6. Methods in house dust mite ecology and biology

What fresh perspectives! Specks of our shed skin delicious boulders, our human pores lubricious dish-shaped living rooms, and particles our largeness elides palpable to mites.

John Updike
'Mites'
New Yorker, *18 July, 1988*

This chapter deals with methods for sampling dust mite populations in the 'wild', rearing mites for experimental purposes as well as some basic techniques for manipulating dust mites in the laboratory. When it comes to methods, scientists are at their least objective and most obdurate. Personal preference for a particular technique, which becomes communicated to co-workers as the 'right way of doing things' can easily become 'the only way of doing things'.

The bulk of this chapter is concerned with methods for estimating the size of mite populations from samples of dust taken in homes (see Colloff, 1991d). As with most methods in field ecology there exists a trade-off between accuracy and use of resources. The methods deemed by their advocates to be the most precise are often the most time consuming and expensive, and may not necessarily yield data that is of higher quality or better suited to the purpose for which it was intended than less exacting approaches. I have attempted to compare different methods according to their relative merits and deficits. The final choice of a method may be formed on little more than personal preference and a feeling that 'it works for me'. There is nothing particularly wrong with this approach, as long as researchers are aware of how the method works within a generally acceptable degree of accuracy and how it compares with other options available.

6.1 Sampling mite populations in homes

The main types of study that involve sampling house dust are surveys, assessment of seasonal population fluctuations and mite eradication trials. Surveys usually involve taking a single set of samples from beds, carpets and/or furniture in many homes within one or more geographical locality. Seasonal population studies and trials of mite control involve repeat sampling of the same objects, over a period of weeks or months, in a relatively small number of homes. The design of a repeated sampling program needs to be different from one with single samples, because the statistics used to analyse single and repeated measurements are different. When a researcher wishes to observe the effect of, say, an acaricide on the house dust mite population over a period of time, the sampling program needs to be controlled and designed to ensure that the sampling itself does not significantly reduce the size of the mite population, thus obscuring the effects of the acaricide.

For example, sampling the same area that had been sampled previously is more likely to result in artificial reduction of mite density due to sampling than if mites were taken from an adjacent area. House dust mite sampling is destructive in that mites are removed permanently from the habitat. There is the prospect of developing reliable mark–recapture techniques for population size estimation (Mollet, 1996) and vital dye marking techniques for mites are available (Woodring and Cutcher, 1967; Moser and Roton, 1970; Mollett, 1996; Thind, 2000). Population reduction due to sampling can be a serious artefact in mite eradication trials and is not always easy to detect. If by the end of a trial the numbers of mites in the homes of the control group have been significantly reduced, this could be explained by sampling artefact, a naturally declining population, an unexpected detrimental effect of a placebo preparation (if used) or because of undeclared intervention by a patient (e.g. someone in a control group has vacuum cleaned their mattress). Sampling house dust mites is an intrusive procedure for the occupants of a home. However much it may be desirable to conduct a preliminary survey to determine an appropriate sampling program or sample size, this may not always be possible: researchers rely on the goodwill and patience of volunteers, and rarely have the option of returning to the sampling site whenever they wish.

Hallas (1998) noted some factors that he considered to be probable sources of (vacuum) sampling variability for dust mite populations. These include:

- dust dynamics, whereby a greater input of dust to floors than beds, combined with the difference in mass between dust from these two sources, gives the impression that mites from floors are present at lower densities than from beds when expressed as numbers per unit weight of dust (see section 6.3 below);
- by-catch sampling, i.e. the inclusion of airborne dust into the sample;
- differential sampling of stages in the life cycle, meaning that non-motile stages are less likely to be removed in the sample. When these represent a high proportion of a large population, only a few active mites may be collected;
- bias induced by variation in humidity – in dry conditions mites are less catchable because they are static, whereas in humid conditions they are more active.

There is evidence that dust dynamics/density can represent an important variable, but there is no published data that demonstrates that immotile stages are any easier to remove from textile fibres than active ones.

6.1.1 Vacuum sampling

Vacuum cleaning has been the sampling technique of choice for dust mites because of its convenience and, to a certain extent, its capacity for standardisation. It has been used in the vast majority of studies. Some authors regard vacuum sampling as an inefficient means of sampling mites, with reports of only 2–10% of the mite population having been removed (van Bronswijk, 1984; Bischoff and van Bronswijk, 1986). Hay (1995) estimated 0.5–1%. Wassenaar (1988a) reported that vacuuming for 5 min. m^{-2} with a cleaner with a mechanical rotary brush head with a beating action recovered significantly more dust but not significantly more live mites. From the perspective of ecological methodology, a relatively low recovery rate is not a bad thing *per se*. What matters is that the recovery rate is consistent and the method reproducible. Methods for quantifying the size of a population are intended to give an estimate of the population in one sample relative to the population in other samples. In the terminology of ecological methodology, a method that relies on counts of individuals is called an 'absolute' estimate, even if all the individuals in the sample unit are not counted. A 'relative' estimate is intended to determine whether the population in one sample is greater or less than that in another sample but without defining by how much (Krebs, 2001).

Bischoff *et al.* (1992a) make the assertion that vacuuming is not reproducible when used in repeated measures sampling designs because the mite population becomes depleted by sampling. But this may only be the case if exactly the same area is sampled on each occasion. A method to avoid this is detailed below (see section 6.2; Figure 6.1).

The power and air flow rate of vacuum cleaners vary considerably and may influence the numbers of mites collected, although there is no published comparative evidence of this that I am aware of. The type of surface influences sampling efficiency. Mulla *et al.* (1975) found 35% of total mites present were removed by the first of three vacuum samplings of a carpet, whereas 80% were removed from a bare floor.

Most dust collecting devices are variations on the same theme (see Figure 6.2). They consist of a tube or

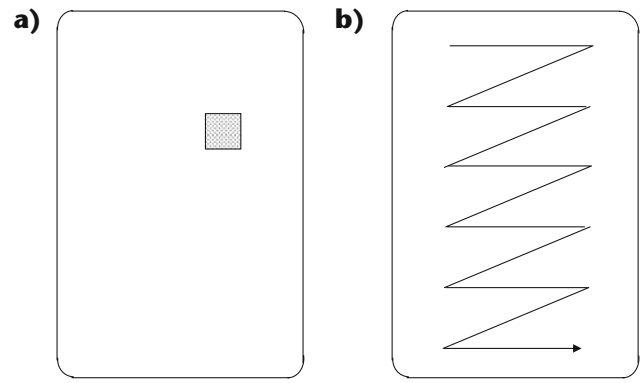

Figure 6.1 Sampling dust from beds. A fixed quadrat **(a)** versus a zig-zag **(b)** sampling pattern; with the fixed quadrat and repeated sampling, the area is likely to be depleted of dust with each sampling, causing an artefact when mite population density is expressed as numbers per gram of dust. With the latter sampling approach and repeated sampling, the same area is unlikely to be covered twice.

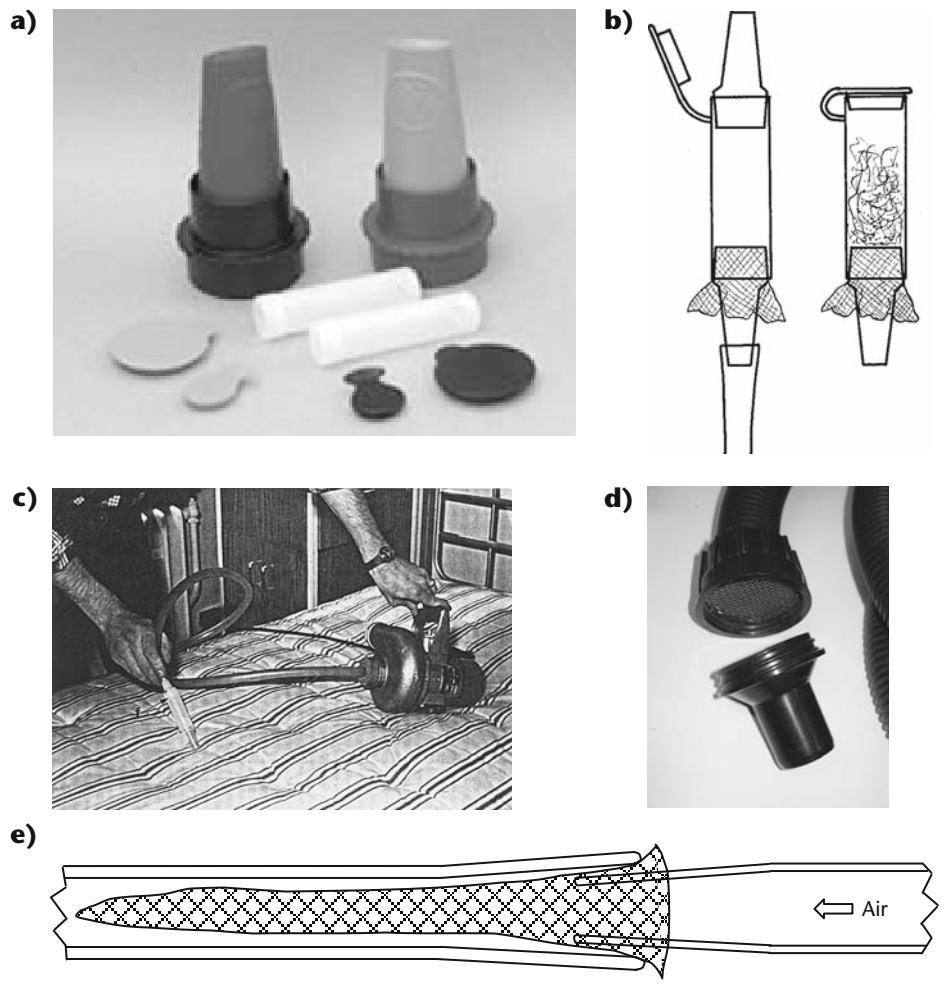

Figure 6.2 Dust sampling devices. **a)** Mitest dust collector (Martin Chapman, Indoor Biotechnologies, http://www.inbio.com). This device is a plastic sleeve containing a dust collector. It fits onto the hose or wand of a vacuum cleaner. After dust has been collected, the bottom of the collector can be capped; **b)** sampling device developed by Sesay and Dobson (1972). A cappable tube is attached to a plastic hose leading to a portable vacuum pump; **c)** an aquarium tube connector is used to hold in place a 25 µm mesh nylon cloth filter that catches the dust; **d)** a simple dust trap on the hose of a vacuum cleaner. The steel mesh supports a piece of tissue paper or nylon cloth mesh on which the dust sample is trapped; **e)** the sampling device developed by Tovey (refer to Sercombe *et al.*, 2005), consisting of a nylon mesh bag inserted into the nozzle of a vacuum sampler tube.

sleeve that can be attached to the end of a vacuum cleaner hose, with a mesh of some description to catch the dust. Typically, after the dust has been collected, the collection tube can be capped, removed and a fresh tube inserted. The simplest device is a piece of nylon gauze inserted into the vacuum cleaner tube.

6.1.2 Comparisons of vacuum sampling and brushing

Brushing of fabrics has been used primarily in places where mains electricity is unreliable or unavailable. Vigorous shaking of items of bedding or clothing into plastic bags has also been used (Hewitt *et al.*, 1973; Dowse *et al.*, 1985).

Abbott *et al.* (1981) took samples from adjacent areas of carpets and mattresses and found the geometric mean number of mites per m^{-2} of mattress was 95 times less, and of carpets 24 times less, when estimated by brushing than by vacuuming. Choice of collecting technique can influence estimates of species-diversity as well as abundance. Stenius and Cunnington (1972) found brushing of mattresses yielded species of Pyroglyphidae, Acaridae, Glycyphagidae and Cheyletidae while vacuuming yielded only the pyroglyphid species *D. pteronyssinus*, *D. farinae* and *E. maynei*. From the above, it is clear that it is not valid to compare quantitative data from studies in which vacuum cleaning was used with data from studies in which brushing was used. The different collecting methods produce markedly different results. Therefore, attempting to compare abundances of mites from studies that have used different sampling techniques may almost be meaningless. Indeed, Blythe *et al.* (1974) stated:

> *An examination of the structure of the mite populations revealed by the two methods [vacuuming and brushing] brings to light serious discrepancies … Compared with vacuum cleaner samples, the brushed samples showed a pronounced bias in favour of the larger instars.*

6.1.3 Trapping

Methods of sampling live mites within fabrics were first developed by Bischoff and co-workers in Mainz, Germany, as part of their research program in dust mite ecology and their need for reliable, reproducible methods to estimate in the laboratory the effects of domestic acaricides on mortality of mites seeded into pieces of carpet of standardised size (Bischoff *et al.*, 1986a; Bischoff, 1988). There have been various subsequent modifications by other researchers and trapping techniques have been used in field studies as an alternative to vacuum sampling. Relative performances of the mobility and heat escape methods were evaluated by Brown (1994).

a The heat escape method

With the heat escape method, the underside of a carpet or piece of fabric is heated slowly on a hotplate and the mites are driven away from the source of heat, due to reduced humidity, and are trapped on a piece of adhesive plastic weighted down on the upper surface of the fabric. The plastic is then peeled off and the mites counted directly under a stereo binocular microscope without further preparative steps. In pieces of carpets seeded with mites, 65% were recovered. The technique is limited to use on fabrics that can be manipulated from both sides and for this reason has been confined to use in laboratory studies. Bischoff and Fischer (1990) found their techniques gave considerably higher estimates of mite density than were achieved by vacuum sampling: in one instance the number of mites extracted from an overcoat was 150 times that achieved by vacuuming. Also they found the heat escape method recovered almost 19 000 mites from a jacket a year after the garment had been dry-cleaned, whereas vacuuming recovered only 50 mites; about the same as had been present before dry-cleaning. The heat escape method also appears to be a far more accurate way of estimating rates of mite re-colonisation. A year after mattresses were treated with an acaricide, Bischoff and Fischer (1990) found mite populations of between 5.5 and 8% of the size of pre-treatment populations, whereas vacuum sampling indicated that no re-colonisation had taken place.

b The mobility test

The mobility test is similar to the heat escape method except no heat is used, so the adhesive plastic is left in situ for 24 hours. Bischoff *et al.* (1986a) and Bischoff and Fischer (1990) recovered 8–30% of mites from mite-seeded fabrics by using the mobility test.

c Heat attractant trapping

Tovey (*pers. comm.*) has developed a modification of the heat escape method to allow collection of mites from fabrics that cannot be manipulated from both

sides, such as fitted carpets and mattresses. A hot water bottle or plastic bag is filled with warm water (ca. 40°C) (the bags inside wine boxes are particularly useful for this) and is placed over a piece of adhesive tape 100 cm^2. The water bag is covered and the 'trap' left for up to 12 hours, usually overnight. With this method the mites move towards the heat source, rather than away from it, because it is mild enough not to decrease humidity substantially but warm enough to increase their mobility.

6.1.4 Comparison of trapping and vacuum sampling

There has been only limited direct comparison of the efficiencies and biases of vacuum sampling and trapping (van Bronswijk, 1984; Bischoff and van Bronswijk, 1986). The most obvious difference is that vacuum sampling collects live and dead mites, eggs and faecal pellets, as well as dust particles, whereas live trapping techniques have been specifically designed to sample only live, mobile mites. Thus live eggs, and the immobile stages in the life cycle will tend not to be collected. What are the consequences for the efficiency of live trapping?

The extraction efficiency of the heat escape method has been estimated at around 65% (Bischoff *et al.*, 1992a, b), using live mites seeded into carpet squares. A growing mite population has a preponderance of immatures in it (see Chapter 5). For example, a growing population of 100 mites may comprise 25 eggs, 35 larvae, 30 nymphs and 10 adults. Bearing in mind that trapping methods depend for their success on the mites being active and moving about, it is possible to estimate the proportion of the population that cannot be trapped at any one time from the numbers of each stage of the life cycle in the total population and the proportion of each life cycle stage that is spent immotile. All of the egg stage is spent immotile, and half each of the larval and both nymphal stages in the pre-moult periods, whereas adulthood is spent active. Thus the number of immotile mites for the population would be 25 + 17.5 + 15 = 57.5. So the trappable component is only 42.5 mites. Therefore, with an efficiency of 65% for active mites, the total population extraction efficiency is only 27.6%. The important additional consequence of this example is that the efficiency of the trapping method will vary according to the 'stage structure' of the population in any home. It will be more efficient in homes that have a higher proportion of adults in the population because it is biased against stages in the life cycle that are inactive. This example demonstrates that the true measure of extraction efficiency of trapping methods, relevant to the field rather than the laboratory, cannot be gauged by measuring the proportion of active mites which are trapped following seeding test carpet squares with known numbers of active mites. Extraction efficiency should reflect the catchability of the total population present in their natural environment.

It would appear, at first sight, that trapping techniques may provide more accurate estimations of the size of the live mite population than vacuum sampling. However, there are a number of problems of extraction efficiency and extraction bias that need to be addressed. It also needs to be tested, rather than presented as an act of faith, whether the extraction efficiency of trapping methods really does represent a significant improvement over vacuum cleaning.

6.1.5 Sampling total mite populations

So far I have dealt with methods for making 'absolute' estimates of partial mite populations. Hay (1995) developed a method for estimating total mite populations. The method was not developed primarily for routine use because it involves taking cores of mattress material and is therefore destructive. Rather, the intention was to compare the total mite populations present with the estimates made by vacuum sampling. Plugs of material were cut out of the mattress, separated into layers and the material teased apart under lactic acid, and the mites removed and counted. Using this technique estimates of 8200–26 800 mites m^{-2} were obtained compared with 3–46 m^{-2} by vacuum cleaning. Core sampling recovered a significantly higher proportion of eggs than did vacuuming.

6.1.6 Partial and total estimates of mite populations

If vacuum sampling estimates only some 0.5–5% of all the mites present, should not a correction factor be applied to samples taken by vacuum cleaning in order to give a more accurate estimate of total mite populations? It would certainly be informative if researchers estimated and reported the efficiency of their sampling devices as a matter of best practice. But the fact remains that vacuum cleaning is the quickest, easiest and most practical method of sampling mites. The heat escape method is not possible, because the heating device has to be placed under the fabric. The coring method involves cutting chunks out of

mattresses and is similarly impractical. Tovey's heat attractant trapping method may prove an alternative to vacuuming, but it requires a repeat visit to collect the sample and is therefore potentially more time consuming than the single visit required for a vacuum sample.

6.2 Dimensions of the samples and units of measurement

With vacuum sampling, various dimensions have been employed. For example, Carswell *et al.* (1982) used a 600 cm^2 quadrat for sampling mattresses; the first sample taken from the area below (not beneath) the pillow, repeat samples taken from 'equivalent' areas. Korsgaard (1983a, b, c) sampled the whole upper area of the mattress in one minute, while Colloff (1987c) used a zig-zag sampling pattern which scanned the whole upper surface of the mattress, but removed mites from a total of 0.25 m^2 of it (calculated from the diameter of the sampling nozzle multiplied by the length of the sampling sweeps). This latter method allows repeat sampling from the same general area but with a minimum risk of artefactual reduction in mite density because exactly the same area within the general area is never exactly covered twice (see Figure 6.1).

Dust from different substrata varies greatly in physical density: that from floors contains particles of grit and sand, while that from mattresses consists mostly of skin scales, which have a very low density. These differences can result in misleading data if numbers of mites from dust samples from mattresses and carpets, expressed per unit weight, are compared. To avoid this problem, Furumizo (1975c) developed a technique called volumetric washing (discussed further in section 6.3 below). Numbers of mites are expressed per unit volume (ml) of sieved dust that has been allowed to settle in ethanol in a measuring cylinder.

Can samples be taken uniformly and accurately from the very different surfaces of a mattress, a carpet and an upholstered chair and the results compared in a meaningful way? The problem is not an easy one to solve. The topography of each of these objects bears no relation to the other. It is likely that the microclimate in each will differ and thus also the distribution and abundance of mites. The easy option is to sample as much of the surface area as possible within a set time and express the numbers of mites or the numbers of species per unit weight. Is this strategy valid?

The topography of a mattress is, on first appearance, deceptively simple: a rectangular object, consisting of springs and stuffing materials, or foam rubber, covered with fabric, with seams and buttons at various intervals. But where does one take the sample – from the sides, the horizontal surfaces, or both? If samples are taken from the top and the mattress is turned over at regular intervals by the owner, what effect would this have on mite populations, and would data from samples from mattresses that are regularly turned be comparable with those from non-turned mattresses? How does one take into account the seams and buttons? Blythe (1976) showed there were greater population densities of mites in areas immediately adjacent to seams than elsewhere on the mattress. These sorts of questions have, to a large extent, been evaded or ignored by house dust mite researchers. Mostly they have chosen sampling units as the weight of house dust recovered by vacuum cleaning, not because it is known to be the most appropriate method scientifically, but because it requires the least effort to standardise.

The argument on whether unit weight or unit area is more appropriate for the expression of mite densities or allergen concentrations in dust samples taken by vacuuming is crucial to the practice of house dust mite ecology. It rests on whether or not the numbers of mites extracted from the dust by vacuuming are dependent on or related to the quantity of dust present in the substrate. In other words, is there any equivalence between the number of dust particles removed by vacuuming and the number of mites or quantity of allergen? The simple answer is that for dead mites and faecal pellets there is some relationship. They are, essentially, components of the dust itself and are likely to be removed with similar ease as any other component. But for live mites there is less relationship: they have sucker-like pulvilli at the ends of their legs which provide them with a good grip on the substrate, making them more difficult to remove than dead mites.

Table 6.1 summarises the advantages and disadvantages of using unit weight and unit area. Of these points, the most significant concerns the use of unit weight for data derived from repeated sampling during the course of a field trial or a clinical trial of house dust mite control. Effective control of house dust mites requires a very high killing efficiency followed by thorough, preferably repeated, vacuum cleaning to remove as much of the allergen pool of dead mites and faecal pellets as possible. If

Table 6.1 Advantages and disadvantages of expressing population density of mites by unit weight and by unit area.

Unit weight (no. mites per gram of dust)	Unit area (no. mites m^{-2})
Easy to use when sampling objects with different topography, e.g. furniture v. carpets	Requires the area to be sampled to be determined, e.g. by use of a quadrat or a set length of sweep of a sampling device
Comparison of data from substrates containing dust of different densities gives misleading results	Data from substrates with different densities of dust is more directly comparable
Use not valid for repeated measures sampling because dust is removed on each sampling occasion	Valid for use in repeated measures sampling
Easy to compare data from different studies: has been used in the overwhelming majority of studies	Few studies have used unit area, and it is difficult to convert from one to the other
Can only be used for samples collected by vacuum cleaning	Can be used for samples taken by any method

data are expressed per unit weight and the density of total mites or allergen concentration compared with baseline values taken prior to commencement of control methods, this will tend to underestimate the reductions because of the amount of dust removed by vacuum cleaning. An example of this effect can be seen from a re-examination of the data gathered during a clinical trial of mite control in which liquid nitrogen was used as a freezing agent to kill mites (Dorward *et al.*, 1988). To compare reductions of mite density per unit weight and per unit area, total mite density from each of four bi-weekly samplings of mattresses following treatment was expressed as a ratio of the pre-treatment density and the mean rate of reduction per mattress estimated from regression analysis, as done by Colloff *et al.* (1989). The grand mean reduction of mite density per gram of dust after 8 weeks was 1.8-fold (range 1.01–4.9) while that of mite density per m^2 was 5.5-fold (range 1.8–13.8). A mite control trial that involves addition of material to the substrate in order to kill mites, for example, wet powders containing acaricides, may also result in a biased estimation of efficacy of reduction. Any residual material not removed by vacuum cleaning may have the effect of 'diluting' mite density or allergen concentration if density is expressed in unit weight. It is only common sense to recommend the data should be expressed per unit area for studies involving repeated measurements of mite population density or allergen concentration in the same home, or the substrate. Such studies involve alterations to the dust load by replicate vacuum cleaning and dust sampling.

Most dust mite surveys have given numbers of mites per unit weight (see Appendix 3; Chapter 8), which makes unit weight the easiest and most convenient way to compare population densities, for the same habitat type, between different published studies. Comparing numbers of mites in bed dust in one study with those in floor dust in another is not likely to be very informative.

But if expressing numbers of mites or allergen concentrations per unit weight of dust is less appropriate than per unit area, why do we see statistically significant correlations between values expressed in this way and other factors under investigation? For mite densities the answer lies partly in the fact that differential determination of the numbers of live mites and the numbers of dead mites has rarely been performed. The efficiency of collection of dead mites is much higher than that of live mites because dead mites have no active method of clinging to the substrate. They are probably extracted with similar efficiency with other similar-sized particulate non-living material in the dust. Takaoka and Okada (1984) showed statistically significant positive correlations between density of total mites (dead and live) per unit weight and density of mites per unit area, thus demonstrating an area–weight relationship (shown in Figure 6.3).

6.3 Extraction of mites from dust samples

Many techniques have been developed for the removal of mites from house dust. These were reviewed by Wharton (1976); Gridelet de Saint Georges (1975) and van Bronswijk *et al.* (1978). The basic types are flotation, suspension and heat extraction. The first two are the most widely used.

6.3.1 Flotation

Flotation involves the exploitation of the difference in density of mites from the aqueous media in which

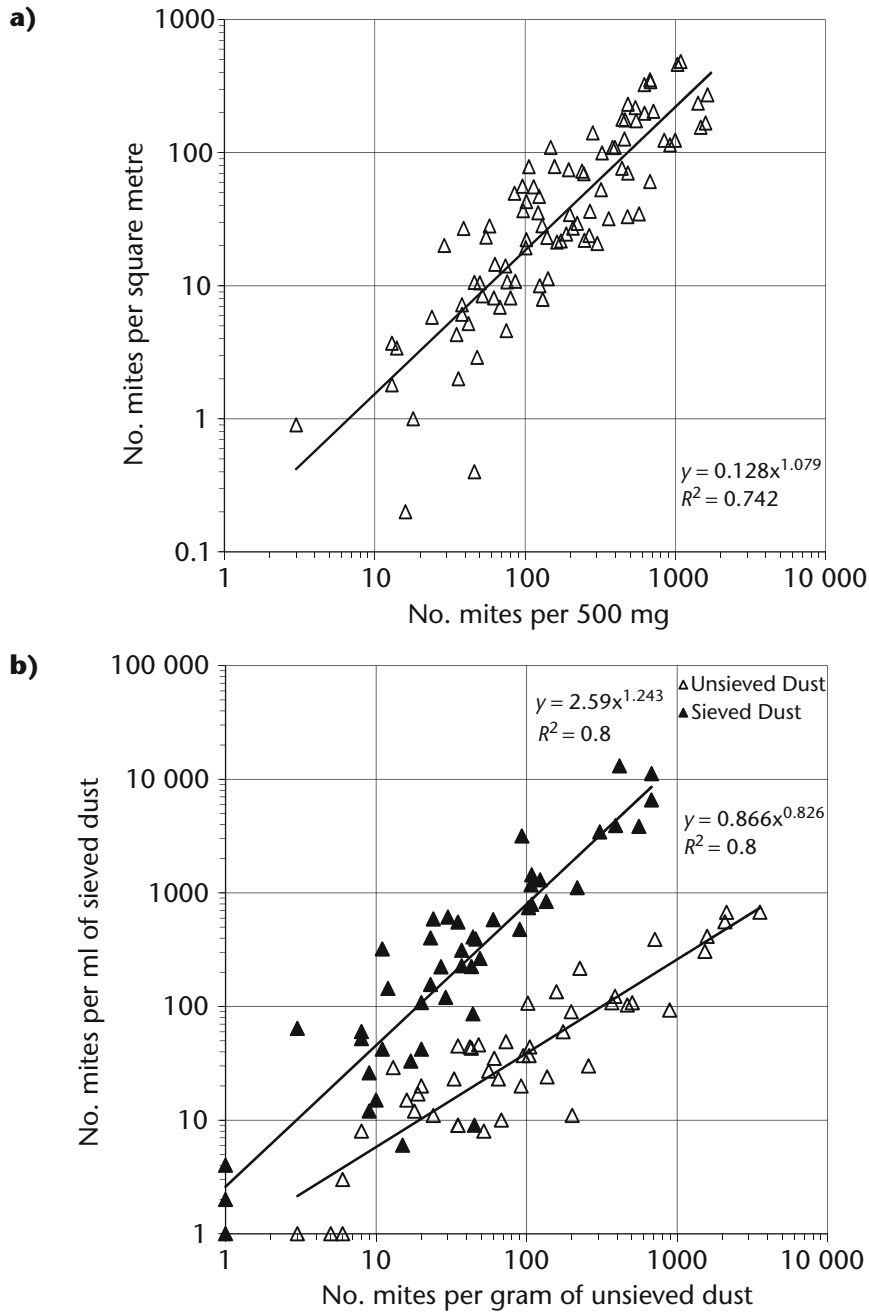

Figure 6.3 **a)** Relationship between density of mites per unit weight of fine dust and per unit area (data from Takaoka and Okada, 1984); **b)** relationship between mites per gram in unsieved (coarse) dust and mites per millilitre of sieved (fine) dust (data from Massey *et al.*, 1988).

they are submerged. The mites float to the surface, become concentrated there, and can be aliquotted off. The major disadvantage of flotation techniques is that they have relatively low extraction efficiency.

The first method for extraction of mites from dust was developed by Spieksma and Spieksma-Boezeman (1967) and involved flotation in lactic acid assisted by centrifugation. It has a relatively low extraction efficiency. Miyamoto and Ouchi (1976) developed a similar method.

Mites have been extracted in saturated sodium chloride solution (Sasa *et al.*, 1970; Shamiyeh *et al.*, 1971; van Bronswijk, 1973; Nourrit *et al.*, 1975; Fain and Hart, 1986; Hart and Fain, 1987; Sakaki *et al.*, 1991). Despite claims of efficiencies of up to 97%, only three of the samples examined by

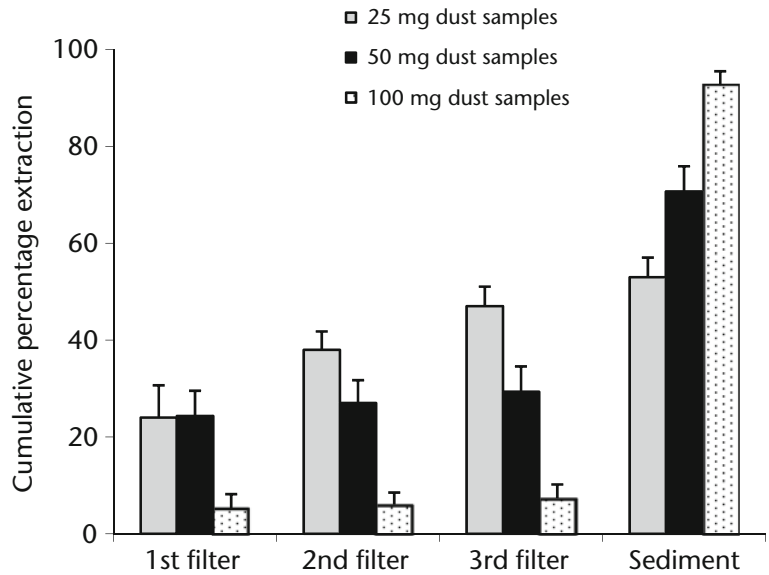

Figure 6.4 Determination of the percentage efficiency (mean ± S.E.) of a saturated NaCl flotation technique for the extraction of mites from dust samples (n = 3 for each sample class; Colloff, unpublished data).

Hart and Fain (1987) were house dust. The rest were mammal nest material, debris in the bottom of museum jars containing pickled mammals and laboratory cultures of mites – none of which have the particulate characters of house dust. Using their technique, which involves exploiting differences in densities between ethanol and saturated sodium chloride solution, I found a geometric mean extraction efficiency for 33 samples of house dust of only 27% (range 3–88%; see Figure 6.4), that the bulk of mites remained in the sediment, and that the proportion extracted increased with increased weight of dust samples. The technique was most efficient for samples that contained little or no fibrous material, but mites got trapped in the dust and remained in the sediment or stuck to the walls of the vessel when the supernatant was poured off. Additionally, quantities of fine dust particles floated to the surface along with the mites. The main advantage of flotation is that it is relatively rapid. Mites are separated from the dust automatically, although a long preparation time may be required. Hart and Fain's (1987) method requires the dust sample be left to stand for 12–24 hours in ethanol before it can be processed further.

Volumetric washing (Furumizo, 1975c) combines aspects of flotation and suspension. The method is apparently highly efficient (96–98% mite recovery). Numbers of mites are expressed per unit volume (ml) of dust that has been sieved free of coarse material and has then been allowed to settle in ethanol in a measuring cylinder. Although it has several advantages, this technique has not become widely adopted, partly because it involves over 20 separate steps to extract the mites and because it is not straightforward to compare numbers of mites expressed in ml with numbers expressed in grams of dust. I have used an approximate conversion figure for sieved dust (based on 32 samples from beds) of 1 ml to 5.5 g. Massey *et al.* (1988) show a log.-linear relationship between numbers of mites per ml and per gram of coarse dust (shown in Figure 6.3b).

Oshima (1964) isolated mites from dust using a separating mixture of ether and tetrachloride. Maunsell *et al.* (1968) used dichloromethane. A method based on flotation at the interface of methylated spirits or ethanol and kerosene was developed by Thind and Griffiths (1979), with subsequent modifications (Thind and Wallace, 1984; Thind, 2000; Thind and Dunn, 2003). Methods using organic solvents obviously have higher occupational health and safety risks compared with methods using sodium chloride solution.

6.3.2 Suspension

Suspension extraction involves placing the dust sample in lactic acid, ethanol or sodium chloride solution, usually in a glass Petri dish, wetting

and dispersing the particles, staining and – in the case of lactic acid suspensions – heating them to macerate the mites. Mites are removed under a stereo binocular microscope. The disadvantages are that it can be time-consuming, especially if large numbers of mites are present, although there are ways to minimise this, for example by pouring the suspension into a fresh Petri dish and examining the film remaining in the first dish. The mites are removed and the process repeated with another fresh dish until all the fluid has been examined. This method is quicker and more reliable than examining all the sample in one dish – as done by Gridelet and Lebrun (1973) and Gridelet de Saint Georges (1975) – there are far fewer particles per unit area in a film of fluid than in a full dish and the mites are much more easily visible. Suspension in viscous fluids such as lactic acid is easier than in alcohol because the particles move around less. The major advantages of suspension techniques are that the entire sample can be examined by eye and, if done correctly, a high extraction efficiency achieved. Also a good picture can be gained of the composition of the dust sample including the size and consistency of particles, the presence of different types of fibres and other components such as feathers and pollen. The suspension technique results in few losses of mites due to transfer from different vessels because the contents of each vessel are examined thoroughly after every transfer.

Arlian *et al.* (1982) described a modified suspension technique whereby 50 mg dust samples were suspended in saturated NaCl with detergent, wet-sieved onto 45 μm mesh and stained with crystal violet, which stains the dust particles but not the mites. This technique has the advantage over lactic acid suspension that live mites are not killed and a very accurate assessment of live and dead mites can be made. Natahura (1989) came up with a similar method, using methylene blue as a stain.

6.3.3 Heat extraction

Heat extraction is a technique borrowed from the Tullgren funnel extraction method, widely used in soil biology, where the sample of dust is placed under a light bulb and the live mites migrate from the dust as the sample starts to lose water (van Bronswijk, 1981; Figure 13.7). It will only extract active stages in the life cycle and is most useful for removing live mites in order to form starter populations for in vitro cultures.

6.3.4 Extraction efficiency

Each procedure differs in the efficiency with which mites are extracted and there is generally an inverse relationship between the amount of 'hands on' time and effort that the method requires and the extraction efficiency. It is desirable to determine the extraction efficiency (number of mites found divided by the number actually present in the sample) of a technique at the beginning of the study. Variation in extraction efficiency between operators can be considerable. However, operators tend to increase efficiency with practice. Additionally, extraction efficiency varies according to species and stages in the life cycle of the mites (shown in Figure 6.5). The extraction efficiencies for intact mites of the same stage were always higher than for damaged mites and efficiency increases with the size of the mites. There was no significant correlation between extraction efficiency and the relative abundance of each stage. Smaller instars and damaged specimens were recovered less efficiently than larger instars and intact specimens. Experimental errors of this sort have obvious implications for population ecology, especially studies of age-structure of populations. It was surprising to find the extraction efficiencies of the very small *Tarsonemus* sp. were similar to those of the much larger *D. pteronyssinus*. This was probably due to an ability to discriminate between the markedly different morphology of tarsonemid mites and pyroglyphid mites and override the factor of size with that of morphology while searching for specimens.

6.4 Mounting, counting and identification of mites

Ideally all mites extracted from the dust samples should be washed, cleared and mounted on microscope slides for identification under a compound microscope. Some researchers have simply counted mites when examining the sample under a stereo binocular microscope (e.g. Walshaw and Evans, 1987); others have taken subsamples of mites for identification (Tovey *et al.*, 1975; Korsgaard, 1982). These approaches have several disadvantages. It is valuable to make permanent preparations of all mites extracted from dust so that the identity of specimens can be checked or re-examined following new developments such as the discovery of concomitant populations of *D. farinae* and its sibling species *D. microceras* in Europe (Cunnington *et al.*, 1987). Permanent

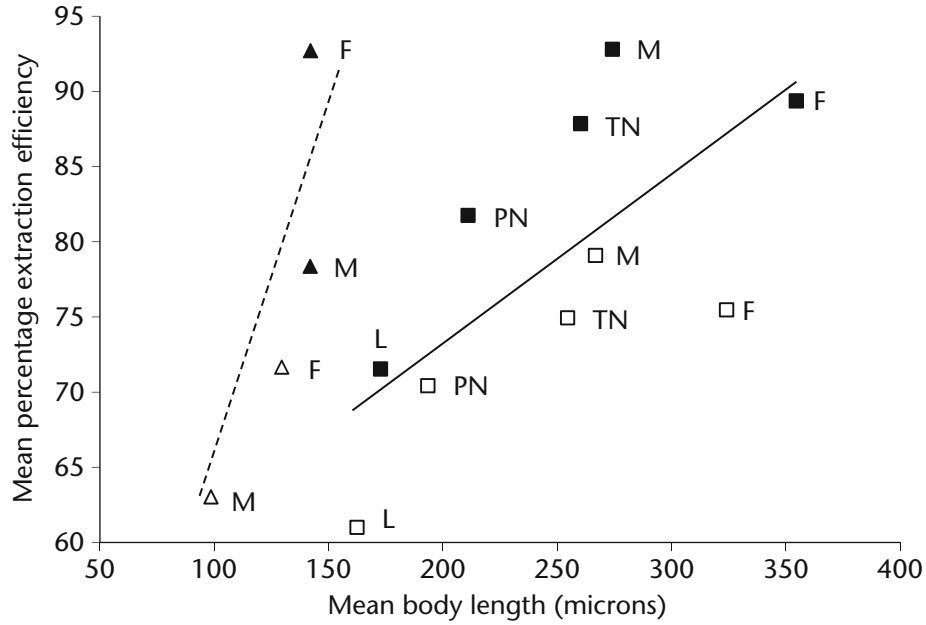

Figure 6.5 Relationship between mean extraction efficiency of *Tarsonemus* sp. (triangles) and *Dermatophagoides pteronyssinus* (squares) and mean body length of specimens (n = 10 specimens of each stage of each taxon). Closed symbols = intact (live) specimens; open symbols = damaged (dead) specimens. L = larvae, PN = protonymphs, TN = tritonymphs, M = males, F = females. (Modified from Colloff, 1991d.)

preparations also allow for subsequent, more detailed analysis of population structure or some other factor at a later date.

6.4.1 Mounting mites on microscope slides

Mounting mites from dust samples on microscope slides one-by-one is extremely tedious. To avoid this, Colloff (1989a) developed a mass-transfer method which allows 200–300 mites to be mounted in a single operation (see Figure 6.6). The mites are frozen in water in a watch-glass (Figure 6.6a) and transferred in ice to a microslide (Figure 6.6b) that has been coated with silicone solution (e.g. 'Sigmacote') and allowed to dry. The water is evaporated off on a hotplate (Figures 6.6c, d) and mountant and cover slip are added in the usual way (Figures 6.6e–h). Aqueous mounting media such as the various modifications of Berlese's gum-chloral medium, e.g. de Faure's or Hoyer's media (see section 6.5.1 and Krantz, 1978, for recipes) have been widely used. They have the advantage that mites can easily be remounted at a later date (refer to Fain, 1980, for method). However, gum chloral-based media have a major disadvantage in that they tend to dry out if the cover slip is not ringed. Also, there are a number of other significant problems associated with these media, especially damage of specimens due to the crystallisation of some components of the medium, as detailed by Upton (1993). Media soluble only in organic solvents, such as Euparal or Canada Balsam, involve rather lengthy preparation stages and are not particularly suitable for mites. They also involve the use of xylene as a solvent, which is undesirable for occupational health and safety reasons. I have achieved good results using polyvinyl alcohol in lactophenol, a modification of Heinze PVA. The original recipe (Heinze, 1952), and the modification of it by Boudreaux and Dosse (1963), contain errors regarding the proportions of the ingredients which render it less than satisfactory. However, the recipe presented in section 6.5.7 is the same as that used at the Central Science Laboratories, UK, and works well.

6.4.2 Counting and identification

Counting of mites is best done at the same time as identifying them. Correct identification of mites is vital. This requires some expertise in basic acarology, but is not as impossible as some clinical researchers imagine. Keys for adults of all pyroglyphid species are presented in Chapter 1 and by Fain *et al.* (1988, 1990). Additionally, there are various courses in acarology available (e.g. at the Acarology Laboratory

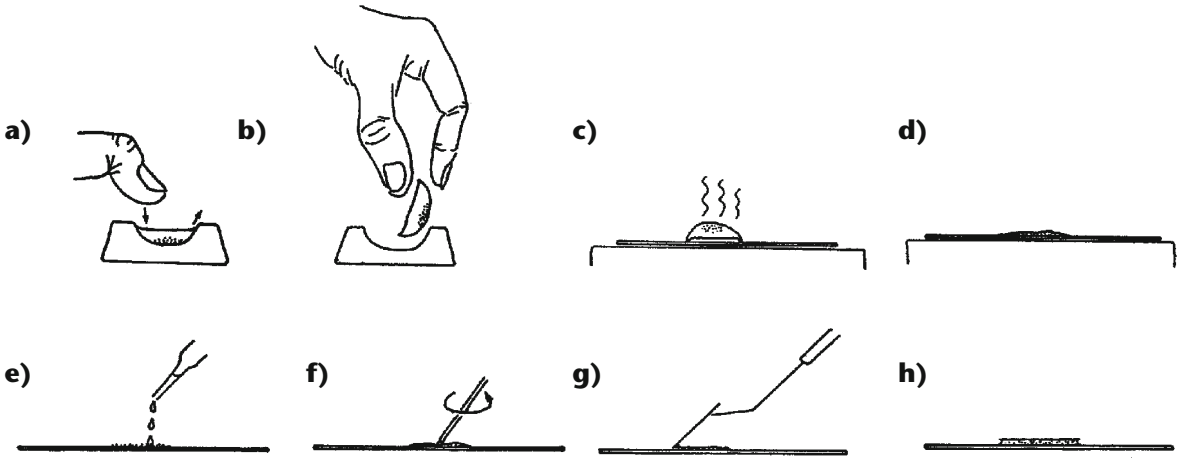

Figure 6.6 The frozen block technique for mounting dust mites on a microslide (modified from Colloff, 1989a). See text for details.

at Ohio State University) that can provide that expertise in a relatively short space of time to persons with little or no previous experience. The determination of immature pyroglyphids to species is more difficult. Only immatures of *D. pteronyssinus*, *D. farinae* and *E. maynei* have been described adequately (Mumcuoglu, 1976). The identification of different stages in the life cycle is relatively easy and can provide important data on the age-structure of populations. Likewise the determination of damaged and intact specimens, using the criteria of Arlian *et al.* (1982), is a useful means of estimating the numbers of mites that were alive or dead at the time of sampling. House dust mite ecology is unusual in that it is concerned not only with the living population but with dead mites – they are part of the allergen pool to which persons with atopy are exposed.

6.5 Laboratory testing of mite control methods

6.5.1 The test substrate

The sampling units should consist of individual pieces of carpet, rather than a single piece of carpet marked into sampling units. The reasons for this are:

- it eliminates the risk of migration of mites from one sampling unit to another;
- the sampling units are easier to handle and seed with live mites;
- the other sampling units are not disturbed during sampling of mite population densities units when sampling;
- the sampling units are easier and more controllable to incubate at a standard temperature and humidity;
- it allows for convenient use of the heat escape method of mite sampling.

The carpet pieces need to be checked with the manufacturer to determine whether they have been treated with a mothproofing insecticide, as this could also have a negative effect on mite populations. If the mothproofing insecticide is a synthetic pyrethroid, it can be denatured by exposure to UV light.

Even if the carpet pieces are new, they can contain mites, depending on where they were stored post-manufacture and for how long. It is thus vital to freeze the carpet squares to kill any live mites, at least to –30°C overnight. The carpet pieces should then be vacuum cleaned thoroughly and the vacuum samples examined to ensure that no live mites are present.

6.5.2 Temperature and humidity control

A major confounding variable in these sorts of experiments is the effect of temperature and humidity on the reproductive rate and mortality of the dust mite population. It is therefore essential that temperature and humidity are controlled constantly and precisely with as little variation as possible. This requires careful monitoring with an electronic thermohygrograph. A climate-controlled room with humidification facilities and shelving for the carpet pieces, as used by Bischoff *et al.* (1986b) is the ideal set-up for such a study. Temperature should be 25 ± 2°C and humidity 75 ± 2% RH. Alternatively, if a climate-controlled room is not available, humidity-controlled incubators

can be used, using the methods for humidity control outlined below (see section 6.6.3).

It is important to equilibrate carpet squares at the required temperature and humidity for at least 24 hours prior to seeding the squares with mites. It is also important to ensure the cultures have been grown at the same temperature and humidity at which they will be exposed in the carpet. If not, the mites will also require equilibration or their reproductive rate and mortality may change after they have been seeded into the carpet.

6.5.3 Seeding the carpet squares with mites

The number of mites seeded into the carpet needs to be known as accurately as possible and not guessed at. The purpose of studies on the efficacy of dust mite control methods is to determine if the control method has an effect on the size of the dust mite population. If the size of the starting population is not known, or based on an estimate of undetermined accuracy, then the study is virtually invalid.

The easiest way of estimating numbers is to take a series of aliquots of mite culture of known weight and count the live mites, calculating the mean and standard deviation, until an acceptably small range is achieved. Researchers can then define, with precise statistical confidence, how many mites are contained within a given piece of carpet at the outset of the experiment. This work is time consuming but essential. It needs to be done shortly before the culture is to be used otherwise the density of mites in the culture may have changed due to water loss by the time they are seeded.

If the mites are sieved from the culture medium prior to seeding (which is the easiest way of getting a high density of live mite material), then a food source needs to be introduced or the mites in the control carpet pieces will die of starvation after seeding, thus confounding the experiment. A suitable culture medium for dust mites (there are many) should be equilibrated at the appropriate test microclimate at least 24 hours before use. Alternatively, mites can be left within their culture medium, though this makes for greater variation (and greater difficulty) when trying to count live mites prior to seeding them.

There are no ideal starting population densities prescribed, but there need to be sufficient mites per unit area so that they will come into contact with each other frequently enough in order to mate. Below a certain population density, the frequency of mating encounters will diminish dramatically and the rate of population increase decline sharply, thus confounding the experiment. As a minimum, something in the order of 10–20 mites cm^{-2} would probably allow for sufficient mating encounters. The mites and the culture medium should be distributed evenly over the surface of the carpet squares and gently brushed in and left for at least 3 days to settle and disperse within the carpet pieces.

6.5.4 Monitoring population growth prior to the mite control treatment

Adequate numbers of carpet pieces need to be used to allow for sufficient replication of treatments and controls. Three pieces for each of treatment and control is probably too few, 20 is too many. Sufficient replicates should be included to allow for pieces that have to be discarded due to lack of mite population increase. It is important to monitor the live mite populations in each piece of carpet prior to assigning the pieces to treatment and control groups. At least three samplings would be ideal. In this way an estimate is gained of the reproductive performance of the mites since the time they were seeded. Any carpet pieces in which the population has declined should be discarded because if it were assigned to the treatment group, it would be impossible to determine whether subsequent decline in mite population density was due to the acaricide or to a continuance of the pretreatment decline.

6.5.5 Mite control treatment

If the treatment is an acaricide, care should be taken to avoid contaminating the control pieces, either through spray drift or storage of control carpet pieces in close proximity to treatment pieces.

6.5.6 Estimating the size of the mite population after treatment

There are two main types of method for doing this: the heat escape method and vacuum sampling followed by suspension extraction (discussed in sections 6.1 and 6.3 above).

a The heat escape method

The underside of the carpet squares is heated on a hotplate at 80°C for 15–30 min. Live mites, if present, move away from the heat source and are captured on strips of transparent adhesive tape laid over the top surface of the carpet square. I have used 3×100 cm^2 strips of tape per square, which were removed after

heating and examined under a stereo binocular microscope for the presence of live mites.

b Vacuuming and suspension extraction

Carpets are vacuum sampled at the recommended rate of 1 m^2 in 2 min. using a filter system or similar to trap the mites (see section 6.1.1). The material adhering to the filter is removed, weighed and the mites recovered, weighed and extracted as follows. The sample is suspended in 50 ml saturated sodium chloride solution, with a wetting agent added. The suspension is then stirred and filtered through a sieve of mesh size small enough to retain mites and their eggs, and stained on the sieve using 1% aqueous crystal violet which stains everything except the mites. After rinsing, the contents of the sieve are transferred, with water, to a 9 cm diameter glass Petri dish. Active, live mites can be seen on the meniscus or below the surface and detected by movement under a stereo binocular microscope.

c Post-treatment monitoring of mite populations

Sampling for live mites should be repeated at sufficient intervals commensurate with the life cycle of the mite at the microclimate at which it has been incubated (ca. 3 weeks), e.g. post-treatment and then 2-weekly on four occasions for treatments and controls.

d Statistical treatment

Appropriate statistical methods for comparing rates of change in mite population density for treatments and controls are essential. It is important to remember that the type of study design described above represents a repeated measures design. Thus only statistics appropriate for repeated measures should be used.

6.5.7 Recipe for Heinze polyvinyl alcohol (PVA) mounting medium

This recipe is from the Central Science Laboratories, UK.

10 g polyvinyl alcohol (PVA);
40–60 ml distilled water;
10 ml glycerol;
25 ml of a 1.5% solution (weight/volume) of phenol in distilled water (e.g. 375 mg phenol in 25 ml distilled water);
100 g chloral hydrate;
35 ml lactic acid.

1. Place PVA powder in a beaker and add water gradually, stirring, while heating the beaker in a water bath on a magnetic hotplate/stirrer at just below boiling point.
2. Add lactic acid and stir.
3. Add glycerol and stir.
4. Mix 1.5% phenol solution and place in a beaker in a water bath on a magnetic hotplate/stirrer. Add chloral hydrate and stir until dissolved.
5. Add chloral hydrate-phenol solution to the PVA solution when the latter is lukewarm.
6. Filter and store in a brown glass bottle to prevent oxidation by UV light.

Note: To dissolve hardened PVA on slide mounts, e.g. for demounting or remounting of slides, use warm lactophenol or, even better, warm 60% aqueous glycerol.

Chloral hydrate is a controlled drug in many countries and may require a licence for research use. An alternative to PVA is a ready-made mounting medium called CMC-10, which is available from Masters Chemical Company Inc., Elk Grove, IL, USA.

6.6 Culturing house dust mites in the laboratory

House dust mites are, by and large, easy to grow in the laboratory providing that sufficient care and attention is devoted to selection of an adequate diet, proper maintenance of microclimate in the culture vessels, prevention of contamination by fungi, cross-contamination with any other mite species, and regular subculturing to prevent overcrowding. There are several reviews and methods papers (Woodring, 1963; Boczek, 1964; Solomon and Cunnington, 1964; Voorhorst *et al.*, 1969; Sasa *et al.*, 1970; Hall *et al.*, 1971; Araujo-Fontaine *et al.*, 1972; Miyamoto *et al.*, 1975; Wharton, 1976; Mumcuoglu, 1977a; James and Mulla, 1978; van Bronswijk, 1981; Castagnoli *et al.*, 1989; Parkinson, 1992).

6.6.1 Basic equipment

The basic equipment requirements are a plastic or glass desiccator, a stereo binocular microscope with a cold light source, a humidity meter and thermometer, suitable culture vessels (shown in Figure 6.7) and a starter culture of the species required. The choice of food may be determined by the final use to which the mites are to be put. Those to be used for making allergen extracts (such as making polyclonal antibodies or doing western blotting) ideally need to be reared on a diet of low intrinsic allergenicity.

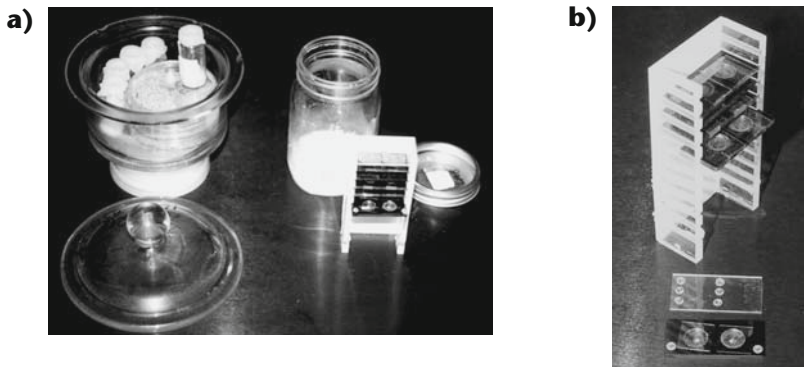

Figure 6.7 Mite culturing equipment. **a)** Desiccator with sodium chloride sludge in the base to control humidity, Kilner jar with NaCl sludge and rack to hold individual Robertson cells; **b)** detail of Perspex rack and individual Robertson cells.

6.6.2 Starter cultures

a Starter cultures from existing laboratory cultures

Starter cultures of various species may be obtainable commercially from manufacturers of diagnostic allergology products. The Central Science Laboratories, UK, has supplied mite cultures commercially. These cultures were developed over 45 years, at what was the Pest Infestation Laboratory at Slough, UK. This laboratory built up and maintained a unique collection of stored products mites and dust mites (Parkinson, 1992). The collection used to number at least 36 species, but at the time of writing, it is no longer clear from the CSL website whether such a service is still offered. The cultures were accumulated as a result of research on the biology and population dynamics of stored products pests such as *Acarus siro* by Solomon (1945, 1946a, 1962) and Cunnington (1965) and *Tyrophagus putrescentiae* by Cunnington (1969). The development of methods to grow these mites in the laboratory (Solomon and Cunnington, 1964) went hand-in-hand with this research and forms the basis of what we know about culturing free-living astigmatid mites today. Other likely sources of mite cultures are research groups.

b Developing a culture from house dust samples

If starter cultures are not available, researchers can prospect for live material in dust taken from houses. Take a series of dust samples from a range of bedding and carpets in different homes and incubate them in the desiccators and see what grows. It may take between a few days to several months before there are noticeable numbers of mites present. When the population is established, gradually add culture medium to replace the dust.

6.6.3 Microclimate

Dermatophagoides pteronyssinus, *D. farinae*, *Euroglyphus maynei* and several of the glycyphagoid species such as *Blomia tropicalis* or *Lepidoglyphus destructor* can be maintained with good population growth at room temperature (ca. 18–22°C) and 75% RH. Population growth can be speeded by increasing temperature to 28–33°C and upping the relative humidity slightly (up to 80%). Humidities in excess of 80% are difficult to maintain mites in because mould growth is encouraged. In fact the easiest way of controlling mould growth is to keep humidity constant at 75% and provide the cultures with weekly aeration and shaking, preferably in a laminar flow hood. Shaking helps prevent the build-up of a mat of fungal mycelia in the culture medium.

The easiest way of maintaining humidity inside the plastic desiccator is by using one of the mixtures of saturated salt solutions recommended by Winston and Bates (1960). Saturated aqueous sodium chloride solution, enough to cover the bottom of the desiccator, is mixed with solid sodium chloride to make a sludge. This will keep the humidity at a steady 75% RH (± 1%) over a very wide temperature range. NaCl mixed 1:1 with KCl will give an RH% around 70–71 between 20 and 30°C, $(NH_4)_2SO_4$ maintains humidity around 80% RH between 10 and 40°C. For further details, consult the reference. Other methods include use of mineral acids or strong alkalis (Solomon, 1951) and pose various risks in relation to occupational health and safety. Another method, safer than strong acids and alkalis, is the use of glycerol solutions (Miner and Dalton, 1953).

6.6.4 Culture vessels

Typically, glass tubes (ca. 2 cm diameter × 5 cm long), 9 cm diameter plastic Petri dishes and cell-culture

bottles have been used. Glass tubes are also normally stoppered with non-absorbent cotton wool. This allows gas exchange between the inside and outside of the tube, but is relatively insecure and mites can escape. Also, the plug becomes full of mites eventually and these may fall off the plug onto the bench when subculturing, thus increasing the opportunities for cross-contaminating cultures. Petri dishes are easy, cheap and secure. Some petroleum jelly or similar grease (high vacuum grease as used for laboratory vacuum pumps and the like is very good) is loaded into a plastic syringe and run round the inside edge of the lid. This produces an escape-proof seal – mites get tangled in the grease. However, the trade-off for good security is poor gas exchange and the cultures have to be aired regularly to prevent CO_2 build-up. This is especially important if using live yeast as a culture medium. Another consequence of reduced gas exchange is that humidity in the culture vessels may come out of equilibrium with that in the desiccators, again, especially if the culture medium is hygroscopic.

Cell culture bottles that have a microporous filter incorporated into the lid allow gas exchange and are relatively mite-proof. Additionally, the small opening to the flask makes it more difficult to get access to the cultures (e.g. if subculturing or adding food material) than does a Petri dish. Doubtless, there are other containers that could be used to circumvent some of these problems.

Small numbers of mites can be raised and observed in modified Robertson cells (Robertson, 1944; see Figure 6.7b). These are black Perspex cells with a central concave chamber milled into them. The lid is a glass microscope slide held in place with a hinge of double-sided adhesive tape. The original Robertson cells had a black filter paper base to provide gas exchange. In the modified versions shown in Figure 6.7b, it was found there was sufficient gas exchange between the top of the cell and the underside of the lid and a filter paper base was unnecessary. A Kilner jar with a Perspex rack can be used to store several cells. Humidity is controlled as for a larger culture vessel, using saturated solutions of inorganic salts.

6.6.5 Food

Mites live in their food; they lay their eggs in it, they defecate in it and they eventually die in it. The particle size of the culture medium can be important in determining population growth rates. As a rule, the smaller the particle size, the poorer the growth. Powdery media tend to encourage more fungal growth than granular media. This can be avoided by adding glass beads to help break up any conglomeration when the culture is shaken.

De-fatted human skin scales, derived from hair collected from a barber's shop, de-fatted in acetone (Spieksma, 1969), ox liver powder, baker's yeast granules, fish food flakes or dried Daphnia, dog biscuits, various cereal preparations including wheat germ, cultures of fungi, and house dust have all been used for culturing dust mites. Yeast and dried Daphnia 1:1 mix is very good for rapid growth of many species. Yeast granules alone give good growth for several species but yeast contains a streptavidin-binding factor, so if the mite extracts are to be used in immunochemical procedures involving streptavidin-biotin binding reactions, the mites must be cleared of culture medium by sieving and/or starving the mites. Axenic culture of house dust mites on chemically defined diets have not been tried but may offer the best prospect for media of low intrinsic allergenicity.

The recent findings of Barber *et al.* (1996) and Sánchez-Monge *et al.* (1996) that cereals contain a series of inhibitors of certain *Dermatophagoides* spp. allergens has some important implications for the choice of culture media in which dust mites are grown. It would appear that use of wheat germ is not advisable in mite cultures that are grown for the purpose of allergen production.

Mumcuoglu (1977a) attempted to rear various mites on simple sugars and achieved some success with *Tyrophagus putrescentiae*, but not *Dermatophagoides pteronyssinus*. Other artificial diets are detailed by Rodriguez and Lasheen (1971), Rodriguez and Blake (1979) and Singh (1977).

6.6.6 Subculturing

Subculturing needs to be done when the culture is starting to get excessively crowded. As a general rule, this can be gauged by looking at the culture medium with the naked eye and seeing if any movement can be detected. If the medium appears to be heaving or shimmering slightly, remove about two-thirds of the contents and replace with fresh culture medium. Be sure to keep the stock culture medium in its own desiccator (with no mite cultures) and at the same temperature and humidity as the mite cultures. Baker's yeast granules that have been stored at dry conditions can absorb a lot of water from the air when placed in humid conditions and may lower the humidity of the

culture vessel dramatically and lethally. Dust mites can survive a relatively short duration at low humidity (Arlian, 1992) and will recover. Gross effects of water loss from mites involve shrinkage of the body: the mites appear shrivelled and flattened dorsoventrally, instead of ovoid. If they appear flattened instead of tumescent then they have probably lost a sizeable quantity of body water and should be moved to a humidity above 75% RH immediately.

Slowing of activity may indicate all is not well with the culture. Normally the mites are moving constantly, couples can be observed in copula and eggs are present. Absence of any of the above may indicate humidity is too low or too high and, as a consequence, the mites are suffering the effects of toxic fungal metabolites.

6.6.7 Isolating mites from the culture medium

This can be done either by picking out individuals with a fine brush or needle or, if many mites are required, by driving them out of the culture medium using a hot light source. When inspecting mites in culture, fibre optic light sources should always be used because the light is relatively cool and will not desiccate them. A hotter light source directed at the middle of the culture will drive mites out to the edge of the vessel where they can be scooped up with a brush. For even larger scale isolation, several cultures can be pooled and sieved through a series of Endecott test sieves (or similar) with meshes of different sizes (e.g. 1 mm, 500 μm, 250 μm, 100 μm) on a sieve-shaker to obtain a mite-rich fraction. The 100 μm mesh will result in a faecal pellet-rich fraction (pellets are ca. 10–50 μm in diameter), though it will also contain some eggs and larvae. Making a faecal pellet-rich fraction using meshes of 50 μm or below is usually not practical because the mesh gets clogged up with pellets, with very little yield. Shimomura *et al.* (1982) described a method of collecting each developmental stage from a culture using sieving.

Having generated a mite-rich fraction, the procedure of Lind and Løwenstein (1983) can be used. In short, the mites from the mite-rich sieve fragment are mixed with saturated sodium chloride in a separating funnel. Funnels must be large enough so that the passage in the tap does not become blocked when drawing off liquid/mites. The culture material will fall to the bottom of the funnel and the mites will float to the top. Repeat twice. This will provide clean mites free of culture medium. Arlian *et al.* (1979b) describe a similar method for separating mites from culture medium using an ethanol suspension. Automatic devices have been described for separating large quantities of mites from the culture medium by Woodring (1968) and Stepien and Rodriguez (1972).

Starving the mites will clear their guts of faecal material and food. This may be important if the culture medium is allergenic, but bear in mind that the faecal pellets and bodies of the mites have different allergen repertoires, e.g. Der p 1 and Der p 3 are mainly found in the faeces but Der p 2 is found mainly in mite bodies. Starving can be done by removing the mites from the culture medium using a hot light and transferring them to a clean vessel without culture medium for 24 hours. Repeating the flotation process will separate faeces from bodies, but the faeces may not then be suitable for allergen investigation because of the high water solubility of group 1 allergens.

6.6.8 Laboratory hygiene and safety

Good laboratory hygiene will keep cultures free of major fungal infection and of cross-contamination. Any cultures found to contain more than one species of mites or with visible fungal growth should be discarded into a bleach bucket. Specific details for keeping cultures sterile are detailed by Parkinson (1992).

Culturing dust mites renders the laboratory worker at risk of becoming sensitised to them. The rule is to ensure that procedures are done in a laminar flow hood or fume cupboard, and that a gown or lab coat, gloves, goggles and a mask are worn. As little skin as possible should be exposed, and culturing operations should be done in a specially designated room away from other people who may not be wearing protective clothing.