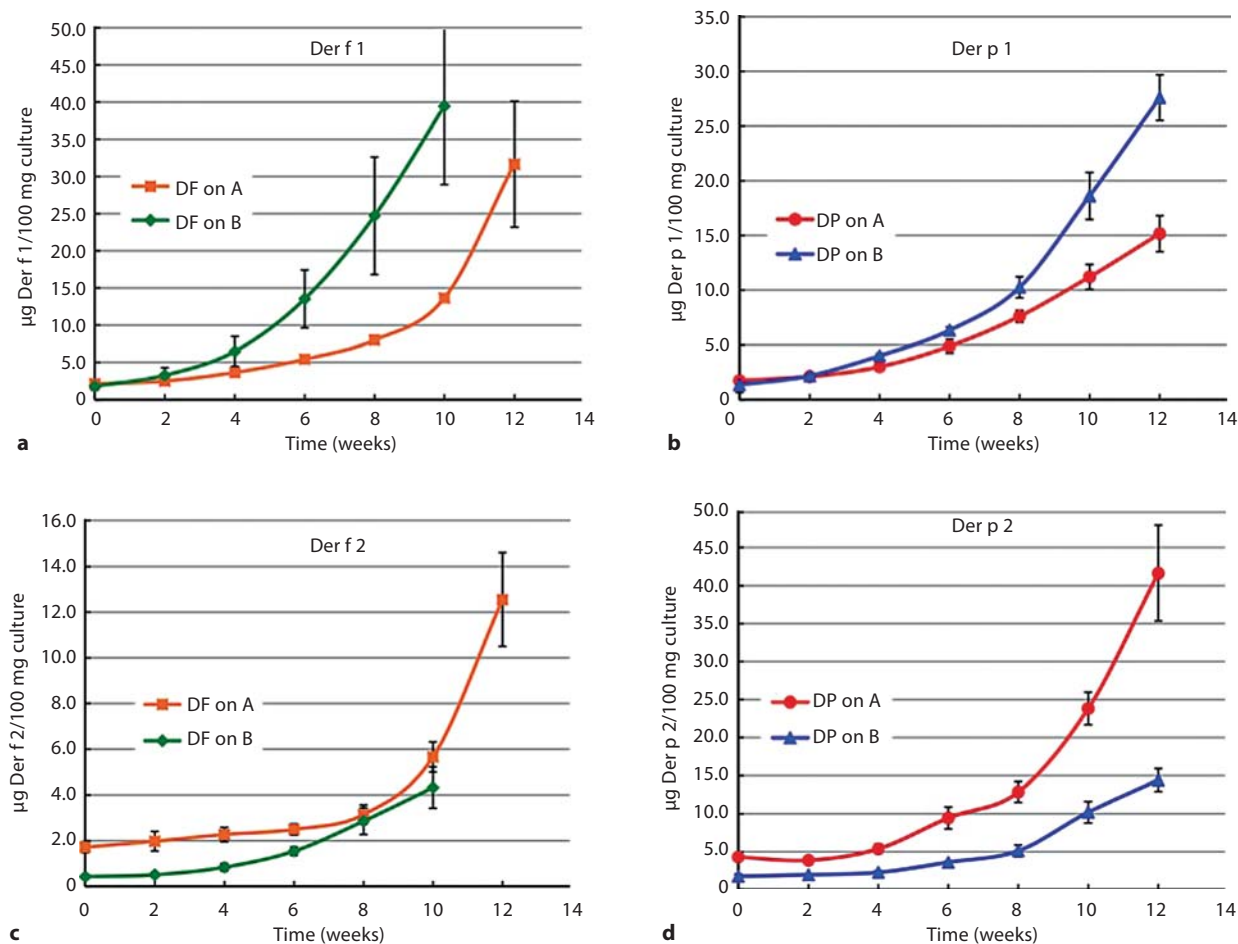


Fig. 2. Group 1 (Der f 1 and Der p 1; **a, b**) and group 2 (Der f 2 and Der p 2; **c, d**) allergen accumulation by *D. farinae* (DF) and *D. pteronyssinus* (DP) cultured on two foods (diets A and B) at room temperature.

10 weeks for *D. pteronyssinus* at which time the food became limited and the populations declined. The difference in the growth rates to peak population density for *D. pteronyssinus* on the two diets (11%) was much less than for *D. farinae* (19%) on the same two diets. Additionally, at the peak on diet A, there were more than 2.5 times as many *D. pteronyssinus* as *D. farinae* in 100 mg of whole culture. Both species of mites achieved similar peak population densities when grown on diet B.

Group 1 and Group 2 Allergen Accumulation

The group 1 and group 2 *Dermatophagoides* allergens and the combined (groups 1 + 2) allergens in all the cul-

tures accumulated exponentially in parallel with the mite population growth and levels continued increasing even after the live mite population densities plateaued or declined (fig. 1, 2).

Until they peaked, *D. farinae* cultures grown on diet B accumulated Der f 1, Der f 2 and combined (groups 1 + 2) more rapidly than did *D. farinae* cultures grown on diet A (fig. 2; table 1). The combined (groups 1 + 2) accumulation rate was 30.9% per week for diet B compared to 19.5% per week for diet A. In spite of the slower accumulation of allergens in diet A cultures (Der f 1 + Der f 2) allergen concentrations were about equal at the peak allergen accumulation times for both foods. On both diets,

Table 1. Live mite population growth and allergen accumulation in *D. farinae* and *D. pteronyssinus* cultured at room temperature on two different foods (diets A and B) in 100 mg of whole culture

	<i>D. farinae</i>		<i>D. pteronyssinus</i>	
	diet A	diet B	diet A	diet B
Live mites				
Time to peak live mite density, weeks	12	8	10	12
Number of live mites at peak	6,511 ± 808	5,131 ± 304	16,998 ± 112	6,639 ± 414
Growth rate k, %/week	19.1	38.5	32.1	21.0
Allergen (groups 1 + 2)				
Time to peak allergen concentration, weeks	12	10	12	14
Allergen (groups 1 + 2) at peak, µg	44.2 ± 10.5	43.8 ± 11.4	56.8 ± 7.9	53.1 ± 2.7
Accumulation rate k, %/week	19.5	30.9	19.9	21.7
Group 1 rate k, %/week	22.0	31.8	19.3	24.5
Group 2 rate k, %/week	15.0	25.0	20.2	17.8
Group 1/2 ratio at peak	2.6	10.1	0.36	2.5

Der f 1 accumulated in cultures at a faster rate than Der f 2 resulting in final group 1/2 allergen ratios of 2.6 and 10.1 for whole *D. farinae* cultures grown on diets A and B, respectively.

Combined allergen (groups 1 + 2) concentrations in *D. pteronyssinus* cultures also reached similar levels on both foods peaking at 56.8 ± 7.9 and 53.1 ± 2.7 µg in 100 mg of whole culture for diets A and B, respectively (fig. 1, 2; table 1). Overall, there was about a 2% difference in the rates of accumulation of combined allergens (groups 1 + 2) for the two diets. Der p 1 accumulated faster than Der p 2 on diet B while the accumulation rates were reversed on diet A resulting in inverted group 1/2 allergen ratios for the two diets, i.e. 0.36 for diet A and 2.5 for diet B.

Both species of mites accumulated combined (groups 1 + 2) allergens at similar rates when grown on diet A (19.5 vs. 19.9% per week; table 1). However, *D. pteronyssinus* allergens accumulated much more slowly on diet B compared to *D. farinae* allergens (21.7 vs. 30.9% per week). For both species, diet B favored group 1 allergen synthesis while group 2 allergen was higher for both species in cultures reared on diet A (fig. 2).

Protein and Endotoxin Contents

Diet A contained a higher amount of water-extractable protein than diet B (20 vs. 8%, data not shown). Consequently, extracts of cultures of both *D. farinae* and *D. pteronyssinus* grown on diet A contained higher concentrations of protein at all sampling times (data not shown). The protein concentrations of extracts of cultures of both species grown on both diets increased slightly over the

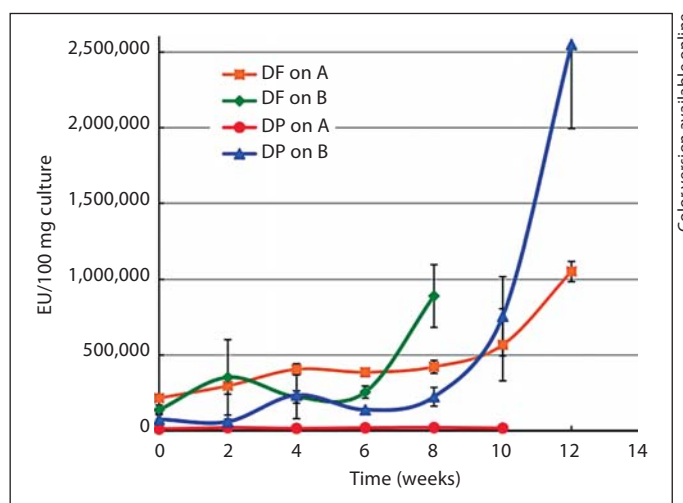


Fig. 3. Endotoxin concentrations of extracts prepared from *D. farinae* (DF) and *D. pteronyssinus* (DP) cultured on two foods (diets A and B) at room temperature. EU = Endotoxin unit.

period of culture but there was much more fluctuation in the protein content of extracts prepared from diet A cultures.

Over the first 8 weeks of growth, extracts of *D. farinae* cultures had generally higher levels of endotoxin than extracts of *D. pteronyssinus* cultures (fig. 3). At the time of peak population density (8 and 12 weeks), extracts of *D. farinae* harvested from cultures grown on both diets had comparable endotoxin levels. In contrast, extracts prepared from *D. pteronyssinus* collected at peak density (10

Table 2. Allergen contents of extracts prepared from whole cultures and washed mites collected at the time of peak population density from *D. farinae* cultures grown at room temperature on two different foods (diets A and B) in 100 mg of whole culture

	<i>D. farinae</i> on diet A		<i>D. farinae</i> on diet B	
	whole culture	washed mites	whole culture	washed mites
Time to peak live mite density, weeks	12		8	
Group 1 allergen				
μg at harvest	31.7 ± 8.5	16.7 ± 6.7	24.7 ± 7.9	4.18 ± 1.00
% lost in washing		47.3		83.1
Accumulation rate k, %/week	22.0	21.7	33.4	30.8
Group 2 allergen				
μg at harvest	12.6 ± 2.0	10.3 ± 2.4	2.86 ± 0.59	0.34 ± 0.07
% lost in washing		18.3		88.1
Accumulation rate k, %/week	15.0	24.8	24.8	27.7
Group 1/2 ratio at harvest	2.6	1.5	9.0	15.7

and 12 weeks) from cultures grown on diet B had 150 times more endotoxin than extracts of *D. pteronyssinus* cultured on diet A. Notably, at the time of peak population density, *D. pteronyssinus* grown on diet B had much more endotoxin than *D. farinae* grown on either food. The amount of endotoxin in extracts prepared from *D. pteronyssinus* reared on diet A was negligible.

Effect of Washing

Since commercial suppliers of mite extracts in the USA are likely to wash their mites prior to extraction (rather than preparing extracts from whole cultures), we washed aliquots from each of the mite cultures at each sampling time and determined the allergen concentration in extracts prepared from these washed mites (data not shown). We then compared the allergen content of the whole spent culture and washed culture material at the time when the live mite populations had peaked and the food was depleted. This represents the time that cultures are ready to be harvested for extract preparation.

Overall, for both species washing removed huge amounts of both group 1 and group 2 allergens (tables 2, 3). For *D. farinae* cultures harvested at the time of peak live mite density (12 weeks for diet A and 8 weeks for diet B), 47–83% of the Der f 1 and 18–88% of the Der f 2 in the whole culture material was lost during washing (table 2). The pattern was similar for *D. pteronyssinus* reared on diet B with 75% of group 1 and 55% of group 2 allergen being lost (table 3).

For both species, the amount of allergen lost by washing was a function of the diet. For *D. farinae*, more than

83% of Der f 1 and Der f 2 allergens were lost during washing of mites grown on diet B while 47 and 18% of these allergens, respectively, were lost by washing aliquots of cultures reared on diet A. Likewise, washing removed 74 and 55% of Der p 1 and Der p 2, respectively, present in *D. pteronyssinus* material grown on diet B. Lesser amounts of these allergens were removed during washing of *D. pteronyssinus* cultures on diet A.

More importantly, independent of diet, the washing process altered the final group 1/2 ratios in the extracts. For both species, generally much more group 1 than group 2 allergen was lost due to washing.

Discussion

In this study we demonstrated that *D. farinae* and *D. pteronyssinus* population growth rates, the rates of accumulation and the amounts of group 1 and group 2 allergens, and the levels of endotoxins in mite cultures and in extracts prepared from them were dramatically influenced by the foods on which the mites were grown. We found that the two species had different population growth rates, allergen production rates, and allergen accumulation dynamics when they were grown on the same food. Likewise, each species responded uniquely to each of the two diets tested. The two diets we compared both supported the development of thriving cultures that depleted their food in 8–12 weeks.

The differences in the population growth and allergen accumulation dynamics between *D. farinae* and *D.*

Table 3. Allergen contents of extracts prepared from whole cultures and washed mites collected at the time of peak population density from *D. pteronyssinus* cultures grown at room temperature on two different foods (diets A and B) in 100 mg of whole culture

	<i>D. pteronyssinus</i> on diet A		<i>D. pteronyssinus</i> on diet B	
	whole culture	washed mites	whole culture	washed mites
Time to peak live mite density, weeks	10		12	
Group 1 allergen				
μg at harvest	11.2 ± 1.1	6.49 ± 1.42	27.6 ± 2.1	6.99 ± 1.43
% lost in washing		42.1		74.7
Accumulation rate k, %/week	19.6	15.1	25.6	28.3
Group 2 allergen				
μg at harvest	23.8 ± 2.1	28.2 ± 5.0	14.4 ± 1.5	6.42 ± 1.52
% lost in washing		–18.5		55.4
Accumulation rate k, %/week	18.2	17.4	18.6	21.6
Group 1/2 ratio at harvest	0.47	0.23	1.9	1.1

pteronyssinus grown on the two different diets were not unexpected because ecological and laboratory studies clearly show that the two species have very different biology. Laboratory studies at comparable relative humidity and temperature conditions show differences in feeding, fecundity, reproduction, population growth rates, and survival between the two species, clearly demonstrating that these mite species are biologically different [20–24]. Despite their clear biological differences, individual research and commercial laboratories typically grow both species on the same diet and at the same climatic conditions (temperature and relative humidity) but these diets and conditions vary among laboratories. The two species do not grow equally well under identical diet and climatic conditions and both parameters influence the dynamics of population growth and allergen production and hence the characteristics of the extracts produced from them [3, 13, 14, 25–27]. The two diets we used in this study further illustrate the biological differences between the two species and how growth media (diet) influence the efficiency of producing mites and the allergen content of culture material. Thus, diet should be an important consideration for researchers and commercial extract producers when culturing mites and comparing allergen data for mite extracts from different sources and in standardizing and characterizing mite extracts.

Commercial and research house dust mite extracts (vaccines) are the aqueous supernatants from crushed, washed mite bodies or spent culture material harvested from thriving cultures after the growth media has been

depleted. The washed mite material harvested from cultures contains predominately all life stages of mites (eggs, larvae, protonymphs, tritonymphs, and adults), exoskeletons of the various developmental stages, egg shells, and the little fecal material that remains in the mite gut. Residual molting enzymes on exoskeletons, other mite secretions such as saliva, most fecal material, and the remaining unconsumed food are washed away. Mite material in spent culture is a much better representation of the mite material in homes. Spent culture material contains all the life stages and exoskeletons but additionally large amounts of fecal matter, molting enzyme residues on the exoskeletons, and partially consumed food with salivary secretions. In the US, the harvesting of mites from cultures to make extracts usually involves the removal of unconsumed food and fecal material by washing or flotation of mites with aqueous solutions [2]. A problem with this is that much allergen is soluble and is washed away; hence the allergen content of washed mites is very different than that of whole spent culture at maturity. In this current study, washing removed up to 90% of the group 1 and 58% of the group 2 allergen present in the same quantity of spent culture. Washed *D. farinae* bodies contained more Der f 1 than Der f 2 on both diets and washing increased the group 1/2 ratio on both diets. In contrast, washing *D. pteronyssinus* removed more Der p 1 than Der p 2 on both diets so that washed *D. pteronyssinus* reared on diet B had group 1 < group 2 while whole cultures of this species grown on this food had group 1 > group 2. Therefore, if the ratio of group 1/2 is important, then not only the

choice of diet but the harvesting protocol utilized is critical.

Previous studies have shown that extracts prepared from washed *D. farinae* contain much more endotoxin than do extracts of washed *D. pteronyssinus*. Trivedi et al. [1] have reported that *D. farinae* extracts from seven different commercial manufacturers have similar, relatively high endotoxin levels in the finished product while seven *D. pteronyssinus* extracts all had low endotoxin levels. This difference in endotoxin content appears to be a consequence of more endosymbiont bacteria present in *D. farinae* than in *D. pteronyssinus* [2] and not due to bacteria present in the culture media. That study included both washed *D. farinae* and washed *D. pteronyssinus* mites grown in our laboratory on the same culture medium (diet A) and under the same climatic conditions (temperature and relative humidity) demonstrating that the differences in bacterial DNA are a function of the mite species rather than of the culture conditions. Our present study demonstrates that diet can also dramatically influence the endotoxin content of house dust mite extracts. *D. pteronyssinus* grown on diet B yielded extracts with endotoxin contents that were much higher than extracts of *D. farinae* grown on both diets while extracts of *D. pteronyssinus* grown on diet A had substantially less endotoxin than any of these other extracts. The significance of the presence of endotoxin in mite extracts remains to

be determined but our study clearly demonstrates that diet also profoundly influences the endotoxin levels in mite extracts.

Conclusion

Clearly, mite-culturing diet directly affects population growth, the dynamics of allergen accumulation, the ratio of the group 1 and group 2 allergens, and the endotoxin contents in cultured mites and the extracts prepared from them. Presumably, diet affects the dynamics of production of the other mite allergens as well. The appropriate group 1/2 ratio which mimics that found in house dust should be the standard for the production of extracts for diagnosis and immunotherapy. Therefore, culture conditions, growth media, and harvesting protocols are all important considerations when developing species-specific house dust mite culture methods for the production of standardized mite extracts for commercial and research purposes.

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