THE HOUSE-DUST MITE *DERMATOPHAGOIDES PTERONYSSINUS* (TROUESSART, 1897),

PRODUCER OF THE HOUSE-DUST ALLERGEN

(ACARI : PSOROPTIDAE)

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DRUK: N.V. DRUKKERIJ V/H BATTELJEE & TERPSTRA — LEIDEN

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE WISKUNDE EN NATUURWETENSCHAPPEN AAN DE RIJKS-UNIVERSITEIT TE LEIDEN, OP GEZAG VAN DE RECTOR MAGNIFICUS DR. K. A. H. HIDDING, HOOGLERAAR IN DE FACULTEIT DER GODGELEERDHEID, TEN OVERSTAAN VAN EEN COMMISSIE UIT DE SENAAT TE VERDEDIGEN OP WOENSDAG 21 JUNI 1967 TE 15 UUR.

door

FREDERIK THEODORUS MARIA SPIEKSMA

geboren te Rijswijk (Z-H) in 1936

LEIDEN — 1967

PROMOTOR:

PROF. DR. D. J. KUENEN

Het allergologisch gedeelte van het onderzoek is uitgevoerd onder leiding van:

DR. R. VOORHORST

Aan Marise

Aan mijn ouders

CURRICULUM VITAE

Frederik Theodorus Maria Spieksma, geboren op 18 mei 1936 te Rijswijk (Z-H), behaalde in 1954 het einddiploma Gymnasium-β aan het St. Stanislas College te Delft. In datzelfde jaar liet hij zich inschrijven als student in de biologie aan de Rijksuniversiteit te Leiden.

Het kandidaatsexamen werd afgelegd in maart 1958. Voor het doctoraalexamen werden onderwerpen bewerkt bij:

Dr. P. Dullemeijer: de anatomie van het skelet en de spieren van de kop van de zeeduivel *Lophius piscatorius* L.

Prof. Dr. H. P. Wolvekamp: de lokomotie bij de rugstreeppad *Bufo calamita* Laur.

Prof. Dr. A. Quispel: inleidend onderzoek over de mogelijkheid van het isoleren van kunstmatig opgewekte mutanten van *Rhizobium*-stammen.

In november 1961 legde hij het doctoraalexamen af, waarna de militaire dienstplicht werd vervuld als reserve officier bij de Geneeskundige Troepen.

In september 1963 werd hij aangesteld als wetenschappelijk ambtenaar bij de afdeling Allergologie van het Academisch Ziekenhuis te Leiden.

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CHAPTER I

INTRODUCTION

1.1 STATEMENT OF THE PROBLEM

This paper is a report of the results of investigations carried out in an attempt to solve the forty-year old problem of the source of the allergen in house dust that causes respiratory complaints in many people. The idea that mites could be the source of the house-dust allergen was put forward by VOORHORST (1962) some years ago. It appeared, however, that relatively little was known about the mites occurring in houses and in house dust.

The study of the mite fauna of house dust in the search for a mite as the possible producer of the house-dust allergen bridges two disciplines of science: biology and allergology. Entering the field of allergology, the biologist encounters many confusing differences of opinion.

To facilitate the understanding of the allergological terms used in this paper, with which most biologists have no reason to be acquainted, a very brief introduction to atopic allergy is provided. For the concept of allergy underlying this introduction, the reader is referred to the work of VOORHORST (1962).

1.2 ATOPIC ALLERGY

Allergic diseases are found in many types and forms. One of the best-known forms, which is also one of the most typical, is hayfever or pollinosis, caused by inhalation of pollen of certain plants. Although the disease has been known for thousands of years, it was first described systematically by Bostock in 1819. The first fundamental investigations were carried out by Blackley in 1873, who demonstrated that hayfever is caused by inhalation of the pollen of grasses. However, not only Gramineae, but also Compositae (*Artemisia, Ambrosia*) and Amentiflorae (*Betula*) produce pollen to which a number of people show allergic reactions.

These allergic reactions in pollinosis, which have a typical seasonal incidence, are characterized by symptoms related to the nose, the conjunctivae, and the bronchi. The predominant complaints are itching and watery discharge in the nose, sneezing, and sometimes nasal obstruction. In the more severe cases there is also itching and burning of the eyes, and heavy tearing. Although they are sometimes seen, asthmatic symptoms are rather uncommon, probably because pollen granules are relatively large (25–100 μ diameter) and by far the greater number of them are caught by

the nasal mucous membranes. To penetrate deeply into the bronchial tree, corpuscular elements must have much smaller dimensions (5 μ or smaller).

All these complaints are caused by a reaction between a substance present in the pollen (allergen) and a substance (reagin) present in the tissues (and also in the blood) of an allergic person. The reaction between an allergen and the reagins bound to the cells gives rise to the liberation of histamine-like products in the tissues.

The reagins have been formed by the allergic patient as a result of earlier contacts with the allergen, and their presence in the tissues can be demonstrated by a reaction of the skin after intradermal injection of the allergen (Ch. VI; 6.2). The type of allergy in which this allergen-reagin reaction is involved is called atopic allergy.

Many of the above-mentioned phenomena of pollinosis are also found in a number of people without an atopy for pollen, although the clinical picture is not always so typical as in hayfever, e.g. the seasonal incidence can be different or altogether lacking. The complaints of these patients are caused by atopic allergens other than that of pollen, but nearly all of them are of biological origin. Some of these atopic diseases are caused by the spores of moulds (*Aspergillus, Penicillium, Alternaria*). Other vegetable sources of atopic allergens are, for example, Radix ipecacuanhae, which is handled by pharmacists; castor beans (*Ricinus*); and various kinds of woods.

Concerning atopic allergens of animal origin, it is known that some parasitic worms (*Ascaris*) contain substances producing sensitization. Mites and insects, such as grasshoppers (*Locusta*), can also be sources of allergens. Other well-known causes of atopic diseases are the skin scales of various mammals, e.g. cats, dogs, cows, horses, etc. It is remarkable that even the scales of the human skin must be added to this series; they give atopic skin reactions the most frequently of all.

1.3 HOUSE-DUST ATOPY

The symptoms observed in patients with an atopy for one of the above-mentioned products can also be caused by house dust. The presence of a distinct allergen in house dust was suggested by KERN (1921) and confirmed by COOKE (1922). This idea was supported soon afterwards by other investigators in different parts of the world, who showed that the typical atopic skin reactions to house dust occur very frequently. In addition to the nasal complaints, as seen in hayfever, patients with house-dust atopy more frequently suffer from asthma attacks because the much smaller dimensions of the dust particles allow them to penetrate deeply into the bronchi.

A very important question was, and still is, what substance or combination of substances in house dust has these allergenic properties? And is this substance also of biological origin?

House dust is a mixture of many compounds, such as epidermal products of man and animals, degeneration products of all kinds of fibrous materials, moulds, bacteria, the remains of food as well as of plants and small animals, and many inorganic components. Until now, however, none of the investigators working on the house-dust atopy problem has succeeded in demonstrating the presence of the house-dust allergen in one of the separate constituents of which house dust is thought to be composed, although in a number of cases other allergens, different from the house-dust allergen, have been found. Is is impossible to discuss all the investigations and arguments here; only the most important points will be briefly indicated.

The epidermal products of cats, dogs, and other domestic animals can be excluded as the cause of the house-dust allergen, because dust from houses without such animals also contains the allergen. The role of the human epidermal products, which contain an allergen to which many people show atopic reactions, has been considered debatable, but on the basis of the allergen content of the human skin scales and the amount of these products in dust, it is impossible that the allergens in house dust and in human skin scales are the same. Moreover, there are many patients who show skin reactions to one of these two allergens and not to the other.

Other constituents of house dust that have frequently been accused of being the source of the house-dust allergen, are the moulds. Again taking into account the amount of mould material in the dust and the allergen content of moulds and house dust, these organisms cannot be the source of the house-dust allergen either.

The role of arthropods as the cause of the house-dust allergen has also been studied. Recently, PERLMAN (1965) concluded that it must be presumed that arthropods and house dust do not have atopic allergens in common.

1.4 MITES AS SOURCES OF ALLERGENS

One of the groups of arthropods suspected of occurring in house dust was the mites. The early literature contains descriptions of several cases of skin diseases in persons handling materials heavily infested with mites, and respiratory complaints have also been reported. CASTELLANI (1912) reported a copra-itch caused by *Tyrophagus putrescentiae* (Schrank, 1781), and LAARMAN (1952) ascribed the same disease to *Cosmoglyphus krameri* (Berlese, 1881). SAMSINÁK (1966) who studied Laarman's material, described the species as *Cosmoglyphus laarmani* Samsinák. In his opinion all records of copra-itch could refer to this species. Other itching diseases due to mites have been reported in the literature as grocer's-itch, coolie-itch, and grain-itch. STORM VAN LEEUWEN, BIEN and VAREKAMP (1924) described a case in which a farmer suffered from severe asthma attacks

after inhalation of dust from oats which appeared to be heavily infested with *Acarus siro* Linnaeus, 1758 and *Glycyphagus* spec. ANCONA (1923) reported 'epidemic asthma' in a number of Italian grain-workers, caused by *Pyemotes ventricosus* (Newport, 1850) living as ectoparasite on the larvae of insects infesting the grain; and DESCHIENS (1951) reviewed a number of cases of respiratory diseases due to mites.

These cases are only a few of the many published reports of skin diseases and asthma caused by contact with mites or mite-containing materials. These reports suggest that mites are a group of animals that can be carriers or producers of substances causing those diseases in man, but it is not known whether in these cases an atopic or another allergic mechanism is involved.

VOORHORST (1962) investigated whether an atopy to mites could be found in atopic patients, and also whether the allergic activity of house dust derives from its possible content of mites. He therefore compared skin reactions to house dust with those to three species of mites in thirty patients atopic to house dust. For this investigation he chose three species of mites that – according to the views of acarologists up to that time – would occur most frequently in houses: *Acarus siro* (= *Tyroglyphus farinae* De Geer, 1778); *Tyrophagus putrescentiae* (= *T. castellanii* Hirst, 1912); and *Glycyphagus domesticus* (De Geer, 1778). The results of this study showed that skin reactions to the three species occurred very frequently in those subjects, but that the allergens in these species were not identical to the house-dust allergen.

These results, combined with the insufficient information about the occurrence of mites in house dust, raise three questions:

1) Do mites occur generally in houses or in house dust, and does this hold for different parts of the world?
2) If so, which species are found most regularly and in the highest numbers?
3) Does one of these species carry or produce a substance with allergen properties identical to the house-dust allergen?

The first and second of these problems lie in the province of the biologist; the solution of the third requires cooperation between the allergologist and the biologist.

CHAPTER II

THE OCCURRENCE OF MITES IN HOUSE DUST

2.1 INTRODUCTION

Reports of the occurrence of mites in houses mention the finding of dense populations in almost every case. Much work has been done on mite infestations, especially of stored food products, and attention has also been paid to mite occurrence of medical importance.

OBOUSSIER (1939) reported finding the following species in houses in Hamburg (Germany): *Carpoglyphus lactis* (L., 1758); *Glycyphagus domesticus* (De Geer, 1778); *Glycyphagus privatus* Oudemans, 1903; and *Tyrophagus longior* (Gerv., 1844). In a brief account, SOLOMON (1961) mentioned some other genera and species as possible inhabitants or invaders of 'houses, shops and other occupied buildings'. He also referred to occasional invasions of Beetle Mites (Oribatei) and Red Spider Mites (Tetranychidae), as well as the occurrence of blood-sucking mites e.g. the Chicken Mite *Dermanyssus gallinae* (De Geer, 1778), and other Gamasides. As the typical House Mite, *Glycyphagus domesticus* is mentioned occurring on damp walls. According to SOLOMON, still other species, particularly associated with food stuffs, have been found about houses: *Glycyphagus destructor* (Schrank, 1781); the Flour Mite *Acarus siro* Linnaeus, 1758 (= *Tyroglyphus farinae* De Geer, 1778); the Dried-Fruit Mite *Carpoglyphus lactis; Thyreophagus entomophagus* (Laboulbène, 1852); *Gohieria fusca* (Oud., 1902); and several species of *Tyrophagus* Oudemans, 1924. The most common predators are mites belonging to the genus *Cheyletus*, especially *C. eruditus* (Schrank, 1781).

PRÍVORA and SAMSINÁK (1957) mentioned some species infesting human beings, and DESCHIENS (1951) reported on mites associated with the respiratory organs in man. Both investigations paid some attention to the houses inhabited by the patients.

Recently, OSHIMA (1964) reported the finding of mites in the dust collected in a number of schools in the Yokohama area. Of these mites, almost 90 per cent appear to belong to the genus *Dermatophagoides* Bogdanow, 1864.

The aim of this part of the present study was to determine which mites occur regularly in houses rather than occasionally, particularly in house dust. A large number of house-dust samples were examined for the presence of mites by an isolation method developed for this purpose (see: 2.2).

The mites were identified according to HUGHES (1961). Parasitiformes

and Oribatid mites as well as the representatives of the Tarsonemidae and Cheyletidae were not identified by species. Unidentifiable mite fragments are mentioned as 'indet'. Special attention was paid to genera of the Acaridiae, particularly to the species of the genus *Dermatophagoides*. This study of the mite fauna of house dust has been published recently (SPIEKSMA and SPIEKSMA-BOEZEMAN, 1967).

2.2 THE ISOLATION OF MITES FROM HOUSE DUST

2.2.1 Introduction

Most of the techniques developed for the isolation of small arthropods find their application in the field of soil science and in studies of stored-food infestations.

MURPHY (1958[a-b]) has provided a comprehensive review of nearly all the important methods used in the study of the arthropod fauna in different kinds of soils. SALT and HOLLICK (1944) described a method for collecting wireworm populations, later modified by RAW (1953) to adapt it for extracting small arthropods from pasture soil. SOLOMON (1958) briefly reported some of the methods used for the recovery of mites from stored food products; and HALE (1964) designed an apparatus for the isolation of arthropods based on the flotation method. SHCHASTNY (1939) used a combination of flotation and centrifugation for the collection of mites and their eggs from flour; MÜLLER (1958) also recovered mites with a centrifugal-flotation method.

During the study of the occurrence of mites in house dust, however, it soon became evident that these methods could not be applied to house-dust samples. For the isolation of these mites, a special method had to be devised by modifying and combining a number of existing techniques (SPIEKSMA and SPIEKSMA-BOEZEMAN, 1967).

2.2.2 General remarks

a) House dust may contain detritus from human and animal skins, from clothes, furniture, carpets, etc.; remains of plants and small animals; and wastage from food and articles used in the household, as well as materials brought in from outside. It is therefore hardly surprising that dust samples from various houses differ widely from one another as to composition and structure. This variation in composition constitutes the main difficulty in the study of the mite fauna in house dust, since it affects the size of the losses of mite material in the several steps of the method to a variable extent.

b) In most of the methods mentioned above, the coarse and the fine materials are removed by a combination of washing and sieving. House dust, however, tangles when wet by water or other liquids because of the hairs and fibrous material in the sample, and should therefore be dry when sieved.

c) The mites isolated from a sample are distinguished more easily if they have been soaked in a lactic-acid solution, which causes them to swell and straighten their legs. This swelling process is accelerated by warming. Because lactic acid 90% has a specific gravity of ± 1.2, it is also suitable for separating mites from various kinds of debris. However, the differences between the specific gravities of the organic and inorganic components of house dust and of the mites are so small, and the lactic acid, even when it is warmed, is so viscous, that good separation cannot be achieved without centrifugation.

d) An important improvement of the techniques of isolating small arthropods consisted of the use of a benzene-water mixture (SALT and HOLLICK, 1944; RAW, 1953). Benzene, and not water, wets the cuticle of most arthropods, and the reverse holds for most soil materials. The wettability of the components of the extremely heterogenous house-dust material, however, differs so widely that the benzene-water method does not result in good separation of the mites: the whole mass of dust accumulates on the water-benzene interface as one lump and only some sand-grains and other small heavier particles sink. Violent shaking does not give any improvement.

e) Since the treatments applied to the dust sample will have killed any living mites, it is impossible to know how many of the mites recovered at the end of the method were alive at the beginning.

2.2.3 Method of isolation

In this section the isolation method is explained step by step, and some remarks are added on the possible loss of mite material and other sources of errors.

a. The amount of dust inspected for mites.

Dust samples are taken from the floor of the living-room with a light-weight vacuum cleaner which is easy to handle, the dust being collected in a paper bag. The amount of dust collected in this way varies from about 5 to about 20 grams.

Since an irregular distribution of the mites in a dust sample would influence the number of mites in the portion inspected, it is advisable to make this portion as big as possible. A 5 gram portion was found to be an adequate quantity in view of both the minimum amount of material collected with a vacuum cleaner and the capacity of the sieving equipment.

However, due to the great differences in the composition of dust samples from various houses, the volume of a 5 gram portion from one house may differ considerably from that of a 5 gram portion from another house. For

instance, when a sample contains much sand (which has a relatively high specific gravity) it will contain less of other dust material than a portion with no sand. These differences in composition will certainly have an influence on the number of mites determined in the sample. Nevertheless, weight is the only suitable criterion for fixing the amount of a portion from a house-dust sample to be inspected for mites.

b. Dry sieving of a portion.

Sieving of a 5 gram portion of house dust to remove the coarse material and the fine dust particles is carried out under dry conditions rather than by a combination of washing and sieving, to avoid tangling or lumping of the fibrous constituents, which would hamper successful separation.

The sieving machine used gives the pile of sieves a circular movement without rotation, at a frequency of about 125 revolutions per minute. Sieving is performed for one hour; but after 15 and 30 minutes the machine is stopped and the dust material is teased apart, because it has a tendency to spread over and then adhere the surface of the sieve.

Several experiments were carried out to determine the best series of sieves for this purpose. A series of 8 round copper sieves with a diameter of 20 cm and a height of 5 cm was chosen, including three plate sieves with round meshes (2.4, 1.7, and 1.0 mm pore size) and 5 wire sieves with square meshes (0.5, 0.25, 0.175, 0.125, and 0.075 mm pore size), which gave nine fractions from each 5 gram portion of dust.

The differences between these nine fractions with respect to the nature of the dust material were not great, except in fraction no. 1, which held all the coarse material, and fraction no. 9, in which most of the fine particles were collected.

In Table 2.1, which gives the averages of eight tests with different dust samples, the distribution of the mites over the nine fractions is shown. On

TABLE 2.1. Percentages of the number of mites found in nine sieve fractions; averages of eight tests.

sieve no.	pore size (in mm.)	fraction number *	mite nos. (in %)
1	2.4	1	2.2
2	1.7	2	3.4
3	1.0	3	3.8
4	0.5	4	2.0
5	0.25	5	16.2
6	0.175	6	31.9
7	0.125	7	29.9
8	0.075	8	9.6
		9	1.0

* Fraction numbers refer to dust held by the sieve of the same number, except for fraction no. 9, which refers to dust passing through sieve no. 8.

the basis of these results sieves 2–7 were omitted, leaving no. 1 (2.4 mm) and no. 8 (0.075 mm), since this gave a satisfactory removal of the coarse and fine material with only a small loss of mite material, i.e. about 3 per cent (see Table 2.1: 2.2 and 1.0 per cent).

c. Suspension of the dust fraction in lactic acid and centrifugation.

About 160 ml lactic acid 90% (s.g. ± 1.2) is added to the remaining dust fraction in a beaker. To accelerate the swelling of the mites and render the lactic acid less viscous, the suspension is heated under regular stirring until it reaches the boiling point.

The suspension is then centrifuged, because the specific gravities of the various constituents of house dust differ so little that good separation of the mites cannot be attained by gravitation only.

The centrifuge is operated at low acceleration, 5 minutes being required to reach 300 *g*. The current is then switched off and no brake is applied; about 7 minutes are required for the apparatus to reach a full stop. This procedure must be repeated three times, because many of the mites settle with the dust material. Since the mites are by now swollen, it is no longer necessary to use lactic acid for the suspension; any solution with a specific gravity of ± 1.2 will be suitable, e.g. a saturated solution of sodium chloride.

To determine the percentage of mites isolated in this way, a number of different dust suspensions were centrifuged five or six times, after each of which a count was made of the mites in the supernatant fractions. The last sediments were resuspended in beakers containing a large quantity of water or salt solution; the greater part of the dust material settled within about 36 hours, leaving any residual mites floating. Very few mites, if any, were recovered from these last supernatant fractions. Comparison of the results of the mite counts showed that after each successive centrifugation, 50 to 70 per cent of the residual mites remained in the sediment, depending on the composition of the original dust sample. This means that 70 to 90 per cent of the total number of mites in a 5 gram sample are isolated after 4 centrifugations.

d. Collection of the isolated mites.

After centrifugation, the supernatant fluid containing the mites is filtrated in a Buechner funnel. Under a stereoscopic dissecting microscope, the mites on the filter paper are collected with a fine needle and carried into a drop of lactic acid on a slide for examination through the compound microscope.

Recognition of the small, whitish mites among the unavoidable dust particles on the filter paper requires some training.

2.3 MITES IN HOUSE DUST

The study of the mite fauna in house dust was originally initiated by

Mrs. M. I. A. Spieksma-Boezeman in 1962. She prepared some of the steps of the isolation method, and one of the most important results of her unpublished preliminary investigations on the mite fauna in house-dust samples is given here.

During the period from May to November 1962, house dust was collected at three-week intervals in a house in Leiden; the numbers of mites are shown in Table 2.2. Hypopi have not been identified. The numbers

TABLE 2.2. Numbers of mites in house-dust samples collected in a house in Leiden during the period from May to November 1962.

date of sampling / genus or group	28-V	18-V	9-VII	27-VII	20-VIII	10-IX	2-X	22-X	12-XI	total	%
Dermatophagoides	8	59	22	50	79	156	132	98	31	635	78
Glycyphagus	2	—	3	9	9	19	25	13	2	82	10
Tyrophagus	1	1	1	11	6	5	7	2	1	35	4
Hypopi	—	—	—	—	—	—	—	1	—	1	0
Cheyletidae	1	1	—	1	1	6	6	5	1	22	3
Parasitiformes	5	3	4	3	4	6	7	4	1	37	5
total	17	64	30	74	99	192	177	123	36	812	100
Oribatei	108	49	75	34	37	35	84	10	15	447	

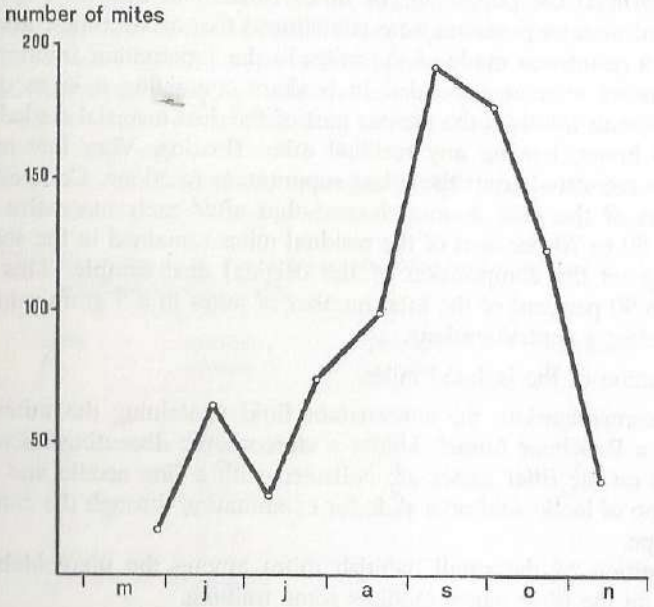

FIG. 2.1. Total numbers of mites (without Oribatei) in house-dust samples collected in a house in Leiden during the period from May to November 1962 (see Table 2.2).

are rounded-off average values of three 5 gram portions from one dust sample. Fig. 2.1 shows the total number of mites (without Oribatei) in each of the samples during that period.

After improvement and standardization of the isolation method, this investigation was repeated for the present study in three houses in Leiden over a period of one year, from May 1964 to May 1965. The rounded-off averages of two 5 gram portions of house dust are given in Table 2.3. The numbers of *Dermatophagoides* in the dust samples from these three houses are shown in Fig. 2.2 (house no. 1 of Table 2.3 and Fig. 2.2 is the same as that of Table 2.2 and Fig. 2.1).

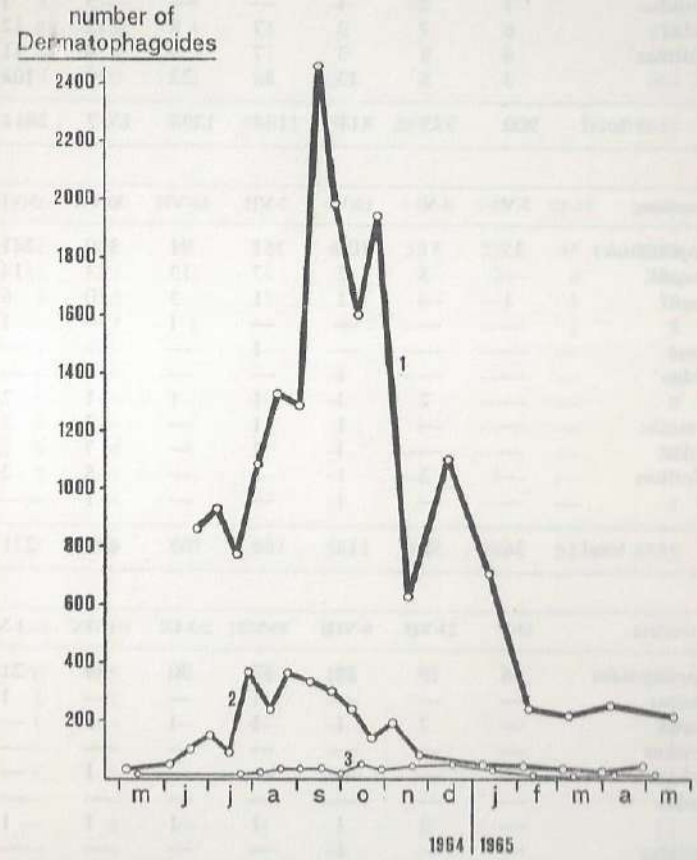

FIG. 2.2. Numbers of specimens of the genus *Dermatophagoides* in house-dust samples collected in three houses in Leiden during the period from May 1964 to May 1965 (see Table 2.3) (house no. 1 is the same as that of Fig. 2.1).

TABLE 2.3. Numbers of mites in house-dust samples collected in three houses in Leiden during the period from May 1964 to May 1965 (house no. 1 is the same as that of Table 2.2).

date of sampling	23-VI	7-VII	21-VII	4-VIII	18-VIII	1-IX	15-IX	29-IX	13-X	27-X	17-XI	15-XII	12-I	9-II	9-III	6-IV	20-V	total	%
Dermatophagoides	859	932	777	1086	1332	1289	2454	1986	1604	1946	632	1105	710	244	218	253	219	17646	94.8
Glycyphagus	10	18	3	12	18	28	19	11	11	14	5	6	6	1	1	16	5	184	1.0
Tyrophagus	12	7	10	6	1	2	1	—	—	—	1	3	6	6	2	13	19	89	0.5
Acarus	1	—	1	—	1	1	1	—	—	—	—	—	1	—	—	6	2	14	0.1
Rhizoglyphus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	0.0
Histiosoma	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	2	0.0
Psoroptidae	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1	—	2	0.0
indet.	1	5	1	8	4	2	—	2	—	—	2	1	1	1	—	—	—	28	0.1
Tarsonemidae	1	2	1	—	—	5	3	4	5	3	4	5	—	1	—	—	—	34	0.2
Cheyletidae	6	7	2	7	8	12	17	11	6	13	5	8	8	3	1	1	3	118	0.6
Parasitiformes	6	9	3	7	9	8	11	11	4	9	3	6	2	1	1	5	1	96	0.5
Oribatei	3	5	13	38	22	10	104	38	14	21	5	29	22	8	9	62	1	404	2.2
total	900	985	811	1164	1396	1357	2611	2063	1644	2006	657	1163	756	265	232	358	250	18618	100

date of sampling	5-V	4-VI	18-VI	2-VII	15-VII	30-VII	13-VIII	25-VIII	10-IX	24-IX	8-X	22-X	5-XI	24-XI	7-I	4-II	4-III	1-IV	28-IV	total	%
Dermatophagoides	35	51	105	151	91	369	241	368	339	303	240	143	194	83	50	47	33	25	45	2913	87.6
Glycyphagus	—	3	2	7	13	13	14	29	32	29	23	15	7	2	6	3	2	2	4	206	6.2
Tyrophagus	1	—	1	1	3	10	6	6	5	9	8	3	2	—	1	1	1	—	1	59	1.8
Acarus	—	—	—	—	1	—	1	2	—	—	—	—	1	1	—	1	—	—	1	8	0.2
Histiosoma	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	0.0
Psoroptidae	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	0.0
indet.	—	2	1	1	1	1	2	—	1	—	—	—	—	—	—	—	—	—	—	9	0.3
Tarsonemidae	—	—	1	1	—	3	3	2	6	1	1	5	—	—	—	2	1	—	—	26	0.8
Cheyletidae	—	—	1	1	—	7	2	6	6	14	6	6	4	1	1	—	—	—	—	55	1.7
Parasitiformes	—	2	1	1	—	5	2	10	7	5	4	3	1	—	—	—	—	1	—	42	1.3
Oribatei	—	—	1	—	—	1	—	1	—	—	1	—	—	—	—	—	1	—	—	5	0.1
total	36	58	114	164	109	409	271	424	396	361	283	175	209	87	58	54	38	28	51	3325	100

date of sampling	13-V	23-VII	6-VIII	20-VIII	3-IX	17-IX	1-X	15-X	29-X	19-XI	17-XII	14-I	11-II	11-III	8-IV	6-V	total	%
Dermatophagoides	16	19	23	37	38	39	21	53	36	44	51	30	15	11	18	15	466	87.6
Glycyphagus	—	—	—	1	—	—	1	—	1	—	—	1	1	—	1	—	6	1.1
Tyrophagus	—	2	1	1	1	3	—	1	1	—	1	1	—	—	—	—	12	2.2
Rhizoglyphus	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	0.2
Thyreophagus	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	0.2
Caloglyphus	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	0.2
indet.	—	2	1	1	1	1	1	—	—	1	—	—	1	—	1	1	11	2.1
Tarsonemidae	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	0.2
Cheyletidae	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	2	0.4
Parasitiformes	1	1	—	1	2	—	—	1	2	—	1	3	—	1	—	—	13	2.4
Oribatei	1	1	—	—	4	1	3	1	—	1	1	2	—	—	2	1	18	3.4
total	18	25	26	41	46	46	26	56	41	47	54	37	17	12	22	18	532	100

The conclusions drawn from these investigations are:

1) Mites occur regularly in house dust, not occasionally.

2) A species of *Dermatophagoides* is represented by much higher numbers than any of the other species found in house dust.

3) There is a seasonal increase and decrease in the numbers of mites, with a maximum from August to October.

4) The numbers of mites in the dust from various houses differ considerably.

2.4 THE MITE FAUNA OF HOUSE DUST

To verify the observations of the mite faunas of the three above-mentioned houses on a broader scale, the dust from 150 houses was investigated. During a period of two weeks in the middle of September, when the number of mites reaches its peak (Figs. 2.1 and 2.2), dust was collected in houses in four places (Delft, Leiden, Oegstgeest, and Noordwijk), all situated in the western part of The Netherlands.

The samples in the paper bags were held at 60°C for 6 hours to kill all living mites. This 'sterilization' prevented any increase of the population during storage of the samples in the laboratory. Silica gel was added to the closed storage vessels to keep humidity at a low level. Six months were required to determine the numbers of mites in all 150 samples.

To give the house-by-house results of this study here would require too much space. The rate of occurrence, the total numbers, and the percentage

TABLE 2.4. Occurrence of mites in 5 gram dust samples collected in 150 houses in September 1965.

genus or group	rate of occurrence *	total number	%
Dermatophagoides	100	9209	69.9
Glycyphagus	61	1224	9.3
Tyrophagus	29	185	1.4
Acarus	19	149	1.1
Rhizoglyphus	5	21	0.2
Thyreophagus	3	4	0.0
Gohieria	20	1542	11.7
Ctenoglyphus	1	1	0.0
Chortoglyphus	1	3	0.0
Histiosoma	1	2	0.0
Psoroptidae	4	17	0.1
Tarsonemidae	29	376	2.9
Cheyletidae	48	290	2.2
Parasitiformes	30	118	0.9
Oribatei	19	44	0.3
total		13185	100

* The rate of occurrence is expressed as the percentage of the number of houses in which at least one representative of the genus or group of mites is found.

of the various genera or groups of mites are shown in Table 2.4. The rate of occurrence is defined as the percentage of the number of houses investigated in which at least one representative of the genus or group is found.

A study of Table 2.4 raises the question of which of these mites are characteristic for house dust and which are occasional invaders. In all likelihood, the occurrence of Parasitiformes, Oribatei, Tarsonemidae, and Psoroptidae in house dust is only occassional or secondary. Representatives of the Cheyletidae are associated with Acaridiae as predators.

The other genera in Table 2.4 comprise mostly Acaridiae associated with stored food products. The genera *Glycyphagus, Tyrophagus, Acarus,* and *Gohieria* are common pests of grain, flour, cheese, etc. The finding of these genera in house dust could be correlated with their association with these kinds of products used in the household. This makes it difficult to decide whether the occurrence of these genera must be considered occassional. These genera are the same as those reported by SOLOMON (1961); they are known to form dense populations, and when this is the case they do not easily escape notice. The dominant species found in this investigation are *Glycyphagus destructor, Tyrophagus putrescentiae* (Schrank, 1781), *Acarus siro,* and *Gohieria fusca.*

The occurrence of the genus *Dermatophagoides* in house dust will be discussed separately in the next section.

2.5 THE OCCURRENCE OF DERMATOPHAGOIDES IN HOUSE DUST

The rate of occurrence of 100 and the number of 9209 of *Dermatophagoides* (see Table 2.4) in the dust of 150 houses is remarkably high. Some species of this genus have been found living in granaries and warehouses in association with stored products (COOREMAN, 1950; SASA and SHINGAI, 1958; HUGHES, 1961; DE LEON, 1963); and some species live in association with birds and mammals (BOGDANOW, 1864; SASA, 1950; TRAVER, 1951; FAIN, 1964 and 1966).

The literature contains only two reports of the finding of a dense population of free-living *Dermatophagoides* in a house: one in a kapok-filled pillow, the other in a feather pillow (BAKER *et al.*, 1956). In 1964 OSHIMA found representatives of this genus in the dust from a number of schools in Yokohama, Japan.

It is astonishing that the abundant occurrence of *Dermatophagoides* in house dust escaped the notice of those who since 1922 have studied house dust for its allergen content. In these allergological studies, however, the arthropod fauna was not investigated with the isolation techniques used in entomology.

The fact that this genus of mite seldom or never occurs as a dense population, as well as its relatively small size, probably also explains why its occurrence in dust was not reported until 1964, when two independent

publications appeared (OSHIMA, 1964; VOORHORST, SPIEKSMA-BOEZEMAN and SPIEKSMA, 1964).

Prof. A. Fain (Antwerp, Belgium) was kind enough to identify the specimens of the genus collected from the Dutch dust samples. Three species were found:

1) *Dermatophagoides pteronyssinus* (Trouessart, 1897)
   (= *Mealia pteronyssina* Trouessart, 1897),
2) *Euroglyphus (Euroglypus) maynei* (Cooreman, 1950) *
   (= *Dermatophagoides maynei* Cooreman, 1950)
   (= *Mealia maynei* Cooreman, 1950),
3) *Dermatophagoides farinae* (Hughes, 1961).

The rate of occurrence, the total number, and the percentage of these three species in the 150 houses are given in Table 2.5. As can be seen from this Table, *D. pteronyssinus* occurs the most frequently and was found in all 150 houses.

TABLE 2.5. Occurrence of three species of the genus *Dermatophagoides* in dust samples collected in 150 houses, September 1965.

species	rate of occurrence	total number	%
D. pteronyssinus	100	8065	87.6
Euroglyphus (=*D.*) *maynei*	53	1033	11.2
D. farinae	2	111	1.2
total		9209	100

Besides its abundant occurrence in houses in The Netherlands, *D. pteronyssinus* has also been recovered from house-dust samples sent to the Department of Allergology in Leiden from other parts of the world: Germany, Switzerland, Norway, Finland, England, Ireland, Spain, Iran, Pakistan, Australia, Hawaii, Brazil, Argentina, Surinam, and the United States of America.

OSHIMA (1964) identified his specimens as *D. scheremetewskyi* and *D. farinae*. After the redescription of *D. pteronyssinus* by FAIN (1966) had been published, OSHIMA wrote to Fain (personal communication) that his *D. scheremetewskyi* is in fact *D. pteronyssinus*. These findings, together with its presence on four continents (Europe, Asia, Africa, and North-America) (FAIN, 1966) make it clear that *D. pteronyssinus* has a cosmopolitan distribution.

* In a recent revision, FAIN (1965) placed *Dermatophagoides maynei* (family Psoroptidae) in the new genus *Euroglyphus* (family Pyroglyphidae). During the initial investigations on the mite fauna in house dust, mites belonging to the genus *Dermatophagoides* were not identified by species. For these reasons the species *Euroglyphus (E.) maynei* is included under the genus *Dermatophagoides* in Figs. 2.1 and 2.2, and in Tables 2.2, 2.3 and 2.4.

Until now, no Dutch house-dust sample inspected for the presence of mites has been found to be free of *D. pteronyssinus*.

The observations on the frequencies of the immature stages of the three species (Table 2.6) suggest that house dust provides favourable conditions for the growth of populations of *D. pteronyssinus* as evidenced by the high number of larvae and nymphs. *Euroglyphus (E.) maynei* is represented mainly by females. *D. farinae* was found in only 3 of the 150 houses (see Table 2.5), but a number of dust samples from the United States of America yielded only this species, *D. pteronyssinus* being lacking.

TABLE 2.6. Occurrence of adults and immature stages of three species of the genus *Dermatophagoides* in dust samples collected in 150 houses, September 1965.

species		rate of occurrence	number	%
D. pteronyssinus	♀♀	95	3307	41.0
	♂♂	91	2549	31.6
	n.	87	1995	24.7
	l.	41	214	2.7
	total		8065	100
Euroglyphus (= *D.*) *maynei*	♀♀	53	836	80.9
	♂♂	21	114	11.0
	n.	18	82	8.0
	l.	1	1	0.1
	total		1033	100
D. farinae	♀♀	2	26	23.5
	♂♂	2	18	16.2
	n.	2	54	48.6
	l.	2	13	11.7
	total		111	100

2.6 SEASONAL PERIODICITY IN THE MITE POPULATION

As can be seen in Figs. 2.1 and 2.2, the mite populations in house dust show a periodic increase and decrease. They reach a maximum in the months of August, September, and October, and a minimum in the months of February, March, and April. An explanation for this periodicity may lie in the climatic circumstances leading to a more rapid growth of organisms such as mites during a period when the temperature and the humidity of the air are relatively high, and a slower growth or decline of the population when it is relatively cold and dry. These circumstances would result in a high number of mites at the end of summer and the beginning of autumn, and a small number at the end of the winter (Ch. V: 5.4 and 5.5).

A comparison of the curve in Fig. 2.1 with curve 1 in Fig. 2.2, which represent the total number of mites (without Oribatei) found in 1962, and the number of *Dermatophagoides* in 1964, respectively, found in the dust from the same house, shows a striking dissimilarity in numbers. This difference may have been caused by improvements in the isolation method, and the fact that the average temperature in the period from May to September in 1962 was about 2.2°C lower than in the same period in 1964 (KNMI, *Maandoverzichten* (monthly reports), 1962 and 1964).

To determine the influence of the season and weather conditions it would be necessary to study the periodicity in the house-dust mite fauna in relation to the climatological circumstances over a period of several years.

2.7 DIFFERENCES IN MITE NUMBERS IN DUST SAMPLES FROM DAMP AND DRY HOUSES

Fig. 2.2 and Table 2.3 show that there are considerable differences in the number of mites in house-dust samples from various houses. It was known that the allergen content of house dust also differs considerably from one house to another, and that the quality of the house, particularly the degree of dampness, determines the allergen content to a great extent (VOORHORST, 1962). It was assumed that if there is a relation between the number of mites and the allergen content in dust, the dampness of the house would have a decisive influence on the number of mites present.

The investigations on this point were carried out in collaboration with Dr. H. Varekamp, who has worked on the correlation between house-dust allergen and dampness of the patients' houses (VAREKAMP, 1958, and 1963; VAREKAMP and VOORHORST, 1960, and 1961; VAREKAMP, SPIEKSMA, LEUPEN, and LYKLEMA, 1966). According to Varekamp's views, and with assistance of the architect M. J. Leupen, the 150 houses mentioned above were selected to form five groups of 30 houses of different degrees of dampness. Dampness of a house has not yet been defined and cannot be measured at any given moment. It depends on many factors, some of which are not observable, and results in the presence or absence of certain signs. The most important factors determining the degree of dampness of a house are the following.

The extent to which surface water is taken up by the soil on which the house is built, and the speed with which water passes through the soil down to a level where it cannot reach the foundation of the house, can be decisive for dampness. A rocky soil will not hold any surface water; sand allows water to pass about 5000 times quicker than clay or peat soils. Houses built on the latter types of soil will certainly have damp foundations, especially when adequate drainage is not provided for. Moreover, dampness of a house can be caused by bad construction, for instance when the outer walls do not keep out ground-water (lack of a 'trass-layer')

or when the walls are permeable to rain-water. Particularly in very old houses, badly constructed and poorly maintained, water penetrates easily and gives rise to a high degree of dampness. Leakages in the roof and water supply or drainage, especially concealed leaks, can also cause the house to become damp. When sunlight and wind have no access to the house (trees, very narrow streets), ventilation and drying are prevented and dampness is not suppressed. The manner in which a house is heated and the extent to which damp air is replaced by dry air by ventilation are also important factors.

If a number of these unfavourable factors are observed in a house, there is a good chance that it will be damp. For confirmation, signs of dampness must be sought, the most important being the growth of moulds; the occurrence of wood-decaying fungi giving rise to wood rot; a musty smell; damp stains on walls; etc.

All these factors and signs, together with reports from the tenants, were taken into account in assigning the 150 houses into five groups of different degrees of dampness characterized as follows: group I: very dry; group II: reasonably dry; group III: somewhat damp; group IV: damp; group V: very damp.

Of these 150 houses, 75 were chosen in Delft, 30 in Leiden, 30 in

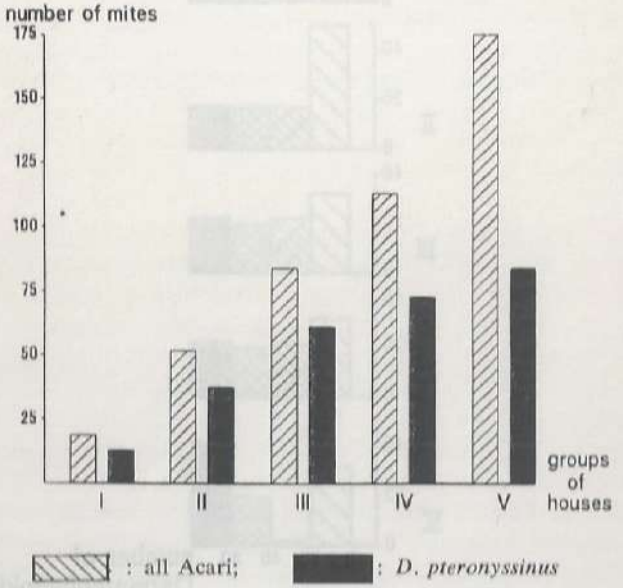

FIG. 2.3. Average number of *D. pteronyssinus* and of all Acari (including *D. pteronyssinus*) in 5 gram dus samples from each of the five groups of 30 houses with different degrees of dampness. I: very dry; II: reasonably dry; III: somewhat damp; IV: damp; V: very damp.

Oegstgeest, and 15 in Noordwijk, such that in each place all groups were represented in equal numbers.

In all these houses dust was sampled in September, when the number of mites reaches a peak.

The diagram in Fig. 2.3 shows the averages of the total numbers of mites and of *D. pteronyssinus* per 5 gram sample for each of the five groups of 30 houses. Fig. 2.4 gives the relative frequency distributions for the five groups.

There is a correlation between the degree of dampness of a house and the number of mites found in its dust. From group I (very dry) to group V (very damp) there is a consistent rise in the average number of mites.

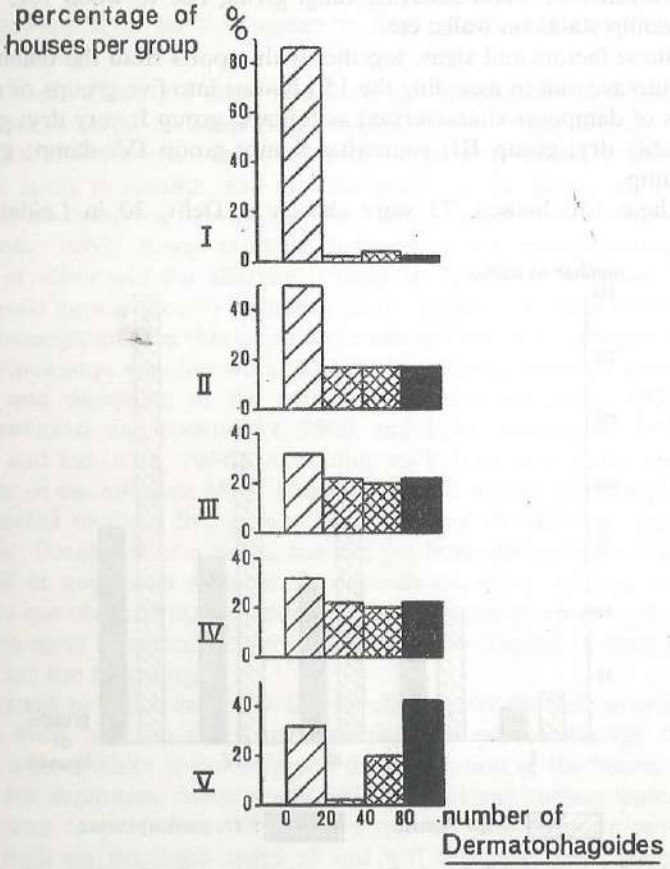

FIG. 2.4. Relative frequency distribution of the houses in the 5 groups with different degree of dampness, according to the number of *D. pteronyssinus* in a 5 gram dust sample.

The number of houses with high numbers of mites increases from group I to group V, whereas the number of houses with low numbers of mites decreases.

It is concluded that, in general, damp houses provide better conditions for the growth of mites than dry ones. Some of the ecological aspects of this phenomenon are discussed in Chapter V (5.5).

CHAPTER III

TAXONOMY AND MORPHOLOGY OF *DERMATOPHAGOIDES PTERONYSSINUS*

3.1 TAXONOMY

The type specimens of several species of *Dermatophagoides* are probably lost or misplaced, especially those of the oldest species. This makes the taxonomical study of this genus very difficult. FAIN is preparing a revision of the genus; a number of his publications are referred to in this short review.

The type species of the genus was described by BOGDANOW in 1864 in Moscow as *D. scheremetewskyi*. The original material is not available, which hampers the study of the genus (FAIN, 1966).

In 1897 TROUESSART (in BERLESE, 1897, 1898) created the genus *Mealia*, with two species: *M. pteronyssina* and *M. longior* (see also TROUESSART, 1901). BAKER and WHARTON (1952) and DUBININ (1953) synonymized the genus *Mealia* with the genus *Dermatophagoides*.

D. pteronyssinus was recently redescribed by FAIN (1966) from paratypes in the original preparations of TROUESSART, the original types being lost. FAIN mentions two synonyms: *Mealia toxopei* Oudemans, 1928, and *Viscopteres saitoi* Sasa, 1948, or *D. saitoi* (Sasa, 1950).

There are three reports of mites identified as *D. scheremetewskyi*, which FAIN's work has shown to be *D. pteronyssinus*. (1) In the material collected by TRAVER (1951) in a case of severe dermatitis ascribed to *D. scheremetewskyi*, FAIN (personal communication) found specimens of *D. pteronyssinus*; (2) the specimens, mentioned by BAKER *et al.*, (1956), found in a feather pillow, appeared to be *D. pteronyssinus* (FAIN, 1966); and (3) OSHIMA (1964) reported the finding of *D. scheremetewskyi* in dust from schools, but after he had seen the redescription of *D. pteronyssinus* he concluded in a letter to FAIN (personal communication) that his specimens belong to the latter species.

According to FAIN (1966), however, *D. scheremetewskyi* and *D. pteronyssinus* may be synonyms. But since the synonymity is uncertain, it is better to keep *D. pteronyssinus* as a separate species, of which there is good type material.

The genus *Dermatophagoides* has long been included in the family Epidermoptidae. FAIN has transferred it to the Psoroptidae. He considers the representatives of this genus to be natural intermediate forms between

the free-living and parasitic Acaridiae and thus the ancestors of all the Acaridiae that parasitize birds and mammals (FAIN, 1963; p. 47).

3.2 MORPHOLOGY

A detailed description of *D. pteronyssinus* is given by FAIN (1966). Only brief mention of some morphologic characters will be made in the present report.

The idiosoma of the female (Figs. 3.1, 3.2, and 3.3) is about 340μ long and about 230μ wide. The cuticle is finely striated. The propodosomal shield is narrow with a rounded posterior and a straight anterior edge. The apodemes of all legs are free. The genital opening is surrounded anteriorly by an epigynium and laterally by genital apodemes. The legs are equal in length and structure, or nearly so.

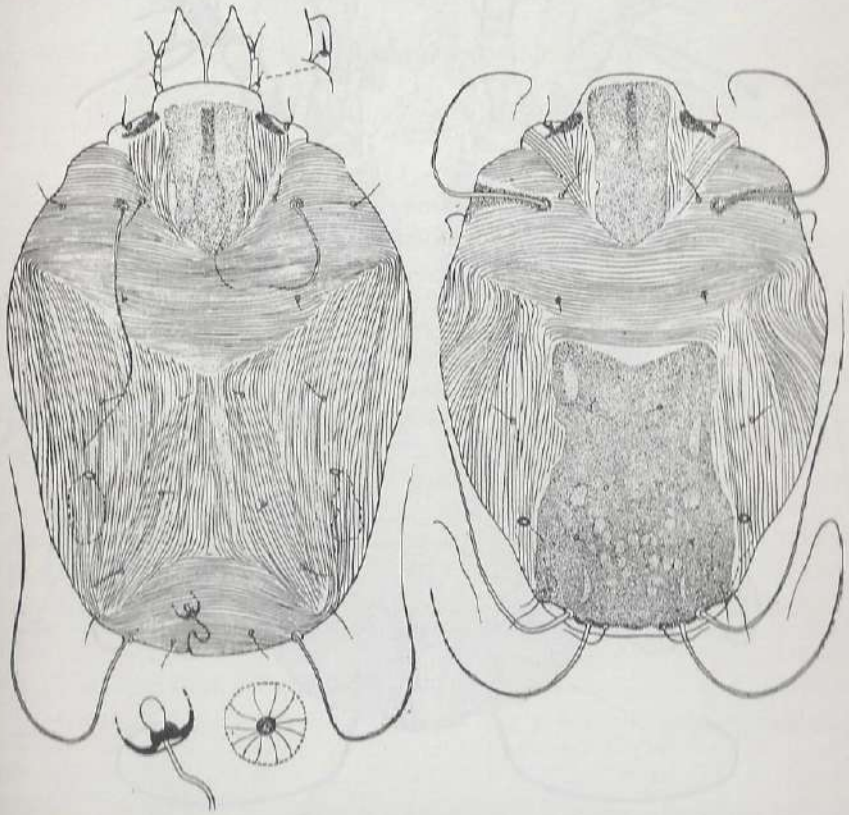

FIG. 3.1. *Dermatophagoides pteronyssinus* (Trouessart, 1897); dorsal view of female (left) and male (right) (after FAIN, 1966).

The male (Figs. 3.1, 3,4, and 3.5) is smaller than the female, the length being about 280μ and the width about 190μ. The striation of the cuticle and the shape of the propodosomal shield are almost the same as in the female. The male has a hysterosomal shield as well. The apodemes of the first and fourth pairs of legs are free; those of the second and third pairs have a tendency to be fused at their medial ends. The apodemes of the third pair of legs are bent sharply at a nearly right angle. The sexual organ is situated between the coxae of the fourth pair of legs. The first and second pairs of legs are equal in length or nearly so; the third pair is considerably stouter and longer than the fourth. The anus is surrounded by

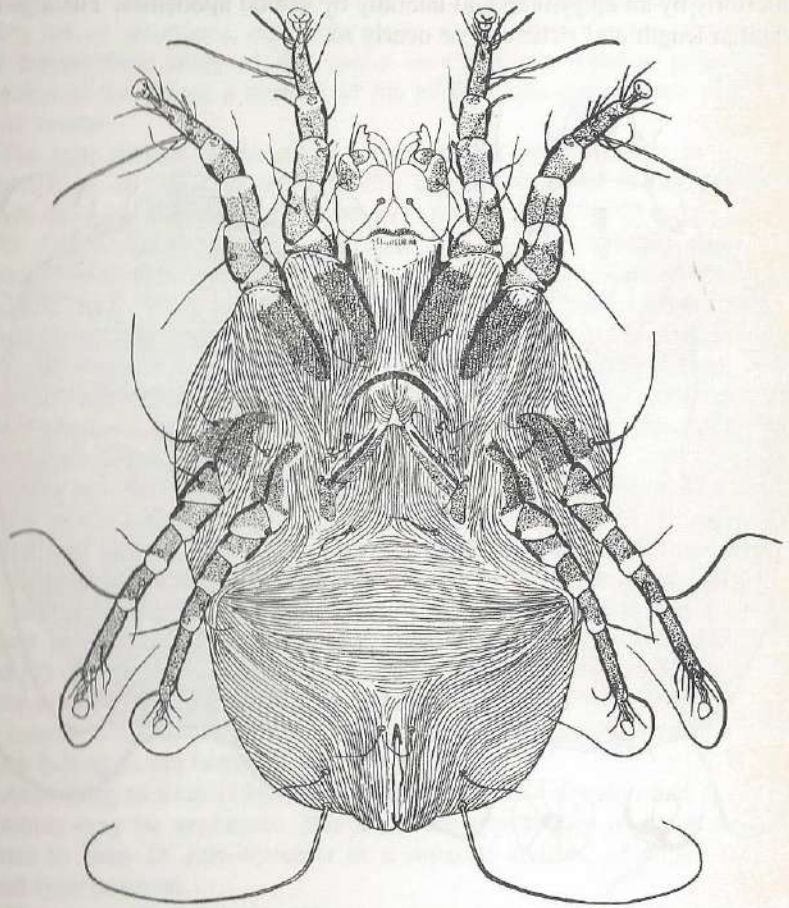

FIG. 3.2. *Dermatophagoides pteronyssinus* (Trouessart, 1897); ventral view of female (after FAIN, 1966).

FIG. 3.3. *Dermatophagoides pteronyssinus* (Trouessart, 1897); female, enlarged by 400 diameters. Photograph by G. Galavazi.

CHAPTER IV

ECOLOGY AND LIFE-CYCLE OF *DERMATOPHAGOIDES PTERONYSSINUS*

4.1 INTRODUCTION

This chapter discusses the results of laboratory experiments designed to study the rate of increase of a mite population in relation to diet, temperature, and humidity, as a means of determining the optimal conditions for *D. pteronyssinus*. The investigations on temperature and humidity were done in a series of eight climate cells ('Dukostaat') at the Department of Ecology of the Zoological Laboratory, University of Leiden. The outer limits of temperature and humidity for mite-survival were not determined.

The life-cycle was studied at temperatures of 25° and 20°C.

Laboratory observations on mating, reproduction, and behaviour are also discussed.

4.2 CULTIVATION OF *D. pteronyssinus* IN THE LABORATORY

Many free-living mites can be kept alive in the laboratory, and those associated with stored food products can be cultivated quite easily.

Mites are very sensitive to desiccation. The required humidity can be maintained in a climate cabinet or in desiccators containing a saturated salt solution. A temperature of 20° to 25°C is suitable for many mites. The kind of food to be used differs from one species to another. Yeast, wheat germ, whole meal flour, and dried heart muscle are acceptable to many free-living Acaridiae.

The mites were reared in small flat-bottomed round glass containers, 5 cm in diameter and 3 cm high (crystallization cups). The rim of the cup was smeared with 'tanglefoot' to prevent escape of the mites and invasion by predators. To avoid airborne contamination by other mites and small insects, larger cups of the same type were inverted to serve as lids. The covered cultivation cups were placed on perforated shelves for free gas exchange.

About 250 mg of a powdered nutrient medium was spread over the bottom of the cup. The mites tend to stay under the food. Inoculation is carried out by placing a number of mites one by one in the cultivation cup, or by adding a small amount of a healthy culture with all stages of development.

The size of the mite population is estimated by counting the live mites under a stereoscopic dissecting microscope.

In some special cases very small cups with a diameter of 1 cm were used, e.g. to study individual specimens. Cells made of perspex or ebonite, as described by SOLOMON *et al.* (1964) and HUGHES (1961), were not used because they develop a strong static electrical charge, which hampers the mobility of the mites, particularly the small larvae.

4.3 FOOD

To find an appropriate nutrient medium for *D. pteronyssinus*, ten products were tested. These products were used to prepare twenty-two different media for estimation of the rate of population increase per medium. Some of the chosen products are used for the cultivation of many other free-living mites (HUGHES, 1961) and in others, according to reports in the literature, representatives of the genus *Dermatophagoides* have been found to occur: albumin tannate (SASA and SHINGAI, 1958); fishmeal (HUGHES, 1961); and house dust (Chapter II). HULL (unplublished observations, in BAKER *et al.*, 1956) cultured *D. scheremetewskyi* in the laboratory and found the most suitable food to be scrapings of cuticle from around human finger nails. For this reason, scales of the human skin were included in this investigation.

Most of the media were prepared both with and without the addition of small amounts of powdered yeast *(Saccharomyces cerevisiae)*, or small amounts of fine dust obtained by sieving house dust. This fine dust was heated to 60°C for 6 hours to kill any mites and mite eggs it might contain. Scales of human skin were obtained from hair collected in barbershops.

The total amount of nutrient material was 250 mg per cultivation cup; the additions, when used, were 50 mg. The powdered material was dusted in a thin layer over the bottom of the cup.

This experiment was carried out in two series. Unfortunately, the results of these two series cannot be compared directly, because in one of them the temperature and relative humidity of the air were not constant due to a small failure in the performance of the climate cabinet. Nevertheless, corrections could be made because two of the media were used in both series. The results of the series performed under inconstant conditions (i.) are corrected on the basis of the results obtained with these two media in both series. The constant conditions (c.) were a temperature of 25°C and a relative humidity of the air of 80%. The temperature and relative humidity during the series of the experiment under inconstant conditions were between 22° and 25°C, and 75 and 85%, respectively.

Five adult female mites were placed in each of the cultivation cups as inoculation.

On eleven media the number of mites showed no increase but, to the

TABLE 4.1. The rate of increase of a population of *D. pteronyssinus* on different kinds of food; i.: under inconstant conditions; c.: under constant conditions.

		Dry powdered nutrient medium (250 mg)		Duration of the culture (in days)	Number of mites after 80 days
		Main constituent	Addition (50 mg)		
1.	i.	HEART INFUSION AGAR		10	
2.	i.	HEART INFUSION AGAR	powdered yeast	10	
3.	i.	POWDERED MILK		20	
4.	c.	DRIED BLOOD PLASMA	powdered yeast	25	
5.	i.	KERATIN		25	
6.	i.	KERATIN	powdered yeast	30	
7.	i.	WHEAT GERM FLAKES		35	
8.	i.	WHEAT GERM FLAKES	powdered yeast	40	
9.	i.	FISHMEAL		45	
10.	c.	POWDERED YEAST		65	
11.	i.	POWDERED MILK	powdered yeast	70	
12.	c.	ALBUMIN TANNATE	powdered yeast	more than 80	40
13.	i.	HUMAN SKIN SCALES		80	45
14.	c.	FISHMEAL	fine dust	80	50
15.	c.	FINE DUST		80	175
16.	c.	FINE DUST	human skin scales	80	225
17.	c.	FISHMEAL	powdered yeast; fine dust	80	275
18.	i.; c.	FISHMEAL	powdered yeast	80	350
19.	c.	HUMAN SKIN SCALES	fine dust	80	450
20.	c.	FINE DUST	powdered yeast	80	500
21.	c.	HUMAN SKIN SCALES	powdered yeast; fine dust	80	750
22.	i.; c.	HUMAN SKIN SCALES	powdered yeast	80	1500

contrary, decreased more or less rapidly. On the other eleven media the number of mites did show an increase during about the first 80 days, and then remained more or less constant.

In Table 4.1 the twenty-two combinations of the ten products are listed according to the rate of increase of the mite population. For the media on which the mites survived less than 80 days, the numbers of days during which living mites were observed are given. For the media on which the mites lived more than 80 days, the numbers of mites counted on the 80th day after inoculation are given. The numbers are rounded-off average values of two cultivation cups.

Of the ten products tested, the best results were obtained with human skin scales, followed by fishmeal and fine dust. It was also found that the addition of powdered yeast favours the growth of the mite population significantly. The medium consisting of human skin scales with an addition of powdered yeast gave the best results of all the combinations.

4.4 TEMPERATURE

For the experiment to determine the optimal temperature for the growth of a population of *D. pteronyssinus* in the laboratory, use was made of thermostatically-controlled cells held at 17°, 21°, 25°, 28°, and 32°C. Relative humidity of the air was held at about 80% by a saturated solution of ammonium chloride on the bottom of the desiccators.

The food was human skin scales to which a small amount of fishmeal had been added. (This medium is not included in the list in Table 4.1 because the temperature experiment was carried out before the optimal nutrient medium was known.)

Three cultivation cups, each containing 20 mites (immature stages, males, and females) per cup, were placed in each desiccator.

From Fig. 4.1, which shows the averages of the rates of increase for three cups at the different temperatures, it is evident that a temperature of 25°C is optimal for the growth of a population of *D. pteronyssinus* in the laboratory.

4.5 RELATIVE HUMIDITY OF THE AIR

To study the requirements of *D. pteronyssinus* with respect to humidity, use was made of climate cells in which the relative humidity was maintained by the intermittent injection of a very fine spray of water triggered by a lithium-chloride cell. The accuracy was ± 3% relative humidity.

Cultures of mites were exposed to four different degrees of relative humidity: 70, 75, 80, and 85%, each maintained in 2 cells. Three or four cultures were placed in each of the eight cells.

The mites were fed on human skin scales with powdered yeast (no. 22 in Table 4.1). The temperature was 25°C. At the beginning of the experiment, each of the cultivation cups contained about 150 living mites.

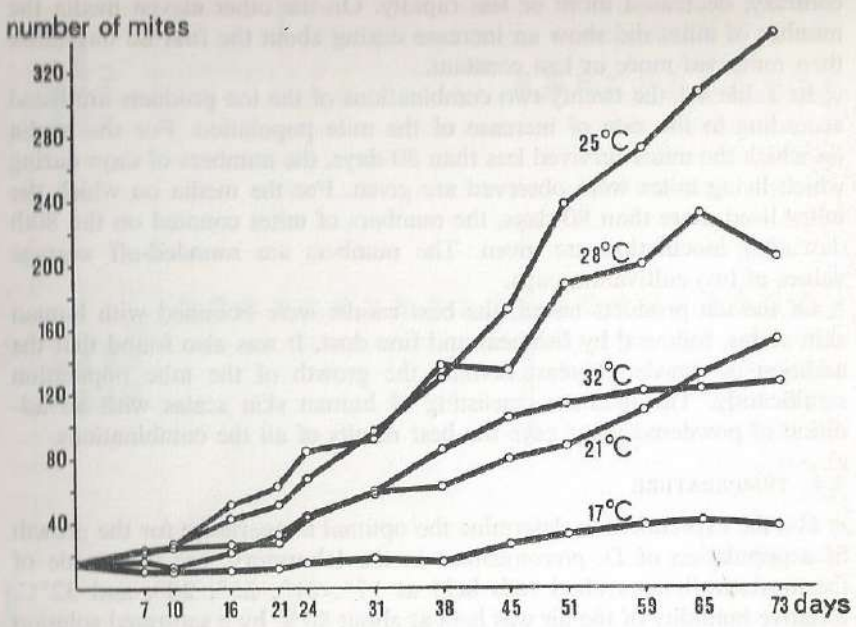

FIG. 4.1. The increase in laboratory populations of *D. pteronyssinus* at various temperatures.

On the 20th day after inoculation, the mites in the cups were counted and the average number per climate cell was calculated. From these numbers the average values for each pair of cells with the same degree of humidity were computed; the results are shown in Table 4.2.

According to this Table, a high relative humidity of the air favours the growth of *D. pteronyssinus,* but the presence of growing moulds coincides with a decrease in the number of mites. This is difficult to explain. Growth of *D. pteronyssinus* may be inhibited by a harmful influence of the moulds. However, an argument against the hypothesis that moulds are harmful is

TABLE 4.2. The increase in laboratory populations of *D. pteronyssinus* at different humidities.

Relative humidity of the air (in %)	Number of mites after 20 days
70	42
75	122
80	775
85	— (moulds)

provided by the result of an experiment in which cultures with growing moulds and a few living mites were shifted from 85 to 80% relative humidity. After a short time the moulds stopped growing and declined slowly, whereas the number of mites increased in the presence of the mould material. Mites in cultures with growing moulds are caught in the hyphes and locomotion becomes impossible. It is not known whether this phenomenon is the main reason for the decline of the mite population or whether it is only a contributory factor.

To determine the real influence of humidity above 80%, it will be necessary to study the growth of populations of *D. pteronyssinus* on sterilized nutrient media inoculated with uncontaminated mite eggs, or with addition of fungicides. Experiments of this kind have not been carried out as yet.

The finding that mite growth is inhibited by moulds is also very interesting from the ecological point of view. Practical experience of flour merchants with stored cereal products has shown that no moulds are found on flour infested with mites and mouldy flour shows no mites. SOLOMON *et al.* (1964) reported on two species of storage fungi antagonistic to mites. Some of the consequences of this possible inhibitory effect of moulds for the domestic occurrence of mites will be discussed in Chapter V (5.5).

The growth of *D. pteronyssinus* populations under laboratory conditions and without sterilization of media and mite eggs, is best promoted by a relative humidity of the air of 80%.

4.6 BEHAVIOUR

During the above-mentioned experiments with laboratory populations of *D. pteronyssinus,* several observations were made on the locomotory behaviour of the mites in the cultures.

Mites of this species have a rather slow but steady way of walking. When they are stimulated by light or heat radiation, their movements become badly controlled. This irradiation has no effect on their walking direction. Many other species run away from light, warmth, or dry conditions, and this reaction can be used in collecting living mites from all kinds of materials, but *D. pteronyssinus* does not show this behaviour.

In the cultivation cups the mites stay on the bottom underneath the nutrient medium most of the time; they seldom climb the walls of the cup. When the powdered nutrient medium is very loose they sometimes do not succeed in staying on the bottom, and then crawl over the surface of the medium. When this happens, the increase of the population in the cup is greatly reduced. The reason for this phenomenon is not known. When a sufficient number of mites remain in a loose medium for a certain period of time, however, the medium becomes firmer and shows a discoloration.

4.7 REPRODUCTION

For a good understanding of the processes of mating and copulation, certain morphological features, particularly of the sexual organs, must be taken into account.

The male of *D. pteronyssinus* is equiped with several organs enabling it to grasp the female during mating. It possesses two adanal suckers, and the fourth pair of legs bears two small suckers on the tarsus. Furthermore, the tarsus of the third pair has two spines, one of which is bifid (see FAIN, 1966). All these organs probably have a function in mating.

The male attaches itself by the two adanal suckers to the posterior part of the back of the female such that their gnathosomata point in opposite directions. The fourth pair of legs of the male grasps the female laterally. The third pair makes regular movements along the dorsal part of the lateral side of the female. The male may remain attached to the female for several hours. In these laboratory observations the moment of copulation could not be determined exactly.

For copulation, the penis of the male, which otherwise points anteriorly (Fig. 3.5), must curve in a posterior direction (Fig. 3.4) to reach the external opening of the bursa copulatrix of the female. This cup-shaped opening is situated at the posterior end of the body a short distance from the medial line.

D. pteronyssinus is oviparous. The female starts laying eggs three or four days after the first copulation. The period of oviposition, which lasts about 20 days, can be followed immediately by another oviposition period after a second mating. A third mating may take place. However, in these laboratory observations, multiple copulation was not observed frequently.

The female produces 25 to 50 eggs during the first oviposition period and 15 to 30 eggs when a second occurs. In the few cases in which eggs were laid after a third copulation, the number was much smaller. In most of the females the production of eggs occurred only during the first 45 days of maturity. The highest total number of eggs laid by one female was 79 in two oviposition periods totalling 27 days. Sometimes 4 eggs were laid on one day, but females were seldom found to contain more than one developing egg. It is therefore supposed that the eggs can develop quickly.

The eggs are laid not in clusters or packets but rather at random along the female's route, preferrably on firm substrate particles.

Reproduction without copulation was not observed in this study.

4.8 LIFE-CYCLE

The postembryonic development of the Acaridiae, to which *D. pteronyssinus* belongs, normally includes three immature stages between the egg and the adult: a larval stage and two nymphal stages. Among the Oribatei,

whose immature stages resemble the Acaridiae, three nymphal stages are formed rather than the two found in the Acaridiae. The three nymphal stages of the Oribatei are called protonymph, deutonymph, and tritonymph. Among many of the free-living Acardiae a non-feeding hypopial form may occur between the two nymphal stages. This hypopus can be regarded as the missing deutonymph; the first and last nymphal stages of the Acaridiae are called protonymph and tritonymph, respectively.

The sequence of the developmental stages of *D. pteronyssinus* in the laboratory consists of an egg, a six-legged larva, a protonymph, a tritonymph, and adult males and females. No deutonymph or hypopus and no heteromorphic males have been found in this species.

The determination of the duration of the several immature stages was carried out at a temperature of 25°C and 80% relative humidity of the air, with human skin scales and powdered yeast as food. These conditions had been established as the best of those tested for the rate of increase of the population in the laboratory.

The egg stage lasts 6 days. The duration of the larval and the two nymphal stages is shown for 65 specimens in Fig. 4.2. The columns represent the number of specimens, distributed according to the duration in days of each of the three stages. The larval stage takes 5 or 6 days, during the last 2 of which the larva rests. From this resting form the protonymph emerges. The protonymphal stage lasts from 4 to about 7 days, including a resting period of about 2 days before the emergence of the tritonymph. This last nymphal stage has a duration of 4 to 8 days (most often 5 to 6 days), and also has a resting form lasting about 2 days. The total immature life from larva to adult lasts 14 to 20 days, with a minimum of 13 and a maximum of 33 days in these 65 specimens. No explanation can be given for the very long duration of the protonymphal stage in one specimen (17 days), and of the tritonymphal stage in four specimens (13–15 days) (Fig. 4.2).

In this laboratory study adult males of *D. pteronyssinus* were frequently observed to attach themselves to the resting forms of the tritonymphs. This habit has also been reported in the literature for other species of the family Psoroptidae. In *Otodectes cynotis* WOODROOFFE (1958) often observed males attached to tritonymphs, and SWEATMAN (1957) almost invariably found adult males of *Chorioptes bovis* attached to female tritonymphs, and observed that unattached tritonymphs did not appear to be able to complete their development.

A number of species of the family Psoroptidae show morphological differences between the sexes, not only in the adults but also in the immature stages, especially in the tritonymphal stage (FAIN, 1963). During these studies, male and female tritonymphs of *D. pteronyssinus* could not be distinguished morphologically. There must, however, be differences between them because from the resting tritonymphs grasped by adult

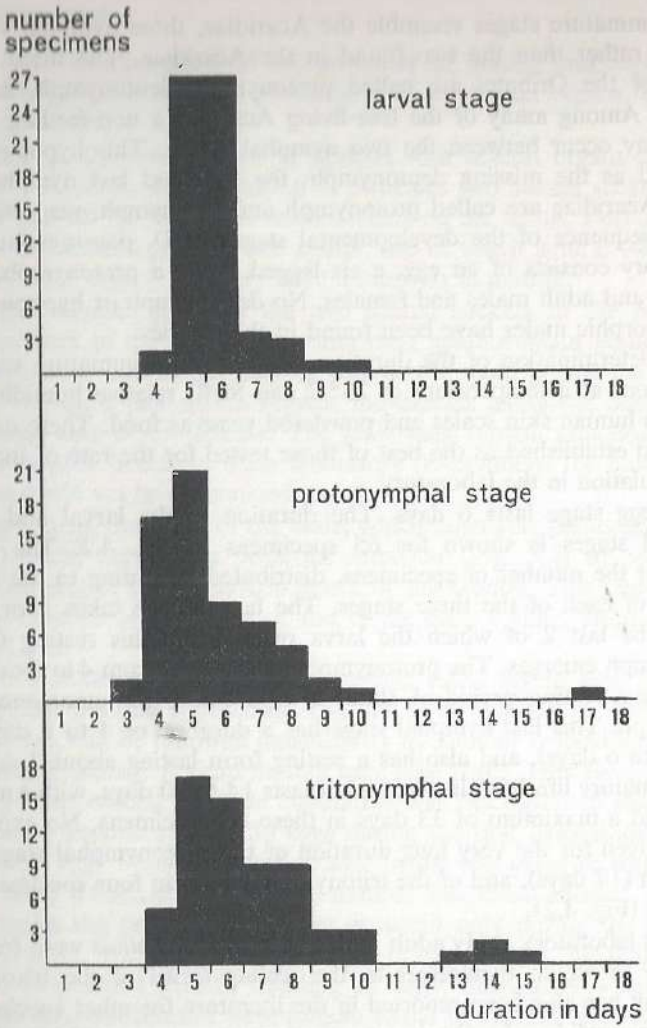

FIG. 4.2. The duration of the three immature stages of *D. pteronyssinus*, determined for 65 specimens.

males, female adults always emerged. Adult males of *D. pteronyssinus* are able to distinguish between males and females before their emergence from the tritonymphal resting form. It was not observed in *D. pteronyssinus*, however, that unattached female tritonymphs were unable to complete their development. All the 65 specimens in which the duration of the immature stages was determined were kept in individual containers, and

both males and females in about equal number emerged from the resting tritonymphs successfully.

Of these 65 specimens, 34 were males and 31 females. No differences between males and females in the duration of the several stages in the life-cycle were observed.

Adult males live 60 to 80 days and females 100 to 150 days. The production of eggs by the female was not observed during the second half of its life, as already mentioned (4.7).

A few observations were also made on the duration of the immature stages at 20°C. At this temperature the larval, protonymphal, and tritonymphal stages last about 9 to 10, 9 to 11, and 11 to 16 days, respectively, including the resting period of 3 to 4 days at the end of each stage. The total immature life has a duration of 29 to 37 days at this temperature. Compared with the life-cycle at 25°C, the development from larva to adult at 20°C requires about twice as much time.

CHAPTER V

ECOLOGICAL CONSIDERATIONS CONCERNING THE OCCURRENCE OF *DERMATOPHAGOIDES PTERONYSSINUS* IN HOUSE DUST

5.1 INTRODUCTION

Although other factors may influence the growth of a population of *D. pteronyssinus,* food, temperature, and the relative humidity of the air are obviously of decisive importance. From its regular occurrence in house dust it can be concluded that in many houses these factors are favourable for the growth of *D. pteronyssinus,* but conditions are generally not favourable for the development of populations of many other species of mites found in relatively small numbers and low frequencies.

For the considerations respecting the relative humidity of the air in houses, use has been made of the studies of M. J. Leupen on the constructional and physical aspects determining microclimatological conditions in houses (LEUPEN and VAREKAMP, 1966).

5.2 FOOD

Because the microclimatic conditions in houses are variable in both space and time, it is improbable that they would create a specific habitat for *D. pteronyssinus* with a relatively narrow preference for temperature and humidity, and not for other species. Moreover, there is no reason to assume that the temperature and humidity requirements of *D. pteronyssinus* differ greatly from those of many other mites. The results of the laboratory experiments (Ch. IV; 4.3) show that a temperature of 25°C and a relative humidity of the air of 80% are suitable for the growth of *D. pteronyssinus*. These conditions apparently do not differ very much from those required for the growth of such species as *Glycyphagus domesticus* (23° to 25°C; 80 to 90% relative humidity), *Tyrophagus putrescentiae* (23°C; 87%), or *Acarus siro* (23°C; 87%) (HUGHES, 1961). But, although these species can be recovered from house-dust samples, their numbers are generally low compared to the numbers of *D. pteronyssinus*. However, if suitable food for these species is available in places in the house with adequate climatic conditions, a dense population develops within a short time.

It therefore seems likely that food is the decisive factor in this respect,

and that house dust contains a material that must be considered to be specific food for *D. pteronyssinus,* whereas food material for the other species of mites is generally lacking in house dust.

According to FAIN (1966), *D. pteronyssinus* has also been found on hides from mammals (TROUESSART, 1901), on the skin of mammals and birds, and in bird's nests. It seems as though *D. pteronyssinus* is attracted by food materials with a high protein content of animal origin.

Its presence on the skins of live animals suggests a tendency towards parasitism. It seems quite possible, however, that in the cases in which mites were collected from the skin of mammals suffering from a skin disease, they had been attracted by the skin lesions rather than having been the primary causers of the disease (FAIN, 1966). Because of its occurrence in house dust with no reports of parasitism in man, it is unlikely that *D. pteronyssinus* is a true skin parasite.

The occurrence on hides narrows the problem of what these mites' food actually is. It may be that the specific food for *D. pteronyssinus* is found among the components of dead skin. It must be kept in mind, however, that hides are not the only form in which dead skin occurs. The live skin of mammals and birds is a tissue that continuously produces horny debris or skin scales as the cells on the outer surface of the skin die.

The assumption that *D. pteronyssinus* uses the horny debris from the skin of mammals or birds as specific food raised two questions. In the first place, could it be demonstrated that *D. pteronyssinus* grows rapidly on a nutrient medium containing nothing or almost nothing but skin scales from mammals or birds? In the second place, could it be demonstrated that the rate of production of this horny debris by human skin is sufficient to provide enough food for a population of mites of the size found in house-dust samples?

As far as the first question is concerned, it is interesting to cite from 'A Manual of Parasitic Mites of Medical or Economic Importance' (BAKER *et al.,* 1956). Concerning experiments on the life-cycle of *D. scheremetewskyi* (there are reasons, however, to doubt that it was *D. scheremetewskyi* indeed, and not in fact *D. pteronyssinus* (Ch. III; 3.1)), it is said (pp. 147-8): 'The mites were kept in a laboratory colony for more than a year (reference: Hull, W.B., 1953. Unpublished observations). After experimenting with various dry foods, such as powdered milk, brewers' yeast, and dried blood plasma, the best food for the mites was found to be scrapings of cuticle from around the fingernails'.

Experiments with various nutrient media reported here (Ch. IV; 4.3), confirm this observation in so far that rapid growth of *D. pteronyssinus* occurred on a dry nutrient medium containing human skin scales with the addition of powdered yeast.

The answer to the second question, about the production of horny debris or skin scales by man, must be sought in the field of dermatology.

Recently, Goldschmidt and Kligman estimated that: '. . . the total body production, assuming a surface of two square meters, is of the order of 0.5 to 1.0 gm daily . . .' (KLIGMAN, 1964). This holds true for the residue of 'crude soft horn' after defatting, washing, and drying. Of this residue, allowing for cell membranes and bound horn fat, about 70% may be considered keratin, again according to KLIGMAN (1964).

Observations on the growth of populations of *D. pteronyssinus* in laboratory cultures show that thousands of mites can live for several months on 150 mg nutrient medium. From these observations it is evident that a production of 0.5 to 1.0 gram (500 to 1000 mg) skin scales daily per inhabitant of a house would provide enough food material for the highest numbers of mites found, even taking into account the fact that people spend parts of the day outside their homes.

The supposition that *D. pteronyssinus* uses human skin scales as the main source of food may also explain why high concentrations of this species are not found in houses. Since the scales are distributed over the entire house, they will occur in relatively low concentrations in house dust.

The number of *D. pteronyssinus* in house dust may be controlled primarily by the existence of places in the house where temperature and relative humidity of the air are favourable for mite growth (see below, sections 5.3 and 5.4), but the development of dense populations may be prevented by the relatively low concentration of the food material in the house or in the dust, although the total amount of skin scales may be more than sufficient.

Other species of mites, mostly associated with stored food products and found incidentally in house-dust samples, may not find their food in the dust. Their presence in dust may be due to migrating specimens. When high numbers of *Tyrophagus* are found in a dust sample, one may expect a concentration, for example in a kitchen cupboard where temperature and humidity have promoted the development of a dense population in a packet of flour.

It should be emphasized that this attempt to explain the occurrence of *D. pteronyssinus* and the relatively small numbers of other species of mites in house dust is based partly on hypothetical considerations. Among other things, it is not quite certain that this species lives exclusively on human skin scales. It is also unknown whether it is the concentration and not the kind of food in the house that is the decisive factor. Moreover, as already mentioned in the introduction to this chapter (5.1), not all the factors controlling the growth of a population have been taken into account. Nevertheless, these considerations do not detract from the value of the hypothesis.

5.3 TEMPERATURE

The optimal temperature of 25°C for the growth of *D. pteronyssinus*,

determined in the laboratory (Ch. IV; 4.4), is very seldom reached in the Dutch climate and never occurs over prolonged periods. The monthly averages of the outdoor day temperatures over a period of 30 years (1931–1960) are shown in Fig. 5.1 (top: solid line). These temperatures were calculated by the *Koninklijk Nederlands Meteorologisch Instituut* (KNMI) from daily observations made at 8:00, 14:00, and 19:00 hours at the weather station in Naaldwijk, situated in the western part of the country.

The temperature of the air in the living-room in summer depends on the outdoor temperature, and, according to incidental measurements by LEUPEN (personal communication), is usually a few degrees higher due to radiation from the sun, household activities, heat production by inhabitants, etc. In winter, however, the temperature of the air in the living-room is kept almost constant by heating (Fig. 5.1; top; dotted line).

The temperature of the materials of the house that provide a substrate on which the mite can live, may differ considerably from that of both the indoor and outdoor air and will show complicated gradients. These differences can be caused by heating systems used in winter, radiation from the sun, nocturnal cooling, evaporation of water, etc. No reliable data are available for the temperatures of the materials serving as the substrate for *D. pteronyssinus*. The average temperatures of these materials may lie somewhere between those of indoor and outdoor air, and it is not likely that inside the house they would reach 25°C.

5.4 RELATIVE HUMIDITY OF THE AIR

The relative humidity of the air depends on the temperature: it drops with rising temperature and rises when the temperature goes down, if the amount of water vapour remains constant.

The monthly averages of the relative humidity by day over a period of 30 years (1931—1960), calculated by the KNMI from observations at 8:00 14:00, and 19:00 hours, are shown in Fig. 5.1 (below; solid line). From the temperature and relative humidity, the absolute humidity can be calculated, e.g. in grams water vapour per m^3 of air (Fig. 5.1; middle; solid line).

When, in winter, the cold outdoor air enters the living-room and is heated to 20°C, the relative humidity of that air will drop sharply, for example from 90 to 40%, without a change in the amount of water vapour. Moisture production by the inhabitants (breathing, evaporation of sweat) and their housekeeping activities (cooking, washing, drying) may increase the humidity indoors, especially in winter when the house is ventilated less than in summer. According to SCHÜLE and LUTZ (1962), measurements have shown that the differences in the amounts of water vapour between outdoor and indoor air are about 2 to 4 gm/m^3 in winter, and 0.5 to 1 gm/m^3 in summer (Fig. 5.1; middle; dotted line). From these data and the observations of the KNMI, the relative humidity of the

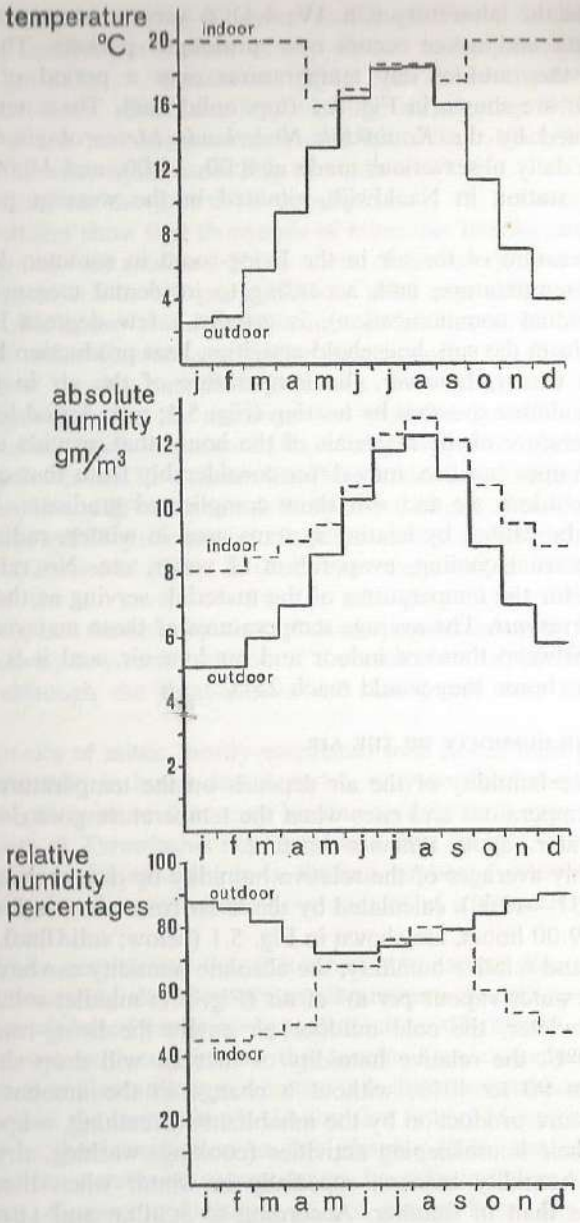

FIG. 5.1 Monthly averages of temperature and humidity of the outdoor and indoor air by day over a period of 30 years (1931—1960). Modification of LEUPEN and VAREKAMP (1966).

indoor air can be calculated (Fig. 5.1; below; dotted line). In summer the relative humidities of the outdoor and indoor air will almost be the same, i.e. about 70 to 80%. During the rest of the year, they differ widely.

It must be kept in mind that Fig. 5.1 is only a schematic representation of temperature and humidity conditions by day, meant to indicate very roughly the relation between these conditions outdoors and indoors during the year.

It has been shown (Ch. IV; 4.5) that *D. pteronyssinus* requires a high relative humidity for the development of a population, and that there is an autumnal peak in the numbers of mites recovered from house-dust samples (Ch. II; 2.6). Because the relative humidity of the indoor air also reaches a maximum at the end of the summer and the beginning of the autumn, it may be concluded that the fluctuations in the numbers of mites are caused by this periodicity of the relative humidity of the indoor air.

5.5 MOISTURE CONTENT OF THE HOUSE

The humidity of the air in direct contact with the building and other materials will be influenced by the condition of these materials, which also serve as the substrate on which the mites live. Most building materials as well as, for example, textiles and also house dust, take up water from the surrounding air. Under stable conditions the moisture content of the materials is in equilibrium with the relative humidity of the surrounding air. In general, the quantity of water taken up to reach this state of equilibrium is small, and the materials still feel dry to the touch.

When sufficient free water is present, the materials can also take up moisture by capillary action. Depending on the kind of material, the amount of water taken up by capillary action can be many times greater than its equilibrium mositure content. This can occur with penetration of the walls by rain, with leakages, or with the penetration of moisture from the soil, all of which cause transport of water in the materials. Under such circumstances the materials feel wet to the touch.

Because the relative humidity of the surrounding indoor air is almost always lower than 100%, the water transported by capillary action will evaporate, causing the formation, just above the wet material, of a very thin layer of air saturated with water vapour. Between this saturated layer and the indoor air there will be a drop in relative humidity. This gradient is steeper the lower the relative humidity of the air, because under stable conditions the zone occupied by this gradient has an almost constant size and does not depend on the relative humidity of the surrounding air. According to LYKOW (1958), measurements have shown that this zone extends about 2 mm above the surface of free water. LEUPEN and VAREKAMP (1966) call this zone the 'boundary layer'.

When no capillary water is present in the material and the moisture

content is in equilibrium with the relative humidity of the surrounding air, no such gradient or boundary layer will exist.

Based on these considerations, LEUPEN and VAREKAMP (1966) have constructed a scheme concerning the relative humidity of the air in the 2 mm thick layer in contact with wet and dry material at three different degrees of relative humidity of the surrounding air, i.e. 40, 60, and 80% (Fig. 5.2).

It is evident that under average conditions in winter and spring, when the relative humidity of the indoor air does not exceed 60%, no *D. pteronyssinus* could be expected to survive on dry material. Only under the higher humidity conditions of summer (80%) is it possible for mites to occur on such a substrate.

On wet material, however, humidity conditions that would permit the growth of *D. pteronyssinus* will be realized within the boundary layer almost throughout the whole year. When the humidity of the indoor air is high (80%) the relative humidity of the air in the boundary layer will be so high that conditions become favourable for moulds. It is possible that under such conditions the growth of moulds inhibits the development of a population of *D. pteronyssinus,* as pointed out in Chapter IV (4.5). It can also be seen from Fig. 5.2 that the zone of 80% relative humidity, which is likely to be preferred by *D. pteronyssinus,* is narrower the lower the humidity of the surrounding indoor air.

This representation suggests that the conditions of relative humidity of the air in direct contact with wet construction material inside the house are almost always favourable for the growth of populations of *D. pteronyssinus,* whereas on dry materials these conditions can become favourable only during late summer and early autumn.

The actual situation in a house, however, will be much more complicated than is represented in this Figure. There will certainly be temperature differences between the material and the indoor air, giving rise to a temperature gradient and, consequently, to a humidity gradient. Also, a flow of air can greatly disturb the conditions in the boundary layer. The effects of irregularities in the surface and the influence of other small dust particles on the surface of the material are not taken into account. And lastly, the data of LYKOW (1958) are based on measurements above a free water surface, and not above wet material.

The schematic representation in Fig. 5.2 of the behaviour of the relative humidity of the air in the boundary layer in relation to the moisture content of the material and the relative humidity of the air in the adjacent space clarifies the occurrence of greater numbers of mites in damp houses than in dry ones (Ch. II; 2.7).

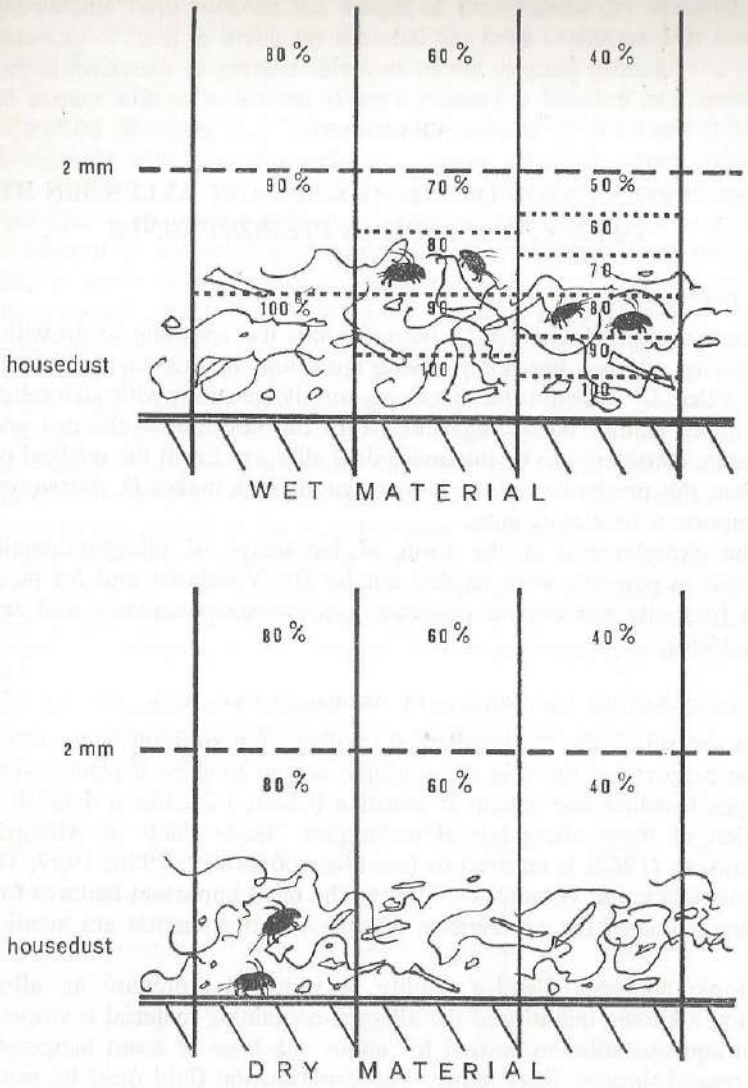

FIG. 5.2. Schematic representation of the relative humidity conditions of the air in the 2 mm layer ('boundary layer') in direct contact with wet and dry material, under stable conditions.
Modification of LEUPEN and VAREKAMP (1966).

CHAPTER VI

THE PRODUCTION OF THE HOUSE-DUST ALLERGEN BY *DERMATOPHAGOIDES PTERONYSSINUS*

6.1 INTRODUCTION

The question of whether *D. pteronyssinus* has anything to do with the house-dust allergen has not yet been answered. In this chapter it will be shown that *D. pteronyssinus* produces an allergen that with allergological techniques cannot be distinguished from the house-dust allergen and is therefore considered to be the house-dust allergen. From the medical point of view, this production of the house-dust allergen makes *D. pteronyssinus* an important species of mite.

The experiments, in the form of bio-assays of allergen-containing material in patients, were carried out by Dr. Voorhorst and his medical team (partially reported in VOORHORST, SPIEKSMA-BOEZEMAN, and SPIEKSMA, 1964).

6.2 PREPARATION AND TESTING OF ALLERGEN EXTRACTS

To determine the atopic-allergen content of a substance, use is made of the property of the skin of an atopic person to show a reaction to the allergen to which the patient is sensitive (Ch. I; 1.2). For a detailed description of these allergological techniques, 'Basic Facts of Allergy' by VOORHORST (1962) is referred to (see also VOORHORST, 1958; 1959; 1960; VAN DISHOECK and VOORHORST, 1959). The most important features for an understanding of the experiments described in this chapter are mentioned here.

Atopic allergens dissolve readily in water. To prepare an allergen extract, a known quantity of the allergen-containing material is suspended in an aqueous solution, stirred for about one hour at room temperature, and passed through filter paper. The experimental fluid must be isotonic with blood plasma and have a pH value of about 6.8. The use of potassium salts must be avoided, because K^+ ions make the injections more painful. Sterility can be procured by heating to 100°C or by filtration through sintered glass bacterial filters, depending on the thermo-resistance of the allergen. The addition of 0.5% phenol can be expected to kill any virus present and to prevent the proliferation of any microbial organism that might accidentally infect the experimental fluid.

The strength of the extract is expressed in percentages, indicating the weight-volume ratio between the weight of the original dry material and the quantity of fluid in which the material has been extracted. For testing, it is often necessary to prepare dilutions of the original extract.

An extract with an unknown allergen content is injected as a series of three tenfold dilutions (e.g. house-dust extracts of 1, 0.1, and 0.01%) simultaneously with a control series of a known standard extract, into the skin on the back of a person who is atopic to the allergen under investigation. After a short time (within 20 minutes) the skin shows a reaction in the form of the so-called 'triple response' of Lewis. This 'triple response' consists of wheal, area of redness, and flare.

The sequence of the injections on the patient's back is varied, and is not known to the doctor who is to judge the strength of the reactions. The degree of the reaction is expressed by plus signs. There are no simple, generally valid rules for the evaluation of the reaction. According to Voorhorst's view, however, for judging many skin reactions, especially when caused by a series of tenfold dilutions, the eye of the investigator is much more reliable than any geometrical method for estimating the diameter or the surface of the wheal or area of redness.

After having tested a number of persons in this way, the number of plus signs for each dilution of the two extracts are counted, and the average number per patient is plotted against the logarithm of the dilutions to obtain a log-dose curve. An example of how the strength of an unknown

TABLE 6.1. Comparison of skin reactions in 13 patients to an unknown house-dust extract (U) with the reactions to the standard house-dust extract (S) (see: Fig. 6.1).

	unknown extract (U)			standard extract (S)		
pat. no.	1%	0.1%	0.01%	1%	0.1%	0.01%
1	++±	++	++	+++	+±	+±
2	++±	++	+±	+++	++±	+
3	+++±	++	+	++±	+±	±
4	+++	+	+	++	±	—
5	+++	++	+	+±	+±	—
6	+++±	++±	+±	++	±	—
7	+++	+++	++	+++	+±	+
8	+++	+++	+±	+	+	—
9	+±	+	—	+	—	—
10	+++	+±	±	+±	+±	—
11	+++±	+++	++	+++	+±	±
12	++++	++±	+	++	+	—
13	++++	+++	++	+++	++	+
total	40	28½	17	28½	16½	5½
average per patient	3.1	2.2	1.3	2.2	1.3	0.4

extract of house dust is determined with respect to a standard extract with a known allergen content is shown in Table 6.1 and Fig. 6.1. The horizontal distance between the curves of the two extracts is one unit of the horizontal logarithmic scale. The unknown extract therefore contains ten times the allergen amount of the standard extract.

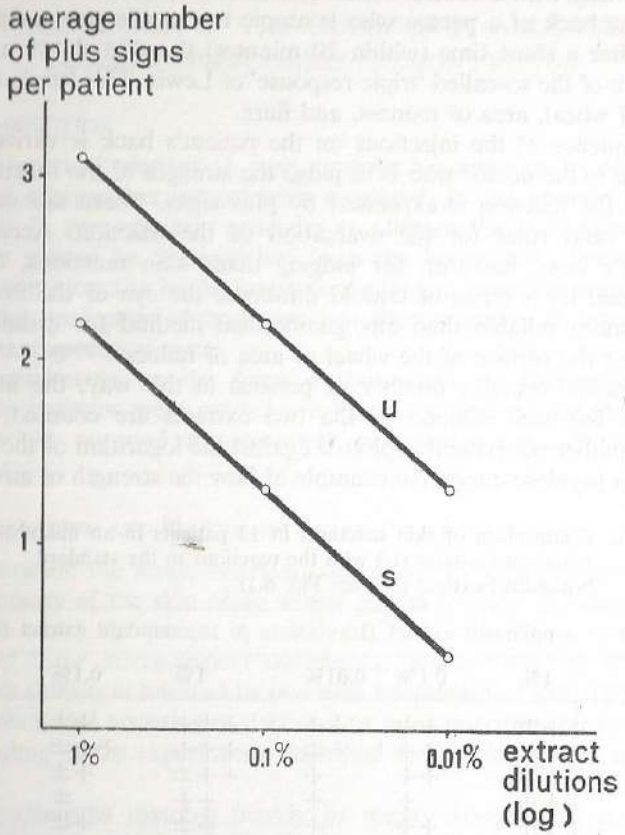

FIG. 6.1. Log-dose curve of two house-dust extracts: comparison of the skin reactions in 13 patients to an unkown house-dust extract (U) with the reactions to the standard house-dust extract (S) (see Table 6.1).
The horizontal distance between the two curves is 1 unit of the logarithmic scale: the unknown extract contains 10 times as much allergen as the standard extract.

6.3. THE NUMBER OF *D. pteronyssinus* AND THE AMOUNT OF ALLERGEN IN A HOUSE-DUST SAMPLE

The number of patients coming to the out-patient clinic of the Department of Allergology with asthmatic complaints due to a house-dust atopy

reaches a peak in the autumn. This autumnal peak is not found in the group of asthmatic patients without skin reactions to house dust (Fig. 6.2). House dust contains more allergen in summer and autumn than in winter and spring (VOORHORST, 1959).

A coincidence was found between the periodicity in the number of atopic patients visiting the clinic, as well as in the allergen content of house dust, and the periodicity found in the numbers of mites in house dust (Ch. II; 2.6). The fact that this coincidence exists, however, does not necessarily mean that there is a causal relation between these phenomena. To demonstrate this, it must be shown that the allergen content of house dust is causally related to the number of mites, and that both do not depend upon a third factor.

To find out whether there is a causal relation between the number of *D. pteronyssinus* and the allergen content, 13 patients were tested with 4 house-dust extracts containing different numbers of mites as well as with

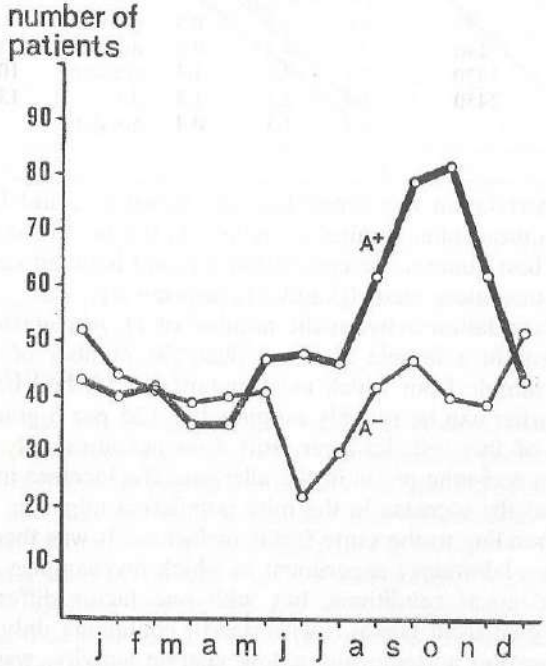

FIG. 6.2. Number of first visits of asthmatic patients to the out-patient clinic of the Department of Allergology, Leiden University Hospital, during the year.
The numbers represent the averages of two successive months, totalled over a period of five years (1961—1965).
A+: Asthmatic patients with skin reactions to house-dust extract.
A—: Asthmatic patient without skin reactions to house-dust extract.

the standard house-dust extract. The results of these skin tests, given in Table 6.2 and Fig. 6.3, show a good correlation between the numbers of mites and the strength of the extracts of the four dust samples. Even quantitatively, the result is not too bad, considering the inaccuracies in the methods of both the skin tests and the mite counts. Moreover, the allergen content is the result of a production process continuing over a period of time before preparation of the extract, whereas the number of mites is determined at a given moment.

TABLE 6.2. Comparison of the skin reactions in 13 patients to extracts of four house-dust samples containing different numbers of *D. pteronyssinus* and to the standard house-dust extract.

House-dust sample	Number of *D. pteronyssinus* per 5 gram dust	Average number of plus signs per patient				Allergen content with respect to standard
		1%	0.1%	0.01%	0.001%	
A	35	1.9	1.1	0.3	not done	0.6 times S
B	240	2.5	1.4	0.4	not done	1.4 times S
C	1330	3.1	2.2	1.3	not done	10.0 times S
D	2450	3.3	2.4	1.5	0.5	15.5 times S
standard (S)		2.2	1.3	0.4	not done	

The least correlation was found between samples A and D; sample D contained 70 times as many mites as sample A, but gave a 26 times stronger reaction. The best quantitative correlation is found between samples B and D, for which the values were 10 and 11, respectively.

From the correlation between the number of *D. pteronyssinus* and the allergen content in a sample of house dust, the number of mites in the original dust sample from which the standard extract had been prepared some years earlier can be roughly estimated at 125 per 5 gram.

The result of this test, however, still does not absolutely exclude the possibility of a non-mite origin of the allergen. The increase in the amount of allergen and the increase in the mite population might be independent processes responding to the same factor or factors. It was therefore necessary to set up a laboratory experiment in which two samples of dust were kept under identical conditions, but with one factor differing, namely mites. House dust from Davos (Switzerland), containing only a few mites per 5 gram portion and showing a low allergic activity, was held for 6 hours at 60°C to kill any live mites and mite eggs. Two 35 gram samples were spread out in dishes placed in desiccators containing a saturated solution of ammonium chloride to maintain an 80% relative humidity of the air. The desiccators were kept in a room with a constant temperature of 25°C. One sample was inoculated with a small spoonful of a full-grown culture containing about 150 living specimens of *D. pteronyssinus;* the

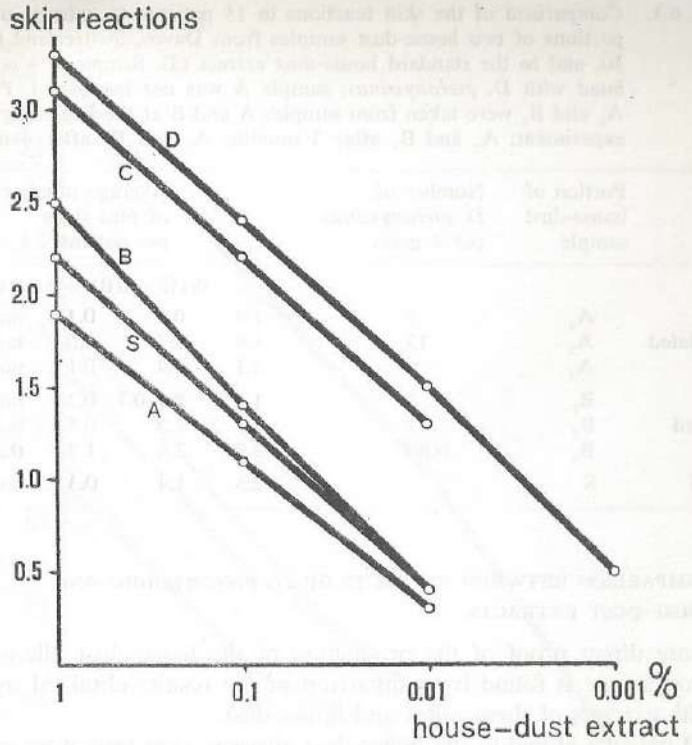

FIG. 6.3. Log-dose curves of 5 extracts: comparison of the skin reactions in 13 patients to extracts of four house-dust samples containing different numbers of *D. pteronyssinus* (A, B, C, and D) and to the standard house-dust extract (S).

other sample was not inoculated and served as control. Both the number of mites and the allergen content were determined in portions of the two samples A and B shortly before inoculation (A_1 and B_1), and three months (A_2 and B_2) and four months (A_3 and B_3) after inoculation. The results of this experiment are shown in Table 6.3 and Fig. 6.4.

The original number of mites in the uninoculated sample showed no increase. The allergen content did not show a significant increase either (A_1; A_2; A_3). The inoculated sample (B) showed an increase in both the number of mites and allergen content after three months (B_2), which became very distinct after four months (B_3). At this time the sample showed an allergic activity about 100 times stronger than that of the original sample and contained almost 1700 mites per 5 gram.

This experiment demonstrates that the allergen content of house dust increases with a rising number of *D. pteronyssinus*, and that in the absence of *D. pteronyssinus* house-dust allergen is also absent.

TABLE 6.3. Comparison of the skin reactions in 15 patients to extracts of three portions of two house-dust samples from Davos, Switzerland (A and B), and to the standard house-dust extract (S). Sample B was inoculated with *D. pteronyssinus;* sample A was not inoculated. Portions A_1 and B_1 were taken from samples A and B at the beginning of the experiment; A_2 and B_2 after 3 months; A_3 and B_3 after 4 months.

	Portion of house-dust sample	Number of *D. pteronyssinus* per 5 gram	Average number of plus signs per patient			
			1%	0.1%	0.01%	0.001%
uninoculated	A_1	5	1.9	0.5	0.1	not done
	A_2	13	1.8	0.7	0.0	not done
	A_3	5	2.1	0.9	0.1	not done
inoculated	B_1	5	1.7	0.7	0.2	not done
	B_2	73	2.3	1.2	0.5	not done
	B_3	1680	3.9	2.8	1.7	0.2
standard	S		2.6	1.4	0.3	not done

6.4 COMPARISON BETWEEN EXTRACTS OF *D. pteronyssinus* AND HOUSE-DUST EXTRACTS

A more direct proof of the production of the house-dust allergen by *D. pteronyssinus* is found by comparison of the results obtained by skin tests with extracts of these mites and house dust.

In 40 patients atopic to the house-dust allergen, skin tests were carried out with an extract of *D. pteronyssinus* isolated from the first successful cultures and at the same time with the standard house-dust extract. The dilutions tested were for the extract of *D. pteronyssinus* 0.001, 0.0001, and 0.00001% and for the standard house-dust extract 1, 0.1, and 0.01% as usual.

All these patients with skin reactions to the house-dust extract also showed skin reactions to the extract of *D. pteronyssinus,* and there was good correlation in the strengths of the reactions to the two extracts. In Fig. 6.5 the strength of the skin reactions to the standard house-dust extract is plotted against the strength of the reaction to the extract of *D. pteronyssinus* in these 40 patients, each dot representing one patient. The strength of the skin reactions is expressed here as the total of the plus signs for the three dilutions per patient.

Fig. 6.6 shows the skin reactions to two distinctly different allergens, *viz.* of house dust and of grass pollen, in 40 other patients with a house-dust atopy. In this case the dots are scattered and a number of patients showed no skin reactions at all to the grass-pollen extract. Comparison of Fig. 6.5 with Fig. 6.6 shows clearly that there is a correlation, both qualitatively and quantitatively, between house-dust extract and extract

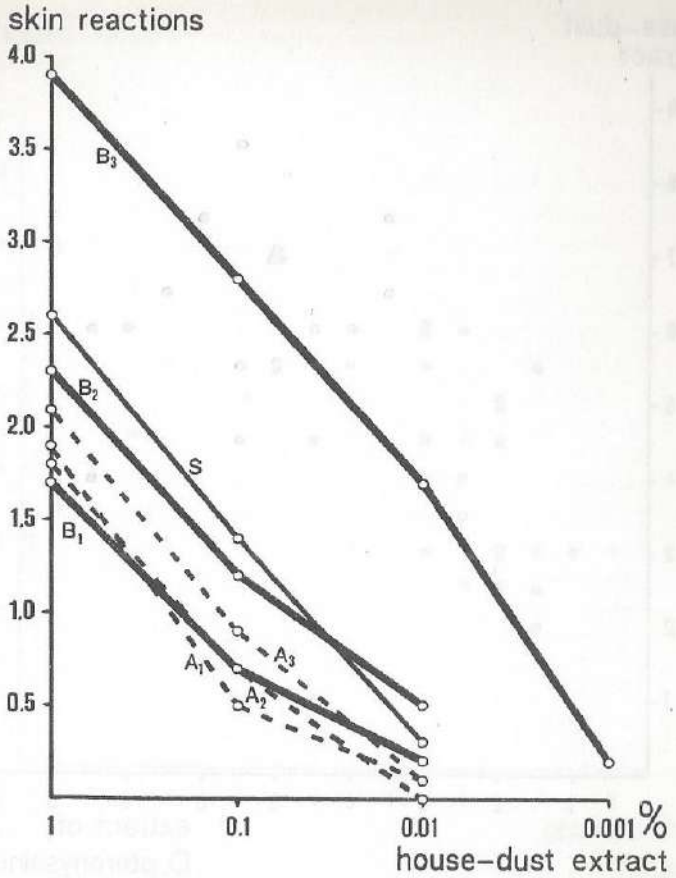

FIG. 6.4. Log-dose curves of 7 extracts: comparison of the skin reactions in 15 patients to extracts of three portions of two house-dust samples from Davos, Switzerland (A and B), and to the standard house-dust extract (S). Sample B was inoculated with *D. pteronyssinus;* sample A was not inoculated. Portions A_1 and B_1 were taken from samples A and B at the beginning of the experiment; A_2 and B_2 after 3 months; A_3 and B_3 after 4 months.

prepared from *D. pteronyssinus,* whereas there is no such correlation between house-dust extract and grass-pollen extract.

In 32 persons showing no skin reaction to house-dust extract, skin tests were performed with extract of *D. pteronyssinus.* None of these persons showed any skin reaction to this mite extract even though 22 of them were atopic to allergens other than that of house dust (Table 6.4).

From these results it can be concluded that *D. pteronyssinus* is the

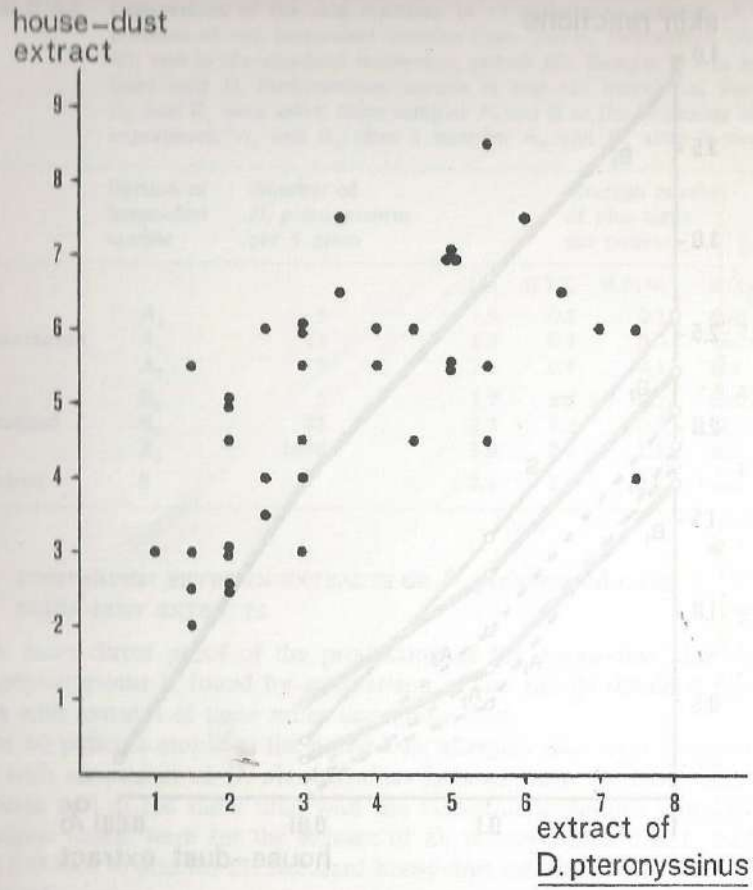

FIG. 6.5. Comparison of the strength of the skin reactions to extracts of house dust (standard) (dilutions: 1%, 0.1%, and 0.01%) and of *D. pteronyssinus* (dilutions: 0.001%, 0.0001%, and 0.00001%) in 40 patients atopic to the house-dust allergen. The strength is expressed as the total of the plus signs for the three dilutions of each of the two extracts. Each dot represents one patient.

carrier of an allergen that cannot be distinguished from the house-dust allergen with the bio-assay technique, which is the only applicable method known for this type of experiment.

6.5 SECRETION OF THE ALLERGEN BY *D. pteronyssinus*

Another important aspect of this problem is the amount of allergen material produced by *D. pteronyssinus*. In other words, could the amount of allergen extracted from a house-dust sample be produced by the num-

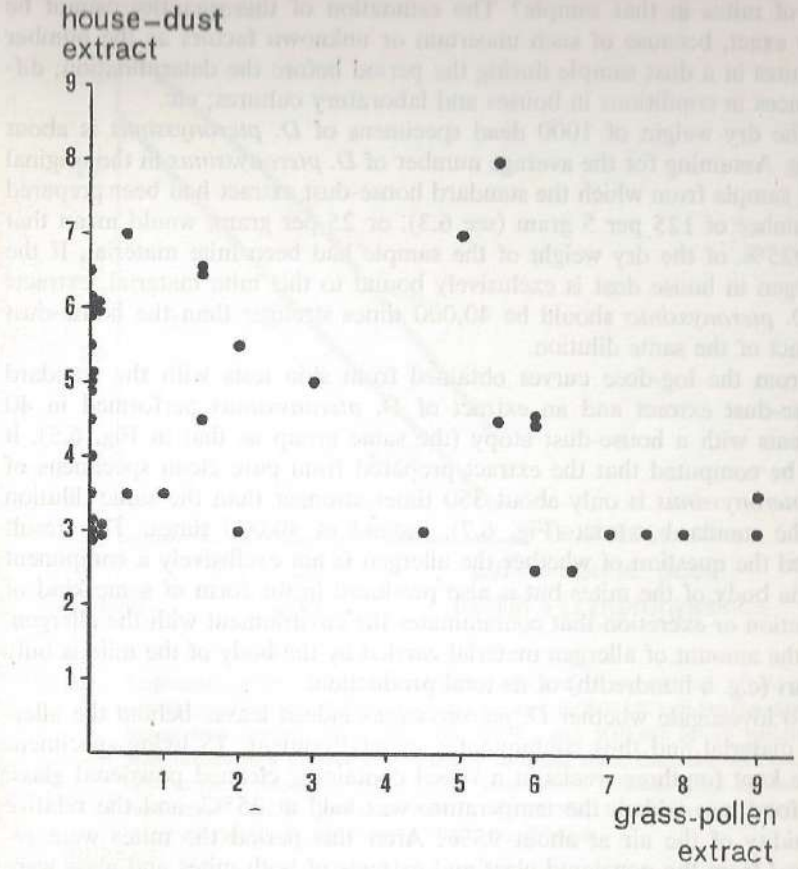

FIG. 6.6. Comparison of the strength of the skin reactions to extracts of house dust (standard) (dilutions: 1%, 0.1%, and 0.01%) and of grass pollen (dilutions: 0.01%, 0.001%, and 0.0001%) in 40 patients (a different group than those in Fig. 6.5) atopic to the house-dust allergen. The strength is expressed as the total of the plus signs for the three dilutions of each of the two extracts. Each dot represents one patient.

TABLE 6.4. Results of skin tests in 32 persons with no reaction to house-dust extract, performed with different allergen extracts and with an extract of *D. pteronyssinus*.

6 persons		16 persons		10 persons	
house dust	neg.	house dust	neg.	house dust	neg.
grass pollen	pos.	human skin scales	pos.	all tested allergens	neg.
D. pteronyssinus	neg.	*D. pteronyssinus*	neg.	*D. pteronyssinus*	neg.

ber of mites in that sample? The estimation of this quantity cannot be very exact, because of such uncertain or unknown factors as the number of mites in a dust sample during the period before the determination; differences in conditions in houses and laboratory cultures; etc.

The dry weight of 1000 dead specimens of *D. pteronyssinus* is about 1 mg. Assuming for the average number of *D. pteronyssinus* in the original dust sample from which the standard house-dust extract had been prepared a number of 125 per 5 gram (see 6.3), or 25 per gram, would mean that 0.0025% of the dry weight of the sample had been mite material. If the allergen in house dust is exclusively bound to this mite material, extracts of *D. pteronyssinus* should be 40,000 times stronger than the house-dust extract of the same dilution.

From the log-dose curves obtained from skin tests with the standard house-dust extract and an extract of *D. pteronyssinus* performed in 40 patients with a house-dust atopy (the same group as that in Fig. 6.5), it can be computed that the extract prepared from pure clean specimens of *D. pteronyssinus* is only about 350 times stronger than the same dilution of the standard extract (Fig. 6.7), instead of 40,000 times. This result raised the question of whether the allergen is not exclusively a component of the body of the mites but is also produced in the form of some kind of secretion or excretion that contaminates the environment with the allergen, i.e. the amount of allergen material carried by the body of the mite is only a part (e.g. a hundredth) of its total production.

To investigate whether *D. pteronyssinus* indeed leaves behind the allergen material and thus contaminates its environment, 75 living specimens were kept for three weeks in a vessel containing cleaned powdered glass. No food was added, the temperature was held at 25°C, and the relative humidity of the air at about 95%. After this period the mites were removed from the powdered glass and extracts of both mites and glass were prepared and tested. The powdered glass was found to be contaminated with the allergen; this is considered to be proof that *D. pteronyssinus* produces and secretes or excretes the allergen. The amount of allergen was about equal to that carried by the mites themselves, but this gives no information about the amount of allergen produced by the mites, because under these unnatural conditions no quantitative results can be expected.

On the basis of these results, it was concluded that for the preparation of extracts containing high concentrations of allergen produced by *D. pteronyssinus*, it would be far more profitable to use full-grown mite cultures instead of clean mites. Extracts of full-grown cultures of *D. pteronyssinus* were tested in 43 patients, and the skin reactions compared with those to the standard house-dust extract. The results of this series of tests, as shown by Fig. 6.8, demonstrate that the extract of the culture is 8,000 times stronger than the standard extract. In these full-grown cultures about 2,500 specimens of *D. pteronyssinus* are found per 250 mg nutrient

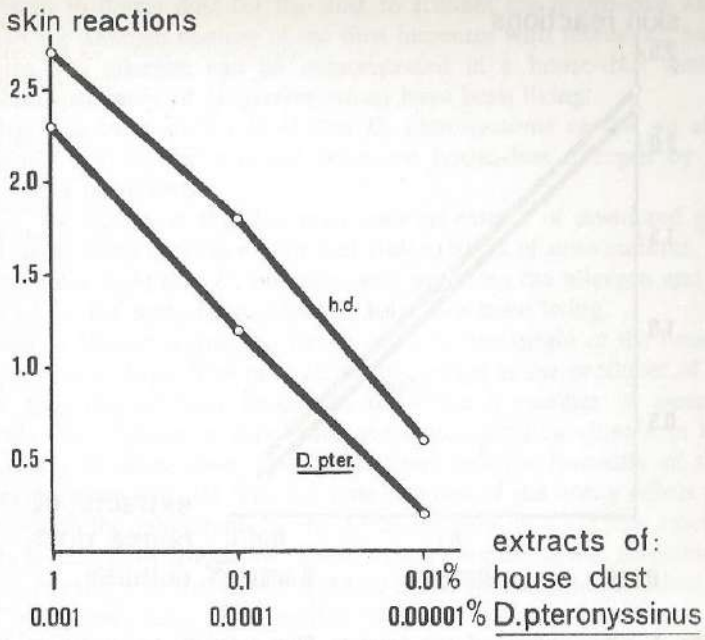

FIG. 6.7. Log-dose curves of 2 extracts. Skin reactions to an extract of *D. pteronyssinus* (dilutions: 0.001%, 0.0001%, and 0.00001%) and to the standard house-dust extract (dilutions: 1%, 0.1%, and 0.01%) in 40 patients. The house-dust extract is 2.9 times stronger than the mite extract, this last extract, however, being 1000 times more diluted. This means that the extract of *D. pteronyssinus* is about 350 times stronger than the standard house-dust extract of the same dilution.

medium in a cultivation cup, or 10,000 per gram. Compared with the assumed number of mites in the original standard house-dust sample of 25 per gram, these cultures contain 400 times as many specimens. This 400 times greater quantity of mites produces 8,000 times more allergen than is found in the standard house-dust sample. This leads to the conclusion that *D. pteronyssinus* leaves behind 20 times more allergen in laboratory cultures than in house dust.

As mentioned above, these are only first approximations, but they show that there is no discrepancy between the allergen content of a house-dust sample and the number of *D. pteronyssinus* living in that sample, with respect to the amount of allergen produced by the mites.

The fact that the human skin scales used as food in the mite cultures also contain an allergen, has no influence on these results. The amount of allergen deriving from human skin scales in these highly diluted extracts

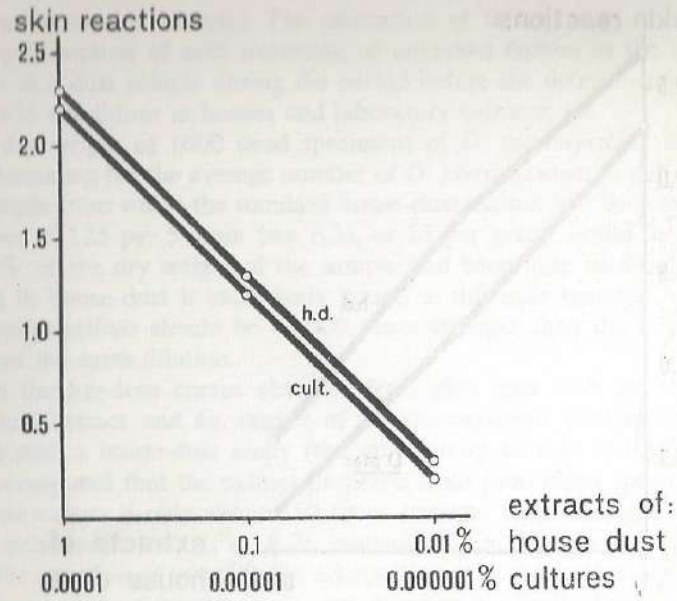

FIG. 6.8. Log-dose curves of two extracts. Skin reactions to an extract of full-grown cultures of *D. pteronyssinus* (dilutions: 0.0001%, 0.00001%, and 0.000001%) and to the standard house-dust extract (dilutions: 1%, 0.1%, 0.01%) in 43 patients.
The house-dust extract is 1.25 times stronger than the extract of the mite cultures, which is, however, 10,000 times more diluted. This means that the extract of cultures of *D. pteronyssinus* is about 8,000 times stronger than the standard house-dust extract of the same dilution.

is so low that no skin reactions could be demonstrated with it in patients atopic to this allergen.

6.6 CONCLUSIONS

In this section the results of investigations on the allergen production by *D. pteronyssinus* are related to its occurrence in house dust as a basis for a new concept of the origin of the house-dust allergen.

As the foregoing has shown (6.3), the number of specimens of the mite *D. pteronyssinus* in house dust shows a peak in autumn coinciding with the peak in the allergen content of house dust and with a maximum in the number of atopic patients visiting the clinic. The differences in allergen content in four house-dust samples showed correlation with the differences in the numbers of mites in the samples, and house dust with a very low allergen content became rich in allergen after inoculation with mites. From these facts it may be concluded that specimens of *D. pteronyssinus* must

be present in house dust for the dust to contain the house-dust allergen, and that the allergen content of the dust increases with increasing numbers of mites. No allergen can be demonstrated in a house-dust sample in which no specimens of *D. pteronyssinus* have been living.

It has also been shown (6.4) that *D. pteronyssinus* carries an allergen that could not be distinguished from the house-dust allergen by means of skin tests in patients.

From the results of the skin tests with an extract of powdered glass in which living mites had been kept and with extracts of mite cultures, it may be concluded (6.5) that *D. pteronyssinus* produces the allergen and leaves it behind on the material in which it is, or has been living.

Based on these conclusions, the concept for the origin of the house-dust allergen is as follows. The mite *D. pteronyssinus* is the producer of a substance that has allergen properties to which a number of people are sensitive. This species of mite finds good ecological conditions in houses, particularly in house dust, as regards food, relative humidity of the air, and temperature (Ch. V). The food may consist of the horny debris or skin scales which the inhabitants of the house produce in sufficient amounts to maintain the populations of mites. Their requirements respecting the relative humidity of the air are fulfilled better in damp houses than in dry ones, and consequently the number of mites in damp houses are higher than in dry houses. Temperature and relative humidity indoors are influenced by seasonal conditions, weather, and household activities. In late summer and early autumn, conditions in general and especially the higher humidity are more favourable for the growth of populations of *D. pteronyssinus* than during the other seasons.

Therefore, the allergen is produced in greater amounts in damp houses, especially during the late summer and early autumn. The mites secrete or excrete the allergen, thus contaminating their environment with it. This environment consists partially of very light dust particles that can become airborne. These light dust particles contaminated with the allergen are inhaled by the inhabitants. Persons sensitized for the allergen will show respiratory complaints after inhalation of these dust particles.

Because the scientific name of the producer of this allergen, *Dermatophagoides pteronyssinus,* is rather cumbersome, a common name is suggested: 'House-Dust Mite'.

CHAPTER VII

SUMMARY

The results of studies by VOORHORST (1962) on various atopic allergens suggested that mites might be the source of the house-dust allergen. This paper is a report on the successful attempt to find a species of mite that could be considered the producer of that allergen.

This research made it necessary to investigate the mite fauna of house dust and to determine which species of mites are to be found regularly. For the isolation of mites from house dust in order to study the mite fauna, a special method was developed. This isolation method is a modification of several techniques used for small arthropods.

The most important result of the study of the mite fauna of house dust was the finding of a little-known species of mite in nearly all the dust samples received from many parts of the world. According to a study by FAIN (1966), this species is *Dermatophagoides pteronyssinus* (Trouessart, 1897).

On the average, this species of mite constitutes about 70 per cent of the total numbers of mites found in the dust collected in houses in The Netherlands. The numbers of mites show a maximum in late summer and early autumn, and a minimum in late winter and early spring. The dust from damp houses was found to have more mites than the dust from dry houses.

In the laboratory, some aspects of *D. pteronyssinus* were studied in relation to food, temperature, relative humidity of the air, locomotory behaviour, reproduction, and life-cycle. Experiments showed that for all tested conditions the best results concerning the growth of a population of these mites were obtained on a dry nutrient medium consisting of human skin scales with powdered yeast, at a temperature of 25°C and a relative humidity of the air of 80%.

Partly in collaboration with LEUPEN and VAREKAMP (1966), a scheme was constructed for the occurrence of *D. pteronyssinus* in house dust as far as these three ecological aspects are concerned. The conditions in houses can be such that they favour the development of populations of *D. pteronyssinus*.

Concerning the role of *D. pteronyssinus* as originator of the house-dust allergen, it is shown in this report that there is a causal relationship between the number of mites and the allergen content of a house-dust sample. It is also demonstrated that *D. pteronyssinus* produces an allergen that cannot be distinguished from the house-dust allergen on the basis of skin reactions in atopic patients and that it contaminates its environment with this substance.

For *Dermatophagoides pteronyssinus* a common name is proposed: 'House-Dust Mite'.

SAMENVATTING

Uit de resultaten van het onderzoek van een groot aantal atopische allergenen concludeerde VOORHORST (1962), dat voor het ontstaan van het huisstofallergeen misschien mijten verantwoordelijk waren. In dit proefschrift wordt de succesvolle poging beschreven om een mijtensoort te vinden, die beschouwd zou kunnen worden als de producent van dit allergeen.

Dit onderzoek maakte het noodzakelijk de mijtenfauna van huisstof te te bestuderen en vast te stellen welke mijtensoorten als regel in huisstof gevonden worden. Om de mijten uit het stof te isoleren, werd een speciale methode ontwikkeld, die bestaat uit een aantal gewijzigde technieken afkomstig van bestaande isolatie-methodes voor kleine arthropoden.

Het belangrijkste resultaat van het onderzoek van de mijtenfauna van huisstof was de vondst van een weinig bekende mijtensoort, die in bijna alle stofmonsters afkomstig van vele delen van de wereld werd aangetroffen. FAIN (1966) heeft de soort gedetermineerd als *Dermatophagoides pteronyssinus* (Trouessart, 1897).

Deze mijtensoort maakt gemiddeld ongeveer 70% uit van het totale aantal mijten, dat werd gevonden in stof dat in Nederlandse huizen werd verzameld. Het aantal mijten vertoont een maximum aan het einde van de zomer en het begin van de herfst, en een minimum aan het einde van de winter en het begin van de lente. In het stof van vochtige woningen werden meer mijten gevonden dan in het stof van droge woningen.

In het laboratorium werden een aantal aspecten van *D. pteronyssinus* bestudeerd, en wel voedsel, temperatuur, relatieve luchtvochtigheid, voortbeweging, vermeerdering, en levenscyclus. Proeven toonden aan, dat van alle onderzochte omstandigheden de beste resultaten wat betreft de groei van een populatie van deze mijten werden verkregen met een droge voedingsbodem bestaande uit schilfers van de menselijke huid met gistpoeder, bij een temperatuur van 25°C en een relatieve luchtvochtigheid van 80%.

Gedeeltelijk in samenwerking met LEUPEN en VAREKAMP (1966) werd een schema opgesteld over het voorkomen van *D. pteronyssinus* in huisstof wat betreft deze drie oecologische aspecten. De omstandigheden in woningen kunnen zodanig zijn, dat zij de ontwikkeling van pulaties van *D. pteronyssinus* begunstigen.

Betreffende de rol van *D. pteronyssinus* bij het ontstaan van het huisstofallergeen wordt in dit proefschrift aangetoond, dat er een causaal verband bestaat tussen het aantal mijten en het allergeengehalte van een huisstofmonster. Ook wordt duidelijk gemaakt, dat *D. pteronyssinus* een allergeen produceert, dat op grond van huidreacties bij atopische patiënten niet onderscheiden kan worden van het huisstofallergeen, en dat aan zijn omgeving wordt afgegeven.

Voor *Dermatophagoides pteronyssinus* wordt als Nederlandse naam voorgesteld: 'Huisstofmijt'.

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STELLINGEN

I De indeling van het geslacht *Dermatophagoides* in de familie Psoroptidae impliceert niet, dat de fylogenetische verwantschap met de Epidermoptidae kleiner is dan die met de Psoroptidae.

FAIN, A. 1963. Bull. Inst. Roy. Sci. nat. Belg., 39 (32) : 46.

II Bij het bestrijden van mijten in woningen zal het droog maken c.q. droog houden van het constructiemateriaal een eerste vereiste zijn.

III De mening, dat astma een typisch psychogeen ziektebeeld is, moet in zijn algemeenheid onjuist worden genoemd.

MENGES, J. L. 1966. Astmapatiënten. Een psychologische bijdrage. Stafleu, Leiden.

IV Atopisch astma in Nederland berust voornamelijk op allergie voor huisstof.

V Tijdens de expiratiefase bij *Lophius piscatorius* L. contraheren de musculus levator arcus palatini en de musculus levator hyomandibularis, gevolgd door contractie van de musculus adductor operculi.

VAN DOBBEN, W. H. 1935. Arch. Néerl. d. Zool. 2(1) : 1.

VI Het locomotiepatroon bij *Bufo calamita* Laur. komt langs reflectorische weg tot stand.

GRAY, J. and H. W. LISSMANN. 1940[a]. J. exp. Biol., 17 : 227.
1940[b]. J. exp. Biol., 17 : 237.

VII Genetische transformatie bij *Rhizobium* opent nieuwe wegen van onderzoek bij de bestudering van de specificiteit tussen *Rhizobium*-stammen en Leguminosen gastheerplanten.

BALASSA, G. 1963. Bacteriol. rev., 27 : 228.

VIII Teneinde het overmatig gebruik van biociden in de praktijk te beperken is, behalve onderzoek naar nieuwe, voorlichting over bestaande andere bestrijdingsmethoden noodzakelijk.

IX In het stelsel van politieke partijen in Nederland zullen er spanningen blijven bestaan, zolang een gedeelte is gebaseerd op geloofsovertuiging, en een gedeelte op maatschappij-beschouwing.

X De dubbelfunctie van hoogleraar-directeur als hoofd van een universitair wetenschappelijk instituut dient te worden gesplitst en uitgeoefend door twee functionarissen van gelijke rangorde in de hiërachische opbouw.