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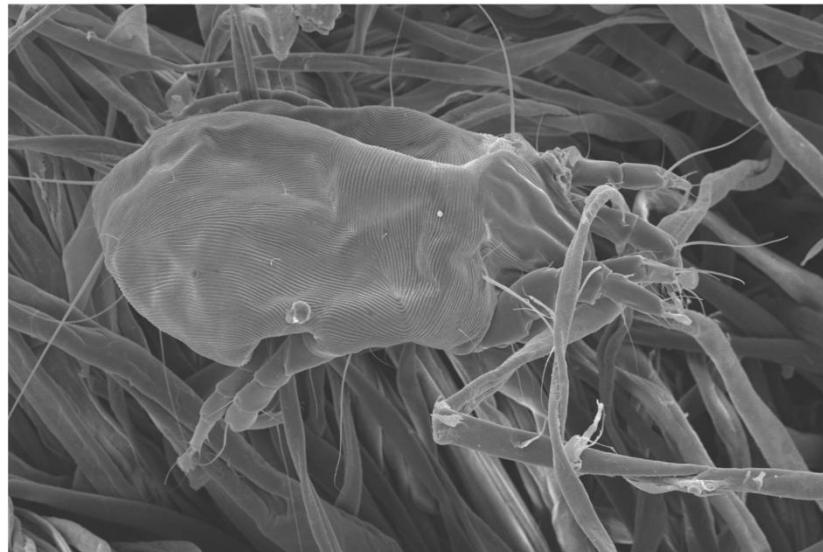
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Author(s)	Clarke, David
Publication Date	2015-03-25
Item record	http://hdl.handle.net/10379/4961

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An assessment of allergenic mite species and allergens in vehicles and homes, with particular reference to dust mite transfer in clothing



A thesis submitted to the National University of Ireland, Galway for a degree
of Doctor of Philosophy

March 2015

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Abstract

Dust mite allergens cause conditions including asthma, atopic dermatitis and allergic rhinitis in people globally. Most studies investigating aspects of mite ecology and epidemiology have focussed on Pyroglyphid mites, which include those species generally most abundant in homes and have formed the basis for allergen sensitisation thresholds over the last two decades. Some studies have highlighted that mite species outside of the Pyroglyphidae family also harbour important allergens capable of causing sensitisation, yet these are regularly left unreported in research examining house dust mites. Additionally, knowledge gaps regarding the presence and quantity of allergenic mites in specific biotopes thus far exist, with no comprehensive research conducted on allergenic mite fauna in Irish homes to date.

The aim of Chapters 2 and 3 of this thesis is to bridge important research gaps in the determination of quantities and species of allergenic mite fauna in previously understudied biotopes. In the first study described in this thesis, mites in dust from 30 homes with pets were compared with 30 homes without pets, with a view to assessing the influence that domestic pets have on allergenic mite densities and species composition within the home. Mite species richness was greater in homes with pets compared to homes without pets, suggesting that the presence of pets can result in a wider variety of epidemiologically important mite species within households.

Vehicles have been greatly understudied with regard to quantifying dust mites and their associated allergens, with no published research thus far concerning mite densities and allergen levels in child car seats – an important void given that young children are most susceptible to developing conditions associated with dust mite allergens. A survey of 106 cars found 12 species of mites, with 80% of driver seats and 77% of child car seats containing mites. There was a significant correlation between the number of mites per gram of dust and the levels of Der p 1 allergen in the car seats sampled, with over 12% of driver seats and 15% of child car seats containing dust mite quantities sufficient to be risk factors for sensitisation and allergic reactions.

Although clothing is the one of the main suspected mechanisms by which dust mites are dispersed and come to colonise new habitats, no research to date has investigated the physical properties that determine quantities transferred. Since dust mites were found in abundance in the vehicle-based survey, the dynamics involved in the transfer of dust mites from car seat material to modern clothing fabrics were hence investigated. Fabric type, mite condition (live or dead) and the applied force between the fabrics were all shown to have a significant effect on the transfer efficiency of house dust mites, while duration of contact was found to have no effect.

Acknowledgements

I would first like to express my sincere gratitude to Dr. Mike Gormally and Dr. Miriam Byrne for all their help, guidance and counselling over the last four years of this study. Their open minded approach was a joy to experience and I consider myself very lucky to have had the privilege to carry out this research alongside them.

I want to say a big thanks to all students in the Applied Ecology Unit, past and present. In particular, to all my fellow PhD students over the last number of years for being great friends, a pleasure to work with and providing moral support; John Staunton, Tracy Hynes, Inga Reich, Brendan Canning, Margaret Hayes, Pamela Boyle, John Carey, Caitriona Maher, and to Gesche Kindermann and Caitriona Carlin. Also a special thanks to Erica Dix, Sarah Liddy and Daniel Burke.

In relation to the statistics used in this thesis, I owe a wealth of praise to Jerome Sheahan. He was always at hand to deal with my detailed queries (day and night) and his appetite for perfection was inspiring; he is one of the greatest assets to the University and an academic of sincere qualities. I will miss the late night chats, the tea and the constant conveyor-belt of cake.

A special thanks to my family and parents who provided every kind of support throughout the duration of my research. I've been very lucky to have family with such a great outlook on everything and their guidance at all times was essential to me completing this work.

Finally, the highest gratitude goes to Elayne. I'll never know how she put up with my constant nagging and wild mood swings. Her support and love was ever present and without her, I would not have had the strength to complete this.

Preface

The literature concerning dust mite research is vast; there are numerous articles related to multiple facets of mite ecology, distribution, physiology and epidemiology written by researchers all over the world. In his book entitled *Dust Mites* published in 2009, Matthew Colloff compiled a comprehensive review of the literature up to that point. As a complete review of the literature since dust mite research began would not be appropriate, the purpose of this introduction (Chapter 1) is to provide a general overview of the most important literature pertaining to the research questions of this thesis. Additionally, this introduction will describe, under separate sub-headings, areas of dust mite research that have either been under-studied or neglected. These sections serve as a preface for chapters 2-4, which are presented in the form of articles. Briefly, they concern:

- Allergenic mites in the microenvironment of pets (Chapter 2)
- The presence and abundance of dust mites and their allergens in child car seats (Chapter 3)
- The dynamics involved in the transfer of dust mites in clothing fabrics (Chapter 4)

Chapter 5 discusses the findings of Chapters 2-4 in light of the relevant research pertaining to each topic referred to in this introduction.

Chapter 1:

General Introduction

1. General Introduction

1.1 Dust mites and their impact on human health.

Dust mites are one of the smallest members of the arachnid family, belonging to the subclass Acari (Fig.1). They are oval to round in shape, white or creamy in colour and at 250 – 300 µm in length, are barely visible to the naked eye. Mattresses, carpets, furniture and textiles are their preferred habitats – areas where shed human skin, their main dietary component, can accumulate easily. Dust mites produce allergens and are widely accepted as being a major contributor in the role of allergic sensitisation in humans worldwide (Platts-Mills *et al.*, 1997). Allergy to dust mites is estimated to affect roughly 65-135 million people, equivalent to 1-2% of the world's population (Colloff, 2009), with young children being most susceptible to sensitisation (Ćustović and Chapman, 1998).

Fig. 1

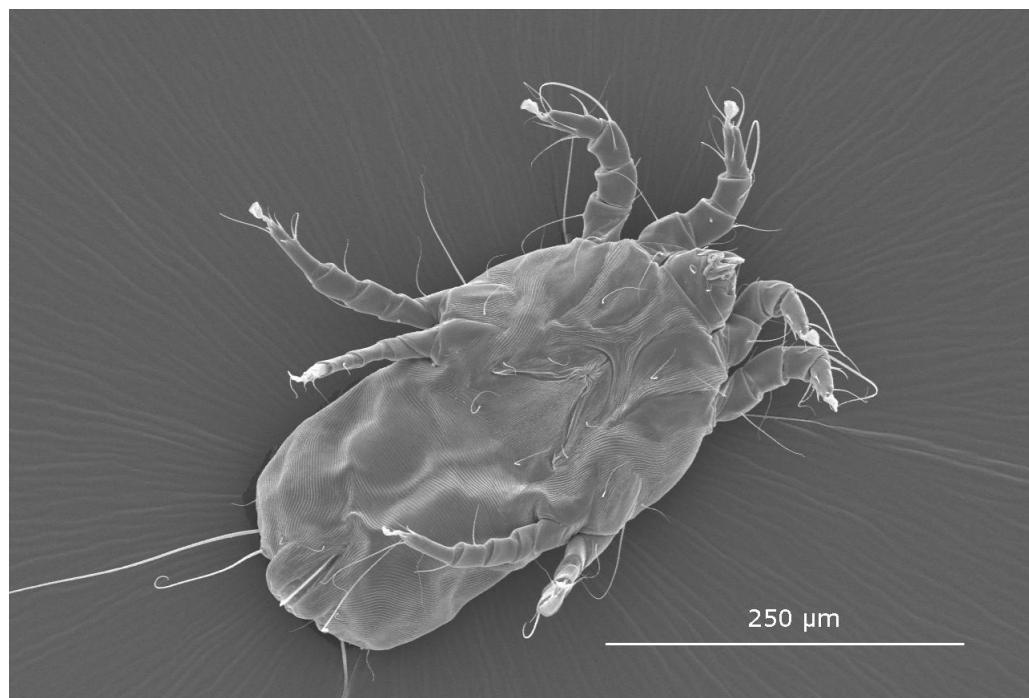


Fig.1. Microscope image of a dust mite specimen (*Dermatophagoides pteronyssinus*). Image courtesy of the CMI, NUI Galway.

In the 1960s, pioneering investigations by Voorhorst *et al.* (1964) in the Netherlands were the first to hypothesise that dust mites were the cause of allergic conditions in humans, while detailed taxonomic studies on the house dust mite *Dermatophagoides pteronyssinus* by Fain (1966) are seen as pivotal in providing a benchmark for taxonomists for later descriptions of other dust mite species (Colloff, 2009). At the time of these discoveries, Voorhorst *et al.* (1964) had difficulty convincing colleagues that dust mites were the source of allergens that caused sensitisation and their findings were met with much scepticism. However, simultaneously in Japan, Oshima (1964) was examining tatami floor coverings for parasites that seemingly caused skin reactions in children. In his findings, he reported the presence of *Dermatophagoides* mites, which helped support the findings by Voorhorst *et al.* (1964) on the other side of the world. Soon afterwards, the idea that dust mites were the cause of allergic symptoms began to disperse through scientific and medical communities, sparking a series of investigations worldwide.

Following on from early research described above, countless studies and trials have demonstrated that dust mite allergy is an independent risk factor for the development of conditions such as asthma (Crisafulli *et al.*, 2007), atopic dermatitis (Teplitsky *et al.*, 2008; Khan *et al.*, 2012), eczema (Celedón *et al.*, 2007), perennial rhinitis (Henszel *et al.*, 2010) and conjunctivitis (Pacciani *et al.*, 2010). In an indoor environment, activities that cause disturbance to reservoir dust such as vacuum cleaning, dusting and bed-making result in settled dust becoming airborne. Small particles such as faecal pellets from dust mites (in the size range of 10 – 40 μm) or other allergen-bearing particles become temporarily suspended in the air and can be easily inhaled (Tovey *et al.*, 1981; De Lucca, 1999). This can cause sensitisation in people who are atopic i.e. people who have a genetic predisposition to develop allergic reactions to common allergens like those derived from dust mites, or other sources such as animal skin scales from cats and dogs (Ownby *et al.*, 2002), cockroaches (Perzanowski *et al.*, 2013) and pollens (Jutel *et al.*, 2005).

When allergens are inhaled by atopic people, they respond by making IgE antibodies which bind with immunologically active cells. This causes the release of mediators such as histamine and leads to the development of localised inflammation. In 1989, risk levels for sensitisation to dust mite allergens were outlined at the First International Workshop on Indoor Allergens and Asthma (Platts-Mills *et al.*, 1989). At this meeting, it was agreed that allergen levels of $\geq 2\mu\text{g/g}$ of dust (equivalent to 100 mites/g of dust) are sufficient to induce sensitisation, while levels of $\geq 10\mu\text{g/g}$ (equivalent to 500 mites/g of dust) are seen as a risk factor for the development of acute asthma. These thresholds have been used by most researchers in the field to date to assess the relevance of their findings with respect to sensitisation (Platts Mills *et al.*, 1989).

1.2 Species and global distribution

In the majority of the literature, the term ‘dust mites’ usually concerns members of the family Pyroglyphidae, of which the species *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Euroglyphus maynei* are most predominant in house dust. *Dermatophagoides pteronyssinus* (commonly known as the European house dust mite) dominates in temperate regions such as Western Europe (Raffan *et al.*, 2005; Zock *et al.*, 2006), while *D. farinae* (the American house dust mite) is more frequent in North America and parts of continental Europe (Sidenius *et al.*, 2002; Macan *et al.*, 2003). *Euroglyphus maynei* is also common in temperate regions (Arlian *et al.*, 2001) and appears to have a widespread geographical distribution, with records in the UK, (Blythe, 1975) continental Europe (Mumcuoglu *et al.*, 1999; Macan *et al.*, 2003) and North and South America, (Morgan *et al.*, 1997, de Oliveira *et al.*, 2003). In rural homes in temperate regions, species of *Glycyphagus* and *Lepidoglyphus* (family Glycyphagidae) traditionally associated with stored products (hereafter referred to as ‘storage mites’) can also be abundant (Chambers *et al.*, 1999), and numerous studies exist highlighting the potential importance of these families of mites in relation to sensitisation (Spieksma, 1997, Warner *et al.*, 1999; Farmaki *et al.*, 2012; Baker and Swan, 2013). The species *Blomia tropicalis* (family Echimyopodidae) has been identified as a particularly prevalent allergenic species

found in house dust in tropical and sub-tropical regions of the world (Arlian *et al.*, 2001; Fernandez-Caldas and Lockey, 2004; Lim *et al.*, 2004; Carvalho *et al.*, 2013).

1.3 Habitats and factors governing survival

Dust mites are found in practically every home, but their predominant habitats within the home are areas that offer plentiful supplies of their main food source i.e. human skin scales. These include mattresses, carpets, rugs, upholstery and clothes –any materials that are fibrous in composition and hence trap and accumulate skin scales more easily. As well as providing a food source, the insulation properties of such materials present ideal living conditions for mites with regard to achieving and maintaining suitable body moisture equilibrium (Colloff, 2009). As skin scales die and fall off the surface of the human body they begin to decay. This process is aided by colonising microorganisms such as bacteria, moulds and fungi that feed on lipids, proteins and carbohydrates present in the skin. It is generally believed that this natural progression creates ideal feeding opportunities for dust mites, as freshly shed skin in its raw state is too tough and dry for mites to consume (Nadchatram, 2005).

However, conflicting evidence exists regarding whether or not the presence of microorganisms on skin scales is essential for digestion of the skin scales by dust mites. Van Bronswijk and Sinha (1973) claim that increased population growth of mites was observed when fed on culture media consisting of defatted skin scales pre-incubated with the mould *Aspergillus amstelodami*. This claim was challenged by Douglas and Hart (1989) who suggested that *Aspergillus amstelodami* may actually be a food source for the mites and that increased population growth occurred for this reason and not due to fungal predigestion of the mite's food source. Hay *et al.* (1992) found specimens of *D. pteronyssinus* encountered a higher diversity of fungi in the wild compared to laboratory cultures, though the densities of individual fungal species in culture were higher in culture than in house dust. Further experiments by Hay *et al.* (1993)

demonstrated that *D. pteronyssinus* cultures bred in the presence of *Aspergillus penicilloides* suffered from detrimental effects including reduced survival rate and lowered fecundity, with these effects worsening with increased density of the fungi. However, the authors also observed poor mite population performance in a fungus-free media environment, suggesting that although fungi in larger densities are inhibitory to mites, they may provide an important nutritional element in mite population growth,

Over the last two or three decades there has been a major shift with regard to building design in developed countries. As humans are confronted with the reality of global warming, their attitudes towards conserving energy and lowering carbon emissions have changed dramatically. In Ireland, regulations such as the Building Control Act (2007) provide guidelines for increasing thermal and energy efficiency of homes by specifying the level of air tightness of homes. Wall cavity insulation, double glazed windows and central heating are now commonplace in modern homes and with a reduction in ventilation and draughts, a more stable indoor environment in relation to temperature and humidity has been achieved. Although these modifications have undoubtedly improved the energy efficiency and level of comfort in homes, the resultant conditions are inadvertently more suited to dust mite propagation (Colloff, 2009).

Relative humidity and temperature are vital to the survival of dust mites and together with food availability, are the main factors that dictate population growth (Arlian *et al.*, 2001). Dust mite bodies consist of 70-75% water by weight, which necessitates constant water regulation for survival and reproduction (Arlian, 1992). As water in liquid form is not available to mites in their natural habitats, they have adapted by extracting water vapour from unsaturated air at humidities ideally in the range of 65-80% while limiting the extent of water loss when humidity drops below 55% (Spieksma, 1997). This is achieved by osmoregulation through the cuticle (Hart, 1998) and by the use of hygroscopic supracoxal glands (Spieksma, 1997; Arlian *et al.*, 1999). In periods of extended drought, mites are thought to create a barrier layer of humid air around the cuticle, thereby preventing further water loss through evaporation. The results of this behaviour

can be further enhanced by individual mites clustering together in groups (Spieksma, 1997), a phenomenon which may be induced by an aggregation pheremone released by the mites (Skelton *et al.*, 2010). The cuticle of pyroglyphid mites is covered with a series of striations (Fig. 2), which, although its function is not fully understood, is thought to play a role in the maintenance of this protective humid air barrier (Colloff, 2009).

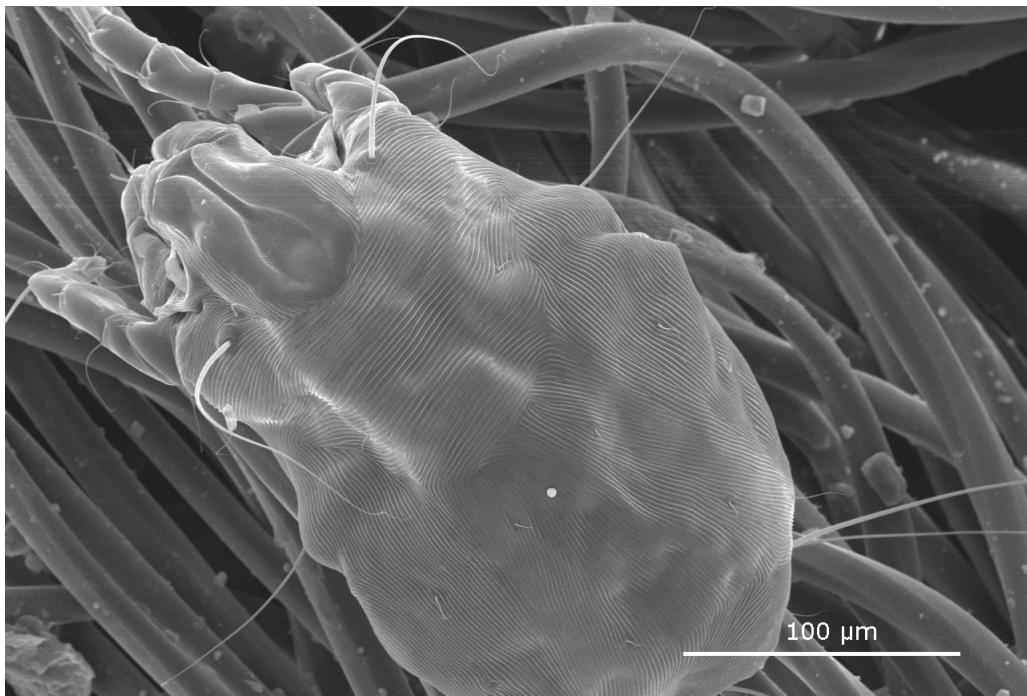


Fig. 2. Scanning electron micrograph of *D. pteronnyssinus* depicting cuticle striations. (Image courtesy of the CMI, NUI Galway)

Temperature is also important in dust mite ecology, although deemed not as critical as humidity (Spieksma, 1997). Optimum laboratory temperatures for the majority of species are in the region of 20-30°C, at which biological activity and population growth are at their greatest (Arlian *et al.*, 2001). Dust mites can survive at temperatures outside this range but generally experience slower growth rates and metabolic activity (Spieksma, 1997; Arlian *et al.*, 2001). In a natural setting, temperature and humidity are rarely constant and mites have to deal with

regular diurnal fluctuations in both (Arlian *et al.*, 2001). In a mattress for example, temperature and humidity will rise and fall depending on the presence of a human occupant, with mites capable of burrowing to seek optimum conditions (Colloff, 2009).

1.4 The importance of storage mites

The vast majority of studies concerning dust mites have focussed on the family Pyroglyphidae, most likely due to the fact that a) they are generally the most predominant family present in house dust (Colloff, 2009); and b) assays for the detection of allergens derived from these species (readily available to researchers) have been developed for over two decades (Luczynska *et al.*, 1989). Despite this, many other species of mites share the dust ecosystem of pyroglyphid mites and over the years, many researchers have highlighted these other mite species as a source for harmful allergens. Table 1 shows a list of all mite genera thus far known to have allergenic properties.

Table 1. Mites thus far known to produce allergens. References refer to authors who first reported the listed species as either having, or suspected of having allergens to which persons can become sensitised. In most cases these allergens have been sequenced and described in future studies, but are not listed here. Table adapted from Colloff (2009) with modifications.

Order	Family and Genus	References	Order	Family and Genus	References
	Pyroglyphidae (house dust mites)			Cheyletidae	
	<i>Dermatophagoides</i>	Spieksma and Voorhorst (1969)		<i>Cheyletus</i>	Morita <i>et al.</i> , 1975
	<i>Euroglyphus</i>	Voorhorst <i>et al.</i> , 1969		Pyemotidae	
	<i>Malayoglyphus</i>	Chew <i>et al.</i> , 1999		<i>Pyemotes</i>	Ancona, 1923
	<i>Stunophagoides</i>	Chew <i>et al.</i> , 1999	Prostigmata	Tarsonemidae	
	Acaridae (storage mites)			<i>Tarsonemus</i>	Korsgaard and Hallas (1979)
	<i>Acarus</i>	Ingram <i>et al.</i> , 1979		Tetranychidae	
	<i>Aleuroglyphus</i>	Miyamoto <i>et al.</i> , 1969		<i>Panonychus</i>	Bernecker, 1970
	<i>Suidasia</i>	Miyamoto <i>et al.</i> , 1969		<i>Tetranychus</i>	Bernecker, 1970
	<i>Thyreophagus</i>	Müsken <i>et al.</i> , 2003			
	<i>Tyrophagus</i>	Spieksma and Voorhorst (1969)			
Astigmata	Glycyphagidae (storage mites)				
	<i>Austroglycyphagus</i>	Chew <i>et al.</i> , 1999			
	<i>Blomia tropicalis</i>	Miyamoto <i>et al.</i> , 1969			
	<i>Gohieria</i>	Mumcuoglu, 1977			
	<i>Glycyphagus</i>	Maunsell <i>et al.</i> , 1968			
	<i>Lepidoglyphus</i>	Spieksma and Voorhorst (1969)	Mesostigmata	Dermanyssidae	
	Chortoglyphidae (storage mites)			<i>Dermanyssus gallinae</i>	Bernecker, 1970
	<i>Chortoglyphus</i>	Miyamoto <i>et al.</i> , 1969		Laelapidae	
	Sarcopidae			<i>Hypoaspis miles</i>	Kronqvist <i>et al.</i> , (2005)
	<i>Sarcoptes</i>	Falk and Bolle (1980)		Phytoseiidae	
	Psoroptidae			<i>Amblyseius cucumeris</i>	De Jong <i>et al.</i> , (2004)
	<i>Psoroptes</i>	Stewart and Fisher (1986)		<i>Phytoseiulus persimilis</i>	Kronqvist <i>et al.</i> , (2005)

Mites of the families Acaridae and Glycyphagidae, commonly referred to as ‘storage mites’ are found globally. Traditionally, they have been identified as a health hazard in an occupational context for farmers, bakers and grain workers (Cusack *et al.*, 1976; Armentia *et al.*, 1997; Fernandez-Caldas, 1997; Chambers *et al.*, 1999), having originally been primarily associated with hay, straw, stored grain, flour and vegetable products (Hughes, 1976; Solarz, 2004). Although long suspected, storage mites are now widely known to produce allergens (Fernández-Caldas, 1997; Franz *et al.*, 1997; Chambers *et al.*, 1999; Solarz *et al.*, 2004) while prevalence of sensitisation to these allergens has also been investigated in numerous studies (Armentia *et al.*, 1997; Radon *et al.*, 2000; Vidal *et al.*, 2004; Celebioglu *et al.*, 2013). The diet of storage mites typically consists of a wide range of foods including stored grain, hay, products such as dried meat, fish and fruit, flour, cereals and cheese (Spieksma, 1997; Chambers *et al.*, 1999; Hubert *et al.*, 2006; Baker and Swan, 2013) while infestations have been observed in products such as cereal-based baby food (Thind and Clarke, 2001) and dog biscuits (Baker and Swan, 2013). A syndrome known as oral mite anaphylaxis (OMA) has recently been designated (Sánchez-Borges *et al.*, 2013), and is characterised by severe allergic symptoms occurring after ingesting foods made with wheat flour contaminated with allergenic mites. Several species of storage mites including *Tyrophagus putrescentiae*, *Tyrophagus entomophagus* and *Aleuroglyphus ovatus*, in addition to pyroglyphid species, have been isolated from contaminated foods in cases where individuals have suffered OMA symptoms. (Matsumo *et al.*, 1996; Blanco *et al.*, 1997; Sánchez-Borges *et al.*, 1997; Hannaway and Miller, 2008; Geller *et al.*, 2009) Although the ingestion of pancakes made from wheat flour have been the source of most cases of OMA, some cases have been attributed to other food types including sponge cake, pizza, and pasta (Sánchez-Borges *et al.*, 2013). It is most likely that the ability of storage mites to thrive on such a variety of food, much of which is commonly stored in kitchens, has led to their successful transition from rural, farm environments to a domestic habitat.

Although species such as *G. domesticus*, *L. destructor*, *A. siro*, *A. farris*, *Tyrophagus putrescentiae*, *Chortoglyphus arcuatus* and *Gohieria fusca* are usually

classed as storage mites, they are regularly found in house dust and have been identified as some of the main allergy causing mites outside of the pyroglyphid family present in house dust (Solarz, 2004; Vidal *et al.*, 2004; Jackson *et al.*, 2005; Skelton *et al.*, 2007; Farmaki *et al.*, 2012; Ahmed *et al.*, 2013). Similar to pyroglyphid mites, temperature and humidity are also vital to their survival, with optimum humidity reported to be higher for storage mites than for pyroglyphids (Vidal *et al.*, 2004). Studies in the north-western region of Spain have found that sensitisation to species including *L. destructor*, *T. putrescentiae* and *A. siro* is extremely prevalent among house dust mite allergic patients (Vidal *et al.*, 1997; Marcos Bravo *et al.*, 1999). Other species such as *Cheyletus eruditus* (family Cheyletidae), which is not classed as a storage mite but is a predatory mite also found in house dust, has also been shown to induce sensitisation in farmers in Germany (Müsken *et al.*, 2000), while cross-reactivity allergens have been found in the storage mite *Tarsonemus granarius* (family Tarsonemidae), suggesting this species could also be of allergenic importance (Xue-Ying *et al.*, 2011).

1.5 Mite fauna of animal environments

Although the focus of most literature dealing with dust mites and their allergens is naturally directed towards human environments, the microenvironments of animals have also been investigated, and have consistently been shown to also harbour mite populations and allergen content. Of these, the majority deal with the allergens derived from pyroglyphid species both from a human sensitisation perspective as well as causing allergic conditions in domestic animals, mainly dogs (Glass *et al.*, 2003; Raffan *et al.*, 2005; Nutall *et al.*, 2006; Cunha *et al.*, 2007) and cats (Loft and Rosser, 2010).

Relatively few studies have reported on mite species composition in the microenvironment or bedding of these animals (Mumcuoglu, 1976; Eaton *et al.* 1985; Randall *et al.*, 2003; Jackson *et al.*, 2005; Farmaki *et al.*, 2012) and interestingly of the few that have, a wide range of mite species seem to prevail (Table 2).

Mumcuoglu (1976) examined dust samples from the bedding of dogs, cats, guinea pigs, hamsters and singing birds in Switzerland and reported a wide range of mite species present. Although no mites were present in bird cages, he reported six mite species from five dog beds and eight species from five cat beds (Table 1). Cages of five guinea pigs and five hamsters were also found to contain five species of mites, the presence of which the author attributed to the use of hay bedding for the animals. A survey by Eaton *et al.* (1985) examined the mite fauna of seven pet beds in rural areas of the UK and reported eleven mite species (Table 1) noting that *D. pteronyssinus* was the most abundant species, present in all but one of the beds sampled. In a more recent study from Greece, Farmaki *et al.*, (2012) examined the species composition and population densities of five mite species, *D. pteronyssinus*, *D. farinae*, *A. siro*, *L. destructor* and *T. putrescentiae*, in houses with mite-sensitised dogs, clinically healthy dogs and households without dogs. They found that although *D. farinae* was more prevalent than *D. pteronyssinus* in the microenvironment of mite-sensitised dogs, there were no significant differences in population abundances or species composition between the housing categories. Parasitic mites such as *Otodectes cynotis* (commonly known as ear mites) infect the auditory canal of mammals and are well documented in cats (Sotiraki *et al.*, 2001; Blot *et al.*, 2003; Lefkaditis *et al.*, 2009), dogs (Ortega-Pacheco *et al.*, 2003; Oh *et al.*, 2004; Souza *et al.*, 2008), foxes (Lohse *et al.*, 2002) and ferrets (Lohse *et al.*, 2002; Le Sueur *et al.*, 2011) while mites of the families Psoroptidae and Sarcoptidae cause mange in a range of mammals (Rodriguez-Vivas *et al.*, 2003; Pan *et al.*, 2006; Singh *et al.*, 2011; Villarroel *et al.*, 2013; Lewis, 2013).

Table 2. Mite species reported from the various studies that examined the dust samples from the bedding of domestic pets.

		References*							
		Farmaki <i>et al.</i> , 2012	Jackson <i>et al.</i> , 2005	Randall <i>et al.</i> , 2003	Eaton <i>et al.</i> , 1985		Mumcuoglu, 1976		
(Sub) Order	Pet bedding type	Dogs	Dogs	Dogs	Dogs	Cats	Dogs	Cats	Guinea pigs / Hamsters
	Species								
Astigmata	Pyroglyphidae								
	<i>Dermatophagoides pteronyssinus</i>	✓	✓	✓	✓	✓	✓	✓	
	<i>Dermatophagoides farinae</i>	✓		✓			✓	✓	
	Acaridae								
	<i>Acarus</i> sp.					✓			
	<i>Acarus farris</i>					✓			
	<i>Acarus siro</i>	✓	✓					✓	✓
	<i>Tyrophagus putrescentiae</i>	✓	✓				✓		✓
	Glycyphagidae								
	<i>Glycyphagus domesticus</i>					✓	✓	✓	
	<i>Lepidoglyphus destructor</i>	✓	✓				✓	✓	✓
	<i>Chortoglyphus arcuatus</i>								✓
	<i>Gohieria fusca</i>						✓		
	Psoroptes sp.					✓	✓		
Prostigmata	<i>Cheyletus</i> sp.					✓			
	<i>Cheyletus eruditus</i> *						✓	✓	✓
	<i>Cheyletomorpha lepidoptorum</i>								✓
	<i>Tarsonemus</i> sp.					✓	✓		
Trombidiformes	Scutacaridae sp					✓	✓		
	Tydeidae sp.					✓	✓		
Mesostigmata	<i>Androlaelaps casalis</i>							✓	
	<i>Kleemannia</i> sp.					✓			
Oribatida	<i>Oribatida</i> sp					✓	✓		

*References are listed in reverse chronological order

Although some varied results have been obtained from these studies, the fact that so few have examined this facet of mite ecology has left scope for further research. From the earlier studies on pet beds, it is evident that the microenvironment of pets can be habitats for a wide range of species, many of which, as mentioned earlier, are allergenic. However, there is little indication as to how the presence of pets may influence the mite composition / abundances in human environments in the home. Excess shedding of skin and hair by pets likely result in ample quantities of food for dust mites. While numerous mite species are associated with the microenvironments of pets, it is unclear whether this phenomenon translates to the human environments which pets occupy. This provided the incentive for Chapter 2 of this thesis

1.6 Dust mites in transport

As the primary requirements for dust mite survival are: a) a suitable substrate where food can accumulate; and b) suitable humidity and temperature conditions, it is unsurprising that the distribution of dust mites is not strictly restricted to buildings. Different modes of transport have been examined mainly in relation to dust mite allergen levels including trains (Colloff, 1987; Uehara *et al.*, 2000), buses (Ćustović *et al.*, 1995; Partti-Pellinen *et al.*, 2000), aeroplanes (Wickens *et al.*, 1997), submarines (Engelhart *et al.*, 1999), taxis (Taketomi *et al.*, 2006) and private cars (Justino *et al.*, 1995; Neal *et al.*, 2002). Further details on allergen levels found in these studies are given in Chapter 3.

Very few studies have reported the species composition and density of mites in either public or private transport vehicles. Colloff (1987) reported ten mite species from 16 out of 22 samples collected from the seats of passenger trains in Glasgow. These included *D. pteronyssinus*, *G. domesticus*, *L. destructor*, *E. maynei*, *T. putescentiae*, *Bakerdonia tarsalis*, *Tydeus interruptus*, *Tarsonemus fusarii* and *Haemogamasus pontiger*. The author noted that *D. pteronyssinus* was the most abundant species found, and attributed the presence of the other species to transportation via household pets and clothing to the seats. In addition to Der 1 allergens, Neal *et al.* (2002) also examined the presence of mites from 139

automobile driver seats in Ohio, USA, and reported a mean of 42.5 - 81.3 mites/g of dust, though information on specific species found in the study was absent.

Compared to the wealth of research conducted in domestic settings, there is a paucity of information with regard to dust mites and indeed allergen levels in transport vehicles. This is surprising given that in developed countries at least, many families today either own a car or travel in a car on a frequent basis. Numerous surveys in many countries have attempted to quantify the time spent by people travelling in various modes of transport, with results depending on multiple factors such as proximity to workplaces and schools, traffic densities and population sizes (Panter *et al.*, 2014). Despite this, it is clear that in the modern world, both adults and children depend on motorised transport to get around and invariably will spend some regular fraction of their lives in a vehicle environment.

In the interest of safety, children are required, when travelling in a vehicle, to be restrained in the correct type of child car seat according to specific height, weight and age guidelines (EU Directive 2003/20/EC). This means that any family with a child under the age of 11 or 12 (approximately) must have a suitable restraining safety seat in their car at all times when travelling with their child. Since child car seats are typically upholstered with polyester material and/or polystyrene foam padding, the external surface of which is similar to regular car seats, these appear to be ideal environments for the accumulation of dust and therefore potentially harbour dust mites and their allergens. This is important considering that exposure to dust mite allergens in infancy and early childhood is widely regarded as being the most important window of sensitisation for children at risk of atopy (Rowntree *et al.*, 1985; Wahn *et al.*, 1997; Celedón *et al.*, 2007). Given that this possibility has not been previously explored, it is not currently known whether child car seats are habitats for dust mites or whether they contain sufficient dust mite allergen levels capable of inducing sensitisation. This topic is the focus of Chapter 3.

1.7 Clothing as a vector for dust mite dispersal

Like numerous other fabric-based textiles such as carpets, upholstery and bedding, clothing has been shown to house populations of dust mites and their allergens. A

study by Bischoff and Fischer (1990) used the heat escape method (application of heat via a hotplate to the underside of a piece of fabric, driving mites away from the heat source) to recover mites from a single knitted jacket and reported over 30,000 live specimens present. A mean density of 8.6 live mites per 100 cm² was recovered from eight out of 15 clothing items by Tovey *et al.* (1995) also by the heat escape method, while an allergen test revealed a mean of 15.9 µg/g of dust of Der p 1 allergen from 35 clothing items. In a study in Israel, Teplitsky *et al.* (2008) examined mite densities in worn clothing, stored clothing and bedding of 19 children diagnosed with atopic dermatitis and reported a number of species including *D. pteronyssinus*, *D. farinae*, *E. maynei*, *B. tropicalis*, *C. eruditus* and unidentified specimens of the orders Astigmata, Mesostigmata and the family Tarsonemidae, but found no significant difference in abundances of these mites between the different materials investigated.

In other studies, clothing has been identified as the probable route by which mites are dispersed to other areas of the home, thereby colonising other biotopes (Colloff, 1987; Mollet and Robinson, 1996). Mollet and Robinson (1996) conducted trials with mites marked with a red dye to determine whether mites were transferred to other locations of the home and family vehicle via clothing. Marked mites were first released onto a fabric covered couch and left overnight. Nine hours later two children were placed sitting on the couch for a period of approximately three hours. Subsequent rinsing of their clothes revealed the presence of marked mites, while vacuum samples from carpets and the family vehicle ten days and 24 hours later respectively also revealed the presence of these mites. Similarly, Neal *et al.* (2002) reported that although low densities of mites and Der 1 allergen were recovered from clothing samples from 10 individuals, 7 of these individuals also had mites in their homes and in their cars. They also found a correlation between the numbers of mites recovered from an individual's clothing and the densities recovered from the homes of the participants.

There is evidence to suggest that clothing can greatly influence dust mite allergen exposure. Separate investigations have found woollen garments to contain higher levels of Der p 1 than other fabrics. De Lucca *et al.* (2000) found that while

wearing a woollen sweater resulted in greater levels of inhaled Der p 1 than wearing items such as cotton t-shirts, cotton sweat shirts and jackets, it also showed an 11 fold increase in allergen exposure compared to background levels. Similar results were observed by Siebers *et al.* (1996) who examined children's clothing and found significantly higher levels of Der p 1 on woollen garments compared to polyester or cotton. These results suggest that woollen garments appear to have a higher propensity to accumulate dust mite allergens than other fabrics such as cotton, though this may be attributed to the fact that woollen sweatshirts and jackets are generally washed less frequently than cotton t-shirts and shirts (De Lucca *et al.*, 2000). A recent study by Tovey *et al.* (2013) measured Der p 1 using personal air pump samplers and found higher concentrations of allergen were collected during than the day than in bed at night, suggesting that clothing may be an important source for dust mite allergen exposure.

To date, research in this area has indicated that: a) clothing can house substantial populations of dust mites; b) mites can be transported to other locations both inside and outside the home via clothing; and c) that clothing type can influence levels of dust mite allergen exposure. However, there is a paucity of data regarding the external variables that influence the transfer of dust mites through clothing. Previous research has revealed some important relationships between dust mites and clothing with regard to possible transportation to other biotopes and allergen exposure, yet the dynamics regarding dust mite transfer via clothing still remains relatively unexplored. This information, which may have implications for the establishment of dust mite populations in new biotopes while also influencing levels of allergen exposure, is the theme for Chapter 4.

1.8 Scope and objectives of this study

Despite the bulk of literature detailing dust mite physiology, ecology and epidemiology, there are many key areas of research in relation to dust mites that remain uncovered. It is now well known that not only pyroglyphid mite species are sources for allergens, but storage mites and mites of other families are also important. Although most dust mite research in the home has naturally focussed exclusively on human environments, little is known about the environments of domestic pets and the influence that pets may have on densities and species composition of mites in the home. Few studies have dealt with the quantification of dust mites and their allergens in cars and of the few that have, none to date have focussed on child car seats, an important biotope given that young children are most at risk of developing allergic conditions. In addition, although it is widely presumed that dust mites are transported between biotopes via clothing, a limited number of studies to date have confirmed this occurrence, while the capacity at which different clothing fabrics pick up and transfer mites due to contact is virtually unknown.

Bearing in mind the above research gaps, the objectives of this study are to:

- Investigate the influence of household pets on the composition and quantity of allergenic mite fauna within Irish homes
- Quantify typical dust mite populations and allergen levels in cars, with particular reference to child car seats.
- Determine the dynamics of dust mite transfer in modern (commonly worn) clothing fabrics.

1.9 Structure of this thesis

This thesis is presented in the format of a series of three articles. Chapter 2 describes, for the first time, the mite fauna of 60 Irish homes, examining and comparing the species composition and densities of mites in two housing categories: a) houses with pets; and b) houses without pets. Chapter 3 describes a survey of 106 cars where dust samples were taken from driver seats and child car seats to quantify dust mite populations, species composition and allergen levels and compare the findings for each seat type. In addition, an assessment of the environmental and behavioural factors affecting mite populations present was performed. Chapter 4 describes experiments that investigate the transference of dust mites from car seat material to three modern clothing fabrics, subject to varying forces applied, durations of contact and condition of the mites (i.e. live or dead). Given that the methods used to collect and analyse dust samples for dust mites and allergen levels were the same for Chapters 2 and 3, there is, out of necessity, some overlap and repetition between the methodology sections of the individual articles. Finally, the questionnaires used in Chapters 2 and 3 are given in the appendices.

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Chapter 2:

The influence of household pets on the composition and quantity of allergenic mite fauna within Irish homes: a preliminary investigation

This paper has been submitted to the journal *Medical and Veterinary Entomology* (November 2014)

2. The influence of household pets on the composition and quantity of allergenic mite fauna within Irish homes: a preliminary investigation

2.1 Abstract

Abstract

Allergenic mites are responsible for inducing hypersensitive reactions in genetically predisposed people worldwide. Mites in dust from 30 Irish homes with pets (dogs n=23, cats n=7) were compared with 30 homes without pets. House dust mites constituted 78% of all mites recorded with *Dermatophagoides pteronyssinus* representing 57 – 72% of mites in furniture and mattresses in both home types compared to only 22% of mites in pet beds. Although storage mites made up just 13% of all mites recorded, they represented 46% of mites recorded in pet beds. Median levels of the dust mite allergen Der p 1 ($\mu\text{g/g}$) in dust samples from mattresses in homes without pets were significantly greater than in mattresses from homes with pets, reflecting the greater densities of *D. pteronyssinus* found in the former home category. Mite species richness was greater in homes with pets (17 species) compared to homes without pets (13 species). This suggests that although the presence of pets can result in a wider variety of epidemiologically important mite species within households, increased competition among mite species may result in a more balanced mite fauna in the home, inhibiting the dominance of any one species and hence lowering allergen associated risks.

Keywords: *Dermatophagoides pteronyssinus*, allergenic mites, homes, pets, Ireland.

2.2 Introduction

Mites are widespread in the home environment of humans. Many are known to produce allergens which are responsible for increases in conditions such as asthma, dermatitis, eczema and allergic rhinitis amongst the worldwide population (Celedón *et al.*, 2007; Crisafulli *et al.*, 2007). The majority of studies investigating dust mites and their associated allergens in home environments have focussed on mites of the family Pyroglyphidae, commonly known as house dust mites. Of the 49 species within this family only 13 have been recorded from house dust, the remainder being inhabitants of bird nests or associated with the feathers of birds (Colloff, 1998). *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are the most commonly reported species, with allergens derived from these regarded as some of the most important in the development and exacerbation of asthma (De Lucca *et al.*, 1999). Suggested sensitisation thresholds for these allergens have been long established (Platts-Mills, 1989), with levels $\geq 2\mu\text{g/g}$ (equivalent to 100 mites/g of dust) sufficient to induce sensitisation, while levels of $\geq 10\mu\text{g/g}$ (equivalent to 500 mites/g of dust) are deemed capable of causing acute asthma in sensitised people.

To date, there have been no published studies (to the best of the authors' knowledge) on the species composition or abundance of mites present in homes in Ireland. Pyroglyphid species usually dominate the acarofauna of house dust, but a variety of other taxa have also been recorded including transient species that do not establish in the dust. Some species of 'storage mites,' mainly pertaining to species of the families Acaridae and Glycyphagidae, are common inhabitants. Members of these families are more often found in greater numbers in, for example, stored grains, flour and cheese (Baker and Swan, 2013), and some are known to produce harmful allergens (Fernández-Caldas, 1997; Chambers *et al.*, 1999; Solarz *et al.*, 2004; Celbioglu *et al.*, 2012). Other mites which are not belonging to these genera but which are also found commonly in house dust are also allergenic. Müskens *et al.* (2000), who investigated sensitisation and potential importance of mite species in atopic farmers in Germany, found 12 of 51 patients tested (14%) were sensitised to the predatory mite *Cheyletus eruditus* (family

Cheyletidae). While the storage mite family Tarsonemidae has been little studied in relation to the production of allergens, Korsgaard and Hallas (1979) suspected that it may be the origin of specific allergens accounting for some of the unexplained cases of allergy caused by the inhalation of house dust. More recent research by Xue-Ying *et al.* (2011) found cross-reactivity allergens in the midgut tissue, gut contents, cuticle and reproductive system of *Tarsonemus granarius*, highlighting this species as being potentially important in triggering IgE antibodies in the human body.

Although allergen producing mites are most commonly reported in human environments, some studies have examined their presence in the microenvironments of domestic pets (i.e. dogs and cats) within homes, most of which have also focussed on pyroglyphid species and their derived allergens. (Randall *et al.* 2003; Jackson *et al.*, 2005; Raffan *et al.*, 2005; Loft and Rosser, 2010). Fewer studies have reported non-pyroglyphid mite species in pet bedding. In addition to *D. pteronyssinus*, *D. farinae* and *Cheyletus eruditus*, Mumcuoglu (1976) found three storage mite species (*Lepidoglyphus destructor*, *Tyrophagus putrescentiae* and *Gohieria fusca*) from five dog beds and five storage mite species (*L. destructor*, *Glycyphagus domesticus*, *Acarus siro*, *Androlaelaps casalis*, *Cheyletomorpha lepidoptorum* and *C. eruditus*) from five cat beds, varying in densities from 30-260 mites/g of dust from dog beds to 40-400 mites/g of dust from cat beds. Pet beds were also examined by Eaton *et al.* (1985) who reported 11 species of mites from seven pet (dog and cat) beds in rural areas of the UK. In Greece, Farmaki *et al.*, (2012) investigated the presence and abundance of three storage mite species *A. siro*, *T. putrescentiae* and *L. destructor*, in addition to *D. pteronyssinus* and *D. farinae* in 40 households with dogs and 25 households without dogs, but found no difference in frequency or abundance of mites between the categories.

What is clear from the aforementioned investigations is that pet bedding can serve as a habitat for a multitude of important mite species, many of which are allergenic (Mumcuoglu, 1976; Eaton *et al.*, 1985; Solarz *et al.*, 2004; Müskens *et al.*, 2000). Nevertheless, it still remains unclear whether the presence of cats or

dogs has any bearing on the diversity and / or density of the permanent mite fauna in human environments within the home. As cats and dogs deposit dander and hair naturally through processes such as grooming and scratching, it is likely that this would result in ample reservoirs of food for skin-scale feeding mites in various locations where pets frequent regularly in the home. Additionally, the movement of pets between outdoor and indoor environments may result in the transport of a range of important allergenic mite species of the families Acaridae and Glycyphagidae, amongst others, into the home, encouraging the establishment of mite populations and facilitating their dispersal between sites throughout the household.

To explore this further, various biotopes of domestic mites within the household were examined. Mattresses, furniture items and pet beds (where applicable) were sampled for mites in: a) homes with pets (i.e. cats or dogs) that were allowed indoors; and b) homes without pets. Densities of mites were quantified and a full species profile was compiled for each biotope sampled. Additionally, Der p 1 allergen levels were measured from excess dust samples and compared between biotopes and home categories.

2.3 Materials and Methods

2.3.1 Collection of dust samples and house dust mite analysis

Dust samples were collected from homes at multiple locations in Ireland between October 2011 and February 2012 and between October 2012 and February 2013. Target samples were categorised into two distinct categories: homes with pets (i.e. homes that had either a dog or cat frequenting the home on a regular basis – abbreviated as ‘HP’), in which pets had either their own specific bedding or had a regular resting place within the home, and homes without pets (i.e. homes that never had any kind of pet animal or bird present – abbreviated as ‘H’). Some of the homes sampled were those of participants who took part in a previous study

investigating the presence of dust mites in child car seats, whom had given prior consent to participate in this study (Clarke *et al.*, 2015). Others were selected through chain referral sampling (Heckathorn, 2002). Thirty homes from each home category were sampled (60 homes in total) in single visits to each home. Samples were taken in both home categories from: a) the mattress or mattresses of an occupant (s); b) an armchair or equivalent piece of furniture e.g. couch from a room that was regularly used by the occupant (s); and c) the bed or resting place of any or all pets residing within the home (HP only). A total of 44 mattresses and 30 furniture items from each home category were sampled, in addition to 34 pet beds, consisting of 23 homes with dogs only (four homes had more than one dog for which an extra sample from a dog bed was collected, resulting in 27 dog bed samples in total) and 7 homes with cats only. Participants were also asked to complete a questionnaire concerning subjects such as physical characteristics of the home and cleaning regimes of the occupants in an attempt to elucidate factors which, on the basis of existing knowledge of mite ecology and population dynamics, may have influenced mite populations present in the samples.

Dust samples were obtained using a portable battery powered vacuum pump (Flite 2 by SKC Inc.). Samples were collected by vacuuming the entire surface area of each item or site in a two minute period, using a zig-zag movement to avoid sampling the same area more than once (Hill, 1998). The dust was collected in a plastic cassette containing a track-etched polycarbonate Whatman membrane filter (diameter 37 mm; pore size 0.4 µm), which was connected to the pump via plastic tubing and a nozzle. Following collection, samples were stored at - 20°C to: a) kill any live mites present, thereby preventing elevated quantities of mites resulting from breeding or oviposition; b) prevent desiccation of the mites collected until ready for counting and identification.

Dust samples were subsequently prepared for mite analysis. Samples were subjected to sieving using a fine mesh (pore size 1 mm) which removed large particles, retaining only the fine dust which consisted mainly of a mixture of shed skin and mites. The mixture was weighed in a pre-weighed container using a microbalance and subdivided into aliquots of 50 mg. A maximum of three

aliquots (150 mg) from each sample was analysed for the presence of mites, depending on the availability of dust collected. Counting and identification of mites was undertaken using a modified approach similar to that described by Cameron and Hill (2002). 50 mg of fine dust was placed in a 50 ml polythene beaker containing 45 ml of tap water. The beaker was covered with Parafilm and inverted 20 times. The Parafilm was removed and the beaker was placed in a freezer at - 20°C for 1 h and 15 min. After this time, an ice block was formed with three distinct layers. These consisted of a top thin layer of ice containing mites that had floated to the surface, the middle layer which was unfrozen and contained water and some dust debris. The bottom layer contained heavier dust debris in ice. The top layer was carefully removed and placed into another 50 ml polythene beaker and allowed to thaw. When fully thawed, the stain methylene blue was added, which adhered to all remaining dust debris in the mixture except the mites. The mixture was then poured onto a gridded no. 5 Whatman filter circle (55 mm diameter) in a Büchner funnel. The suction operation of the Büchner funnel allowed removal of excess liquid and thus retained any mites present in the sample on the filter circle. The gridded filter circle was subsequently examined for presence of mites under a stereobinocular microscope at 40x magnification. Mites were removed from the filter circle using a fine dissection needle and placed into a drop of Hoyer's medium on a microscope slide. When all mites present were isolated and transferred to the drop of mounting medium on the slide, a coverslip was then applied. Mite specimens were carefully identified under a phase contrast microscope at 100x magnification with the aid of identification keys (Evans and Till, 1979; Baker, 1999; Colloff, 2009). In keeping with standard practices in the field, mite counts are reported as mites/g of dust.

2.3.2 Der p 1 analysis

In addition to mite analysis, samples with a minimum of 50 mg of dust remaining ($n = 38$) were analysed for Der p 1 allergen content by Airmid Health Group Ltd. Samples were extracted in phosphate buffer saline of pH 7.4, which contained 0.05% Tween 20 (PBS-T). Following centrifugation, supernatants obtained were stored at -20°C prior to Der p 1 allergen measurement using a two-site

monoclonal antibody ELISA (Luczynska *et al.*, 1999). Four dilutions (1 : 2, 1 : 14, 1 : 98, 1 : 686) of each sample were added to the plate, with mean values from a Der p 1 standard curve taken as the final result for allergen concentration. Absorbance values were expressed in micrograms per gram of dust ($\mu\text{g/g}$), with a limit of detection of 0.025 $\mu\text{g/g}$

2.3.3 Statistical procedures

The statistical packages IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, 2011) and Minitab 16 Statistical Software (2010) were used to analyse the data. Nonparametric statistical methods (Mann-Whitney U test) were used to test for differences between the medians of groups tested, while chi-squared analyses were used to test for measures of association between biotopes and home categories in relation to the presence or absence of mites and in relation to sensitisation thresholds

2.4 Results

89% of mattresses, 87% of furniture items and 97% of pet beds sampled contained mites (92% overall). The distribution of mites found at each biotope is shown in Table 1.

Table 1. Distribution of domestic mites found in the various biotopes within the home

	Number of mites	Percentage of total
Mattresses	3077	72.8
Furniture	804	19.0
Pet beds	348	8.2
Total	4,229	100.00

A total of 4,229 specimens were found in 182 samples of dust from a combination of mattresses, furniture and pet beds, with 19 species of mites identified (Table 2). Distinct specimens that could not be identified to species level but were clearly belonging to a specific genus, sub-order or order are also listed. Eleven out of the

19 mites identified to species level (55%) are known allergen producers (Solarz *et al.*, 2004; Colloff, 2009; Beroiz *et al.*, 2014). The pyroglyphid house dust mite species *D. pteronyssinus* was the most dominant species with 2478 specimens found (59% of the total).

2.4.1. Species richness and mite densities in biotopes sampled

While overall species richness (Table 3) was greater in HP (17 species) than H (13 species), higher median and mean mites/g of dust were found from biotopes sampled in H (Table 3 and Fig. 1a). Sixteen of the 17 species in HP were also present in pet beds (Table 3), while pet beds also contained the highest mean number of mite species per sample (Fig. 1b). *Tyrophagus putrescentiae* and Oribatida sp. were found (albeit in small numbers) in pet beds and furniture items of HP only, while *Chortoglyphus arcuatus* and *Gohieria fusca* were found only in mattresses of H. (Table 2) In addition, *Tyrophagus palmarum*, *A. casalis*, *Otodectes cynotis* (family Psoroptidae) and Eriophyidae sp. were found exclusively in pet beds. The species *O. cynotis* and Eriophyidae sp were found in cat bedding only, while *A. siro* and *C. eruditus* which were present in dog beds, were absent from cat beds (Table 4).

Chapter 2: Influence of pets

Table 2. Mite orders, families and species and their abundance in home with pets (HP) and without pets (H). Mattresses = M, Furniture = F Pet beds = PB.

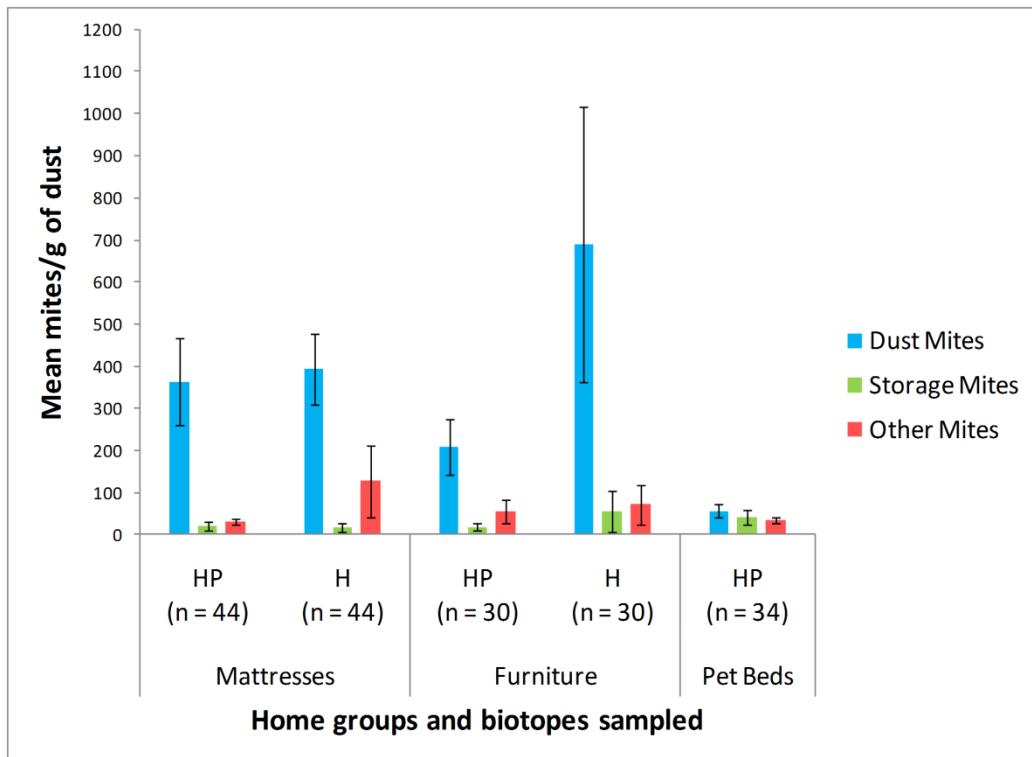
(Sub) Order	Families and species	Homes with pets (HP)			Homes without pets (H)		Total (% of total)
		M	F	PB	M	F	
Astigmata	Pyroglyphidae (House dust mites)						
	<i>Dermatophagoides pteronyssinus</i> *	754	254	77	1110	283	2478 (59)
	<i>Dermatophagoides farinae</i> ?*	5	0	0	10	0	15 (<1)
	<i>Dermatophagoides</i> sp.	187	60	23	250	51	571 (14)
	<i>Euroglyphus maynei</i> *	64	4	8	126	13	215 (5)
Astigmata	Acaridae (Storage mites)						
	<i>Acaridae</i> sp.	0	3	5	1	0	9 (<1)
	<i>Acarus</i> sp.	0	0	14	0	0	14 (<1)
	<i>Acarus farris</i> *	11	2	57	0	3	73 (2)
	<i>Acarus siro</i> *	0	0	9	0	0	9 (<1)
	<i>Tyrophagus</i> sp.	1	3	8	2	2	16 (<1)
	<i>Tyrophagus palmarum</i>	0	0	3	0	0	3 (<1)
	<i>Tyrophagus putrescentiae</i> *	0	1	3	0	0	4 (<1)
Astigmata	Glycyphagidae (Storage mites)						
	<i>Glycyphagidae</i> sp.	12	13	11	3	3	42 (1)
	<i>Glycyphagus domesticus</i> *	1	4	27	2	1	35 (<1)
	<i>Lepidoglyphus destructor</i> *	3	7	8	10	1	29 (<1)
	<i>Chortoglyphus arcuatus</i> *	0	0	0	60	0	60 (1)
	<i>Gohieria fusca</i> *	0	0	0	9	0	9 (<1)
Astigmata	Psoroptidae						
	<i>Otodectes cynotis</i>	0	0	3	0	0	3 (<1)
Prostigmata	Tarsonemidae (Storage mites)						
	<i>Tarsonemidae</i> sp.	11	6	2	16	2	128 (3)
	<i>Tarsonemus granarius</i> *	1	1	2	107	1	21 (1)
	<i>Tarsonemus scaurus</i>	5	8	10	80	5	108 (3)
Prostigmata	Cheyletidae						
	<i>Cheyletus eruditus</i> *	27	10	8	78	9	132 (3)
Mesostigmata	Mesostigmatid sp. **	0	2	1	1	1	5 (<1)
Mesostigmata	Laelapididae						
	<i>Laelapidae</i> sp.	0	0	4	0	0	3 (<1)
	<i>Androlaelaps casalis</i>	0	0	3	0	0	3 (<1)
Oribatida	Oribatid sp.	0	1	6	0	0	7 (<1)
Trombidiformes	Eriophyidae sp.	0	0	1	0	0	1 (<1)
	Unidentifiable mites	63	37	55	67	13	235 (6)
	Species richness	10	11	16	12	10	19
	Total no. of specimens	1145	416	348	1932	388	4229

* indicates species known to produce allergens. ** denotes specimens that were distinctly different from other species from the same order, yet could not be identified to species level due to missing appendages or imperfections. Otherwise, specimens that could not be identified to species level but cannot be described as being distinctly different from other species of the same genus are included under the genera to which they belong. Species richness totals are calculated as the total number of species found at each biotope. Specimens of a particular genus or other higher taxon which occur where there are no other species relating to that genus or higher taxon occurring, these are included as being distinct and contribute to the species richness for that biotope. Species marked with "?" indicate that although most taxonomic characteristics of that species were present, a defining feature was either missing or obscured, preventing complete confirmation of the species.

Table 3. Species richness and densities of mites found in each biotope in houses with pets (HP) and houses without pets (H).

Group	Biotope	N	Median species /sample (IQR)	Mean species /sample ($x \pm SE$)	Max species /sample	Total no. of species found	Median mites/g (IQR)	Mean mites/g ($x \pm SE$)
HP	Mattress	44	1 (1-2)	1.9 ± 0.2	6	10	141 (47-371)	471 ± 128
	Furniture	30	1 (1-3)	2.0 ± 0.3	7	11	120 (70-375)	280 ± 69
	Pet beds	34	2 (1-3)	2.4 ± 0.2	5	16	63 (33-172)	131 ± 28
	Dogs	27	2 (1-2)	2.0 ± 0.3	5	12	47 (127-177)	35 ± 135
	Cats	7	4 (2-5)	3.4 ± 0.5	4	13	78 (63-144)	33 ± 33
Total		108	2 (1-3)	2.1 ± 0.1	7	17	96 (38-326)	311 ± 58
H	Mattress	44	2 (1-3)	2.2 ± 0.2	5	12	280 (138-526)	538 ± 120
	Furniture	30	2 (1-2)	1.8 ± 0.2	4	10	283 (136-491)	818 ± 331
	Total	74	2 (1-3)	2.0 ± 0.1	5	13	281 (135-522)	651 ± 151

(a)



(b)

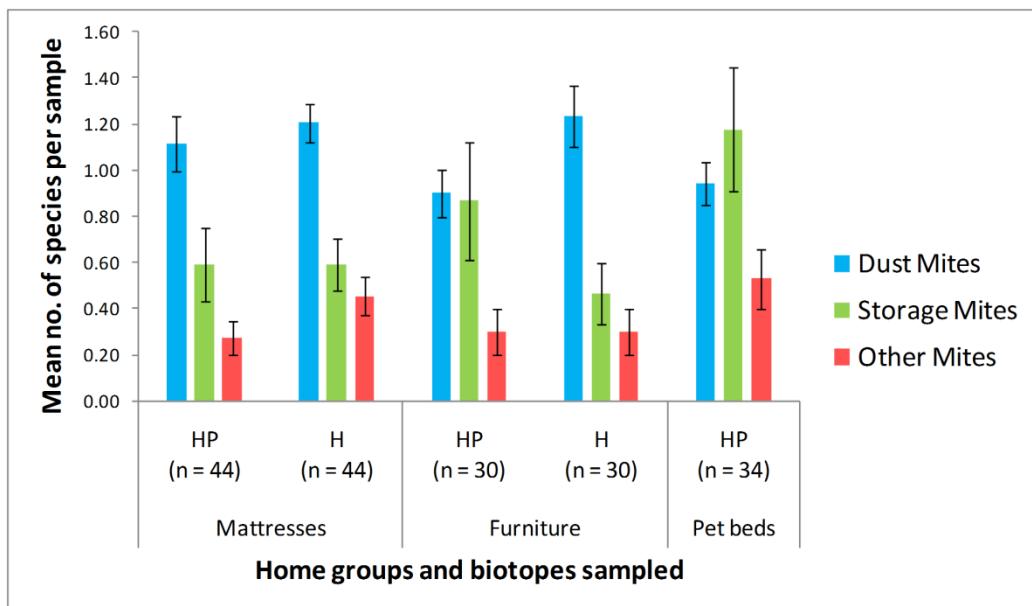


Fig. 1. (a) Mean densities (with standard error bars) of mites (mites/g of dust) belonging to the categories ‘dust mites’ (members of the Pyroglyphidae), ‘storage mites’ (members of the Acaridae and Glycyphagidae) and ‘other mites’ (all other species, genera and orders) found at each biotope in homes with pets (HP) and homes without pets (H), and (b) mean number of mite species per sample (with standard error bars).

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Table 4. Mite species richness and abundance in dog beds and cat beds

(Sub) Order	Species	Dog beds (n = 27)		Cat beds (n = 7)	
		No. of specimens	(% of total)	No. of specimens	(% of total)
Astigmata	Pyroglyphidae (House dust mites)				
	<i>Dermatophagooides pteronyssinus</i> *	56	21	21	26
	<i>Dermatophagooides farinae</i> ??*	0	0	0	0
	<i>Dermatophagooides</i> sp.	18	7	5	6
	<i>Euroglyphus maynei</i> *	8	3	0	0
Astigmata	Acaridae (Storage mites)				
	<i>Acaridae</i> sp.	5	2	0	0
	<i>Acarus</i> sp.	14	5	0	0
	<i>Acarus farris</i> *	56	21	1	1
	<i>Acarus siro</i> *	9	3	0	0
	<i>Tyrophagus</i> sp.	7	3	1	1
	<i>Tyrophagus palmarum</i>	1	<1	2	3
	<i>Tyrophagus putrescentiae</i> *	1	<1	2	3
Astigmata	Glycyphagidae (Storage mites)				
	<i>Glycyphagidae</i> sp.	11	4	0	0
	<i>Glycyphagus domesticus</i> *	15	6	12	15
	<i>Lepidoglyphus destructor</i> *	2	<1	6	7
	<i>Chortoglyphus arcuatus</i> *	0	0	0	0
	<i>Gohieria fusca</i> *	0	0	0	0
Astigmata	Psoroptidae				
	<i>Otodectes cynotis</i>	0	0	3	4
Prostigmata	Tarsonemidae (Storage Mites)				
	<i>Tarsonemidae</i> sp.	1	<1	1	1
	<i>Tarsonemus granarius</i>	0	0	2	3
	<i>Tarsonemus scaurus</i>	7	3	3	4
Prostigmata	Cheyletidae				
	<i>Cheyletus eruditus</i> *	8	3	0	0
Mesostigmata	Mesostigmatid sp.**	0	0	1	1
Mesostigmata	Laelapidae				
	<i>Laelapidae</i> sp.	1	<1	3	4
	<i>Androlaelaps casalis</i>	1	<1	2	3
Oribatida	Oribatida sp.	3	1	3	4
Trombidiformes	Eriophyidae sp.	0	0	1	1
	Unidentifiable mites	43	16	12	15
Species richness		12		13	
Total no. of specimens		267		81	

* indicates species known to produce allergens. ** denotes specimens that were distinctly different from other species from the same order, yet could not be identified to species level due to missing appendages or imperfections. Otherwise, specimens that could not be identified to species level but cannot be described as being distinctly different from other species of the same genus are included under the genera to which they belong. Species richness totals are calculated as the total number of species found at each biotope. Specimens of a particular genus or other higher taxon where no other species relating to that genus or higher taxon occur are included as being distinct and contribute to the species richness for that biotope. Species marked with "?" indicate that although most taxonomic characteristics of that species were present, a defining feature was either missing or obscured, therefore confirmation of the species was not possible.

While median mites/g of dust from mattresses and furniture in HP were lower than those from mattresses and furniture in H (Table 3), no significant differences were detected (Mann-Whitney U test: $U = 765.5$, $Z = -1.69$, $P = 0.0918$ and $U = 323.5$, $Z = -1.87$, $P = 0.0625$ respectively). Of all the mattresses sampled, 55% and 75% in HP and H respectively exceeded the lower threshold of sensitisation of 100 mites/g of dust ($\chi^2 = 4.03$, $df = 1$, $N = 88$, $P = 0.045$), while 20% and 21% respectively exceeded the upper threshold of 500 mites/g of dust ($\chi^2 = 0.07$, $df = 1$, $N = 88$, $P = 0.787$). Of the furniture items sampled, 37% and 73% in HP and H respectively exceeded 100 mites/g of dust ($\chi^2 = 8.15$, $df = 1$, $N = 60$, $P = 0.004$), with 10% and 17% respectively exceeding 500 mites/g of dust ($\chi^2 = 0.57$, $df = 1$, $N = 60$, $P = 0.448$). While the median mites/g of dust from dog beds were lower than that from cat beds (Table 3), no significant differences were detected (Mann-Whitney U test: $U = 72$, $Z = -0.96$, $P = 0.338$). Of all the pet beds sampled, 21% exceeded the lower threshold of 100 mites/g of dust, while none exceeded the upper threshold of 500 mites/g of dust.

Chi-squared analyses were also used to test for measures of association between biotopes (i) within each home category and (ii) between biotopes in different home categories in relation to the presence or absence of mites. Results of these analyses are displayed in table 5. No significant associations between any of the categories tested were detected.

Table 5. Chi-squared tests for measures of association between biotopes (i) within home categories and (ii) between home categories. HP = Homes with pets (homes with cats and dogs combined), H = Homes without pets, HD = Homes with dogs only, HC = homes with cats only

Chi-squared test	X ²	df	N	P-value
Within Homes				
HP				
Mattresses v Furniture	2.68	1	74	P = 0.102
Mattresses v Pet Beds	5.26	1	78	P = 0.220
Furniture v Pet Beds	0.50	1	64	P = 0.482
H				
Mattresses v Furniture	0.16	1	74	P = 0.692
Between Homes				
Mattresses				
HP v H	1.40	1	88	P = 0.237
HD v H	2.17	1	81	P = 0.141
HC v H	0.38	1	51	P = 0.539
Furniture				
HP v H	0.00	1	60	P = 1.000
HD v H	0.04	1	53	P = 0.850
HC v H	0.35	1	37	P = 0.552
Mattresses and Furniture (combined)				
HP v H	0.76	1	148	P = 0.384

2.4.2 Der p 1 analysis

Median levels of Der p 1 ($\mu\text{g/g}$) from mattress samples were significantly greater (Mann-Whitney U test: $U = 20$, $Z = -2.04$, $P = 0.041$) in H than HP (Fig. 2). Similarly, this trend (while not significant) was also reflected in the furniture samples (Mann-Whitney U test: $U = 5$, $Z = -1.03$, $P = 0.302$). Of all the mattresses tested, 78% and 100 % in HP and H respectively exceeded the lower threshold of sensitisation of $2\mu\text{g/g}$ ($\chi^2 = 2.48$, $df = 1$, $N = 19$, $P = 0.115$), while 44% and 60% respectively exceeded the upper threshold of sensitisation of $10 \mu\text{g/g}$ ($\chi^2 = 0.46$, df

= 1, N = 19, P = 0.498. Of the furniture items tested, 67% and 80% in HP and H respectively exceeded 2 μ g/g ($\chi^2 = 0.24$, df = 1, N = 11, P = 0.4621) with 67% and 60% in HP and H respectively exceeding 10 μ g/g ($\chi^2 = 0.52$, df = 1, N = 11, P = 0.819). However, Der p 1 levels were low in the pet beds tested (Fig. 2).

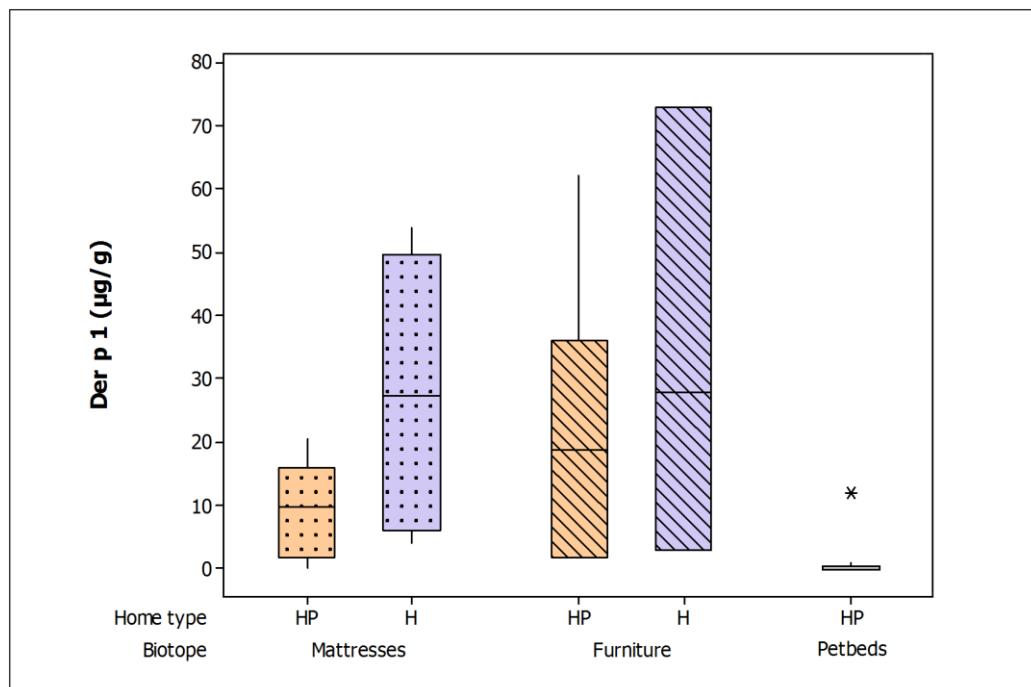


Fig. 2. Median (IQR) levels of Der p 1 (μ g/g) from each biotope in homes with pets (HP) and homes without pets (H): Mattresses HP (n = 9): median = 9.8 (1.8-16.0) μ g/g; Mattresses H (n = 10): median = 27.3 (6.1-49.9) μ g/g. Furniture HP (n = 6): median = 18.9 (1.8-36.0) μ g/g; Furniture H (n = 5): median = 27.9 (3.1-73.1) μ g/g . Pet beds HP (n = 8): median = 0.01 (0.0-0.43) μ g/g.

2.5 Discussion

Mite species diversity was higher in homes with pets than homes without pets, predominantly as a result of the high mite diversity found in samples from pet beds. Since previous records of the house dust mite species *Euroglyphus maynei* in Ireland are not known, this may be the first confirmed record of this species in Irish homes. *Dermatophagoides pteronyssinus*, the most abundant species found, was present in all homes sampled in this study, occurring in 77% of mattresses, 77% of furniture items and 71% of pet beds in HP and 91% of mattresses and 87% of furniture items in H. These are higher frequencies than those reported from comparable biotopes from homes in the UK (22% - Jackson *et al.*, 2005), Greece (43% - Farmaki *et al.*, 2010) and the US (30% - Randall *et al.*, 2003). Climate has previously been shown to affect mite numbers, with generally higher densities found in humid, temperate climates in contrast to dry, arid ones (Crisafulli *et al.*, 2007). An optimum temperature of 23°C for population growth in laboratory cultures has been observed for *D. pteronyssinus* (Arlan *et al.*, 1990) compared to a higher temperature of 27°C for *D. farinae* (Furumizo, 1975), which may explain why the former species was found in abundance in this study. The temperate climate of Ireland predominantly generates cool, moist and humid conditions, likely creating more favourable temperature and relative humidity optima for *D. pteronyssinus*.

Median and mean mite densities were lower in biotopes from HP than in H, while median Der p 1 allergen levels were significantly lower in mattresses of HP than in H. The frequencies of mattress and furniture samples exceeding the lower sensitisation threshold of 100 mites/g of dust were significantly lower in HP than H. Additionally, the frequencies of samples exceeding the upper sensitisation threshold of 500 mites/g of dust and, in relation to Der p 1, samples exceeding 2 μ g/g of dust and 10 μ g/g of dust, were all lower in HP than H for both mattress and furniture samples. This suggests that the presence of pets in homes may, for some reason, have resulted in both lower mite densities and Der p 1 allergen levels in the home. A similar observation was reported from a study in Liverpool

(Raffan *et al.*, 2005) which suggests that more frequent cleaning and vacuuming may be carried out by dog owners as a result of excess build-up of skin and hair shedding around the home, inadvertently reducing mite densities in the process. This also seems to be reflected in the data from bedrooms in this study. For bedrooms of HP, 43% of participants had vacuumed the room within the previous two months, coinciding with lower mite and Der p 1 densities than samples from H, with only 18% of bedrooms having been vacuumed during this time. However for living rooms the reverse was true, with 18% of living rooms vacuumed within the previous two months in HP compared to 23% in H, with higher mite and allergen densities in the latter.

Predation by cheyletid species could, in theory, reduce house dust mite populations, though any significant reduction is unlikely given that their abundances are lower than other predator-prey equilibria (Colloff, 1991). Since *C. eruditus* was more abundant in mattress and furniture samples from H (87 specimens found) than HP (37 specimens found), it is unlikely that predation alone was responsible for lower mite densities in the latter. Increased competition between the greater number of mite species from HP (17) could be a possible explanation for lower overall mite densities in this home category. Although storage mites seem to exhibit a much more varied palate (consisting of stored grain, hay, dried meat, fish and fruit, flour, cereals and cheese) than pyroglyphids, pyroglyphid mites are not exclusive skin-scale feeders and are known to ingest a variety of food types in vitro, including various fungi which are part of the normal constituents of house dust (Hay *et al.*, 1992). It is likely that in the wild, increased competition for food resources among species would be a limiting factor for the dominance of any one family or species of mites, possibly explaining why lower densities of pyroglyphid mites were found in HP compared to H biotopes. This certainly seems like a strong possibility in pet beds at least, where pyroglyphid mites were not as dominant as in samples from other biotopes (Fig. 1(a) and (b)). Treatment of pets with insecticides such as Fipronil is known to be an effective acaricide (Coleman and Atwell, 1999), while flea control agents have been associated with lower levels of Der p 1 in the microenvironments of dogs (Raffan *et al.*, 2005) and cats (Loft and Rosser, 2010). However, respondents in this study

indicated that none of the pets examined in this study were treated with any kind of insecticide or acaricide, thus ruling out any acaricidal effect on lowering mite densities and allergen levels in samples from HP.

Conditions governing the propagation of domestic mite populations in each specific biotope in the home are complex. Temperature and humidity are vital to the survival and development of domestic mites (Spieksma, 1997), and the suitability of each biotope for mite propagation is most likely heavily reliant on these factors. Additionally, seasonal and climatic variability is well known to affect mite numbers (Arlian *et al.*, 1990; Solarz, *et al.*, 2004), with peak mite densities and allergen levels usually occurring in late summer or early autumn (Arlian *et al.*, 1982; Crisafulli *et al.*, 2007; Farmaki *et al.*, 2010). Since samples were collected between the months of October and February, some of the coldest months of the year in Ireland, it is quite possible that greater densities of mites may have been observed during peak periods of warmth in summer and early autumn. It is important to note that while quantifying mite densities of a particular biotope is regarded as a reasonable estimate of potential allergen exposure at that point and time, the nature of fluctuating mite densities at different times of the year may dramatically change the significance of mite densities with regard to sensitisation risks. As mite allergens are known to exist in an environment long after mite bodies have disappeared (Colloff, 2009), these are likely to be a more accurate assessment of exposure risks in terms of accumulation of these allergens over time.

Median levels of Der p 1 measured in this study were, by threshold standards, high (median Der p 1 levels ranging from 9.8 µg/g to 28.9 µg/g), exceeding those from comparable biotopes in various other studies worldwide (Randall *et al.*, 2003; Raffan *et al.*, 2005; Loft and Rosser, 2010). This indicates that risk of exposure to sensitising levels of Der p 1 in the homes sampled appears to be quite prevalent. However, it is suspected by some authors that in environments of high or very low allergen levels, there exists a tolerance inducing or prohibitive effect on sensitisation (von Hertzen and Haahtela, 2009), with reportedly lower instances of allergen related illness in children exposed to high and low allergen concentrations compared to medium levels, following a bell shaped curve pattern

of exposure rates and clinical disease (Cullinan *et al.*, 2004; Tovey *et al.*, 2008). This concept of tolerance is interesting in the context of pets in the home. A recent study by Virtanen *et al.* (2014) found that indoor exposure to a dog in childhood reduced the risk of developing type 1 diabetes compared to outdoor dog exposure, indicating that exposure to a wider variety of microbiota associated with a dog may play a role in developing immunity to a wide range of clinical diseases. It may be that in the case of mites, the presence of a pet in the home may have a balancing effect on the overall mite diversity and general microbiota of the home, thus inhibiting the dominance of any one mite species and hence lowering mite abundance and allergen levels in HP. However, the interactions between mites, allergens, pets and the various other microbiota found in a home in relation to allergic disease is likely to be multifaceted, and should require further research.

Although pyroglyphid mites are the most widely researched in the context of the role they play in allergic responses, it is important to note that many species of storage mites have long been known to have allergenic properties (Pichler *et al.*, 2001) and for this reason should not be underestimated with regard to their role in sensitisation. The storage mite species *C. arcuatus*, *G. fusca*, *A. farris*, *A. siro*, *G. domesticus*, *L. destructor* and *T. putrescentiae* (Table 2) are all known allergen producers (Solarz *et al.*, 2004; Skelton *et al.*, 2007; Colloff, 2009; Celbioglu *et al.*, 2012; Beroiz *et al.*, 2014). *Cheyletus arcuatus* has been studied in relation to its allergenicity in regions such as Colombia (Peurta *et al.*, 1993), Spain (López-Conde *et al.*, 2005; Boquete *et al.*, 2006), and Poland (Solarz, 2004). The storage mite species *A. farris* and *G. domesticus*, both allergen producers, were frequently found in pet beds in this study, with fewer specimens found in mattresses or furniture items. *A. farris*, a species usually associated with field habitats, e.g. hay, was found frequently in dog beds, presumably transported there via the coats of the dogs through contact with hay.

The occurrence of outdoor mite specimens in pet beds (Table 2) highlight the role pets could play in the introduction of a range of mite species from various ecological niches into the home. Such species included mites of the suborder Oribatida and the families Laelapidae and Eriophyidae. Oribatid mites are soil

living decomposers commonly found in woodlands and typically feed on dead plant material and associated colonising fungi (Gan *et al.*, 2014). The family Laelapidae (order Mesostigmata) are free-living vertebrate ectoparasites found in various substrates such as bird and mammal nests, soil and leaf litter (Kavianpour *et al.*, 2013) while Eriophyid mites (order Trombidiformes) are phytophagous, associated only with plant matter (O'Connor *et al.*, 1999). Although species of the families Acaridae and Glycyphagidae are commonly found in house dust, they are traditionally associated as pests of products such as stored grain, cereals and hay (Spieksma, 1997; Chambers *et al.*, 1999; Solarz, 2004) in rural or farm environments. While out in such habitats, cats and dogs presumably would have picked up these species on their coats before being deposited in the animals' respective beds or furniture resting place within the home, though it is possible that human occupants could have introduced these into the home also. The species *A. siro*, which has previously been identified in the microenvironment of dogs (Jackson *et al.*, 2005; Farmaki *et al.*, 2012), is a common pest of stored grains and animal feeds (Chambers *et al.*, 1999) and was associated with dog bedding only in this study. Similarly, although present in all human biotopes in both home categories, *C. eruditus* was present in dog bedding but not in cat bedding, though the species' presence in cat bedding has been noted previously (Mumcuoglu, 1976). Only one specimen of *O. cynotis* (a parasitic mite found in the auditory canal of cats, dogs and other carnivores) was found in a sample from a cat bed and was most likely a parasitic host of the resident cat, while a single Eriophyid specimen was also found in a cat bed, likely picked up by the animal via some source of outdoor vegetation. Excluding *A. siro* and *C. eruditus*, most of these mite taxa do not pose any known human sensitisation risks but some species such as *A. casalis* (family Laelapidae), a mite commonly associated with poultry and wild birds, are thought to be associated with dermatitis in humans (Rosen *et al.*, 2002).

2.6 Conclusions

In summary, a large proportion of the homes sampled in this study contained diverse and abundant mite populations and to the best of the authors' knowledge, this is the first comprehensive study of resident mite populations in Irish homes. A high diversity of known and potentially allergenic species found in pet beds indicates that they could be foci for a host of mite allergens. However, as little is known regarding the potency of storage mite allergens, it is unclear whether the same sensitisation thresholds of mite populations and allergen densities that apply to typical pyroglyphid species also apply to storage mites. Progress with immunoassay methods for detecting storage mite allergens has been achieved (Dunn *et al.*, 2008). However, recent findings of cross-reactivity allergens or possible unique, undescribed allergens in mite species such as *C. eruditus* (Müsken *et al.*, 2000) and *T. granarius* (Xue-Ying *et al.*, 2011), both of which were found in this study, exemplify the need to re-evaluate the relevance of sensitisation to these and other mite species with regard to population densities and their associated allergens within the home. Finally, although evidence from this preliminary study suggests pets may inadvertently reduce overall mite densities and allergen levels in a home, the relationship between pets, mites, allergens, other microbiota of the home and clinical disease is likely complex and undoubtedly requires further research. To better understand these dynamics, future studies should adopt a clinical research approach involving greater sample sizes consisting of a variety of sample categories, which could include homes with both dogs and cats, as well as other types of household mammalian and avian pets.

2.7 Acknowledgements

The authors would especially like to thank all participants whose willingness and kindness made this study possible. Thanks to Airmid Health Group Ltd. who carried out the allergen analysis on dust samples and Dr Anne Baker, Natural History Museum, London, for advice on mite identification. Also a special thanks

to the National University of Ireland Galway College of Science Fellowship for providing the funding to undertake this research.

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Chapter 3:

Child car seats: a habitat for house dust mites and reservoir for harmful allergens

This paper has been accepted for publication in the journal *Annals of Agricultural and Environmental Medicine* (January 2015)

3. Child car seats: a habitat for house dust mites and reservoir for harmful allergens

3.1 Abstract

House dust mites produce allergens which can cause or aggravate diseases such as asthma, eczema and rhinitis. The objectives of this study are to quantify typical house dust mite and Der p 1 allergen levels in child car seats and to determine external variables that may influence mite populations in cars. Dust samples were collected from the child car seats and driver seats of 106 cars using a portable vacuum sampling pump over a two minute sampling period. Mites were counted and identified and results were expressed as mites per gram (mites/g) of dust, while Der p 1 content of samples were measured by enzyme-linked immunosorbent assay (ELISA). Questionnaires were completed by participants to identify environmental and behavioural effects on mite populations. Results were analysed using General Linear Model (GLM) procedures. Twelve species of mites, of which nine are known to produce harmful allergens, were recorded from 212 dust samples. Over 80% of drivers' seats and over 77% of child car seats harboured dust mites with a significant correlation ($P = 0.001$) between the mites/g of dust and Der p 1 content recovered from each seat. A mean of 53 mites/g of dust per seat was recovered, with a mean Der p 1 level of $1.1\mu\text{g}/\text{g}$. Over 12% of driver seats and 15% of child car seats contained house dust mite levels sufficient to be risk factors for sensitisation and allergic reactions. Child car seats and driver seats are habitats to a range of mite species which can be present in sufficient concentrations to cause or aggravate allergen related illnesses in individuals who are genetically predisposed.

Keywords: house dust mites, allergens, child car seats.

3.2 Introduction

House dust mites, which are ubiquitous in the home (particularly in mattresses, carpets and upholstery) produce allergens, and are widely recognised as one of the leading factors in the development of house dust atopy, which can cause or aggravate symptoms of asthma (Asman *et al.*, 2010; Hallas *et al.*, 2011; Tovey *et al.*, 2013), eczema (Henszel *et al.*, 2010) and rhinitis (Arlian *et al.*, 2001). To date, the majority of studies investigating house dust mites and their associated allergens have been carried out in the home (Solarz *et al.*, 1998; Arlian *et al.*, 2001; Henzel *et al.*, 2010) or in public buildings such as libraries and hospitals (Solarz *et al.*, 1998) schools (Einarsson *et al.*, 1995) and hotels (Wickens *et al.*, 1997). Fewer studies have examined different modes of transport as foci for dust mite populations, with many of these focussing primarily on allergen levels in public transport vehicles. Of these, a Japanese study of passenger trains recorded high mite antigen levels corresponding to >100 mites/m² (Uehara *et al.*, 2000) in contrast to low mite allergen concentrations reported for public transport systems in Helsinki (Partti-Pellinen *et al.*, 2000); for aeroplanes in New Zealand (Wickens *et al.*, 1997); and for buses and trains in Manchester (Ćustović *et al.*, 1995). In Brazil a significantly greater percentage of taxis (42%) harboured sensitising levels ($\geq 2\mu\text{g/g}$) of Der 1 allergen compared to private cars (5%) (Taketomi *et al.*, 2006) while another study in the same country reported low levels of Der p 1 and Der f 1 from 60 private vehicles (Justino *et al.*, 1995). Of those few studies which examined mites in vehicles, ten mite species were reported from 16 out of 22 samples collected from the seats of passenger trains in Glasgow, with *Dermatophagoides pteronyssinus* being the most abundant species found (Colloff, 1987). In addition, a US study of automobile driver seats recorded 42.5 - 81.3 mean mites/g of dust from 139 automobile driver seats, with 23% of samples bearing sensitising Der 1 allergen levels exceeding 2 $\mu\text{g/g}$ (Neal *et al.*, 2002).

A recent survey conducted by the UK Department of Transport found that people spend an average of one hour per day travelling with 64% of trips being made by car (National Travel Survey, 2012), while in the U.S. time spent travelling by car has been estimated at more than 18 hours per week (Arbitron National In-Car

Study, 2009). The Irish Central Statistics Office reported that 69% of commuters and 62% of children travel to work and school by car respectively, with the average person in Ireland spending seven hours per week travelling in their car (CSO Travel Report, 2012). With recent changes in European law with respect to child safety in vehicles (EU Directive 2003/20/EC), it is now compulsory for a child to use the correct child seat or booster cushion when travelling in a motorised vehicle. Upholstered seats in vehicles typically consist of polyester and / or cotton. These materials easily accumulate shed human skin scales and other organic detritus which form the main food component of house dust mites.

As of yet there have been no attempts made to quantify typical house dust mite numbers, determine species composition or measure house dust mite allergen levels in child car seats. This indicates an important knowledge gap that currently exists, given that young children are particularly susceptible to becoming sensitised to house dust mite allergens (Marks *et al.*, 1995; Platts-Mills *et al.*, 1997). In the present work, both house dust mite populations and Der p 1 allergen levels in child car seats and driver seats were quantified. In addition, the densities and ranges of house dust mites and allergen levels in both seat types were compared, with a view to assessing through questionnaires, the environmental and behavioural factors which influence house dust mite densities in cars.

3.3 Materials and Methods

3.3.1 Collection and Analysis of Dust Samples

Dust samples were collected from both child car seats and driver seats from 106 cars at six different locations in the west of Ireland from May - July 2011. Information leaflets outlining the research were distributed to five participating educational establishments and subsequently sent to parents prior to the scheduled sampling days. In addition to collecting dust samples from cars, a questionnaire was given to each participant to elucidate those factors which may influence house dust mite populations within the car environment. Sampling was carried out using a battery powered pump, model Flite 2 made by SKC Inc. Air flow was set

to the maximum 26 L/min. Two samples, one from the driver seat and one from the child car seat were taken from each car. Samples were taken by vacuuming the entire surface area of each seat over a two minute period. Dust was collected in a plastic cassette containing a track-etched polycarbonate membrane filter (Whatman) of diameter 37 mm and pore size 0.4 µm, which was connected to the pump via plastic tubing. Dust samples were subsequently stored at -20°C to: a) kill any live mites present, thereby preventing artificially elevated numbers of mites as a result of breeding; and b) preserve collected mites until counting and identification commenced. House dust mite extraction was carried out using a modified approach described previously (Cameron and Hill, 2002). Mites were counted and carefully identified with the aid of identification keys (Baker, 1999; Colloff, 2009) under a phase contrast compound microscope at 100x magnification.

3.3.2 Der p 1 determination

In addition to house dust mite analysis, 71 samples (37 driver seats, 34 child car seats) with sufficient dust remaining (50 mg) were analysed for allergen content (Airmid Health Group Ltd.). Dust samples were extracted in phosphate buffer saline pH 7.4 containing 0.05% Tween 20 (PBS-T). Samples were centrifuged and supernatants obtained were stored at -20°C before being subjected to Group 1 *D. pteronyssinus* (Der p 1) allergen measurement using a two-site monoclonal antibody ELISA [24]. Four dilutions of each sample were added to the plate (1 : 2, 1 : 14, 1 : 98, 1 : 686). For determining the concentration of Der p1 in the samples, at least two points of the dilution series should fall within the linear part of the standard curve. The mean of these values was used as the final result for Der p 1 concentration. Results of the absorbance were expressed in micrograms per gram of dust (µg/g), with a detection limit of 0.025 µg/g for Der p 1.

3.3.3 Statistical procedures

Mite data were analysed using IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, 2011) and Minitab 16 Statistical Software (2010). Although individual mites were counted from each sample results for the purpose of reporting means, medians and exposure thresholds are expressed as mites/g of dust. At each combination of environmental and behavioural input variables used in the analysis mite counts per gram in a random gram of dust tended to have a Poisson distribution, so for the purpose of the inferential analysis it was decided to transform to a variable defined as the square root of mites/g of dust. Using this as a response variable throughout the entire analysis (rather than a direct Poisson or related regression technique), it was possible to conduct General Linear Model (GLM) procedures while adhering reasonably well to the assumptions on which these rely. Correlation coefficients and their significance were computed for several pairs of variables and GLM analyses were conducted to assess the effects of a number of input variables (environmental and behavioural factors) on the response variable.

3.3.4 Selection of input variables

One of the primary aims of this study was to determine the variables that influenced house dust mite populations in driver seats and in child car seats. Over 30 questions were included in a questionnaire in an attempt to obtain as much information as possible about the physical and behavioural characteristics of the participants which may have influenced mite populations. After preliminary analyses, these were reduced to four input variables for the purpose of the statistical analyses. These four variables were selected on the basis of existing knowledge of house dust mites where possible. The input variables that were used (separately) in the statistical analyses for the driver seat and child car seat data were as follows: 1) time spent by driver or child in the car per week; 2) age of the driver or child car seat; 3) time since the car was last vacuumed; 4) whether or not the driver or child was atopic (i.e. predisposed to developing hypersensitive allergic reactions). The variables concerning time spent in the car per week were partitioned into five categories: <1 hr; 1 - <3 hrs; 3 - <6 hrs; 6 - <10 hrs; and >10

hrs per week. The exact age, in years, of the driver seat was determined from the registration number plate on each car. However, only an approximate age (in years) of child car seats could be determined from the questionnaire to respondents. This was more difficult to determine since a number of child car seats were only a few months old whereas others were used by several children of the same or different families over many years. For this reason, child car seat age was divided into the following three categories for the purpose of data analysis: <1 yr; 1 - <3 yrs; and > 3yrs. For the input variable ‘time since the car was last vacuumed’ two categories < 2 months and \geq 2 months were chosen, which coincide closely with the natural lifecycle of a typical house dust mite at optimum conditions (Colloff, 2009).

3.4 Results

3.4.1 Descriptive statistics

Mites were identified to species level where possible but in some cases (due to missing appendages or imperfect specimens) were listed as members of a specific family or order. A total of 1060 specimens were found in 212 samples of dust, with over 12 species of mites from seven different families identified (Table 1). Nine out of the 12 species (75%) are known allergen producers (Müsken *et al.*, 2003; Colloff, 2009). The pyroglyphid house dust mite species *D. pteronyssinus* was the most predominant species with 830 specimens found in total. Records of the pyroglyphid species *Euroglyphus maynei*, the second most abundant species (71 specimens found) and *Dermatophagoides farinae* (seven specimens found) have, to the best of the authors’ knowledge, not been published previously for Ireland. Of the pyroglyphid species identified, various life stages were recorded including larvae (3%), protonymphs (9.1%) and tritonymphs (12.3%). Of the 347 female pyroglyphid specimens identified, 45 (13.0%) were gravid specimens (Table 2).

Chapter 3: Child car seats

Table 1. Species of mites found in the 106 cars sampled in this study.

Family/Order	Species	Specimens found		
		Driver seats	Child car seats	Total (% of total)
Pyroglyphidae	<i>Dermatophagoides pteronyssinus</i> *	464	366	830 (78.3%)
	<i>Dermatophagoides farinae?</i> *	4	3	7 (0.7%)
	<i>Euroglyphus maynei</i> *	38	33	71 (6.7%)
Acaridae	<i>Acarus farris</i> *	7	4	11 (1.0%)
	<i>Acarus</i> sp.	2	4	6 (0.6%)
	Acaridae (unidentified)	2	7	9 (0.8%)
	<i>Tyrophagus</i> sp. *	6	15	21 (2.0%)
	<i>Lepidoglyphus destructor</i> *	0	2	2 (0.2%)
Glycyphagidae	<i>Glycyphagus domesticus</i> *	1	7	8 (0.8%)
	<i>Gohiera fusca</i> *	2	2	4 (0.4%)
Cheyletidae	<i>Cheyletus</i> sp. *	8	4	12 (1.1%)
Tarsonemidae	tarsonemids (unidentified)	9	11	20 (1.9%)
Mesostigmata	mesostigmatids (unidentified)	8	5	13 (1.2%)
Oribatida sensu lato**	oribatids (unidentified)	0	1	1 (0.1%)
	unidentifiable desiccated mites	20	25	45 (4.2%)
Total		571	489	1060

* Indicates species known to produce allergens. Species marked with “?” indicate that although most taxonomic characteristics of that species were present, a defining feature was either missing or obscured, preventing complete confirmation of the species. ** The suborder Oribatida (formerly order Oribatida, now within the order Sarcoptiformes) has recently been re-classified to include the kohort Atigmatina, together with the families Pyroglyphidae, Acaridae and Glycyphagidae (Krantz and Walter, 2009). Hence, ‘Oribatida sensu lato’ refers to oribatids without astigmatid mites.

Table 2. Gender and life stages of identified pyroglyphid house dust mite specimens.

Species	Adult Male (% total)	Adult Female (% total)	Gravid Female (% females)	Protonymph (% total)	Tritonymph (% total)	Larva (% total)	Gender / life stage unknown (% total)	Total (% total)
Driver seats								
<i>D. pteronyssinus</i>	143 (30.8)	184 (39.7)	22 (11.0)	41 (8.8)	69 (14.9)	10 (2.2)	17 (3.7)	464 (91.7)
<i>E. maynei</i>	12 (31.6)	16 (42.1)	1 (0.5)	0 (0)	3 (7.9)	3 (7.9)	4 (10.5)	38 (7.5)
<i>D. farinae</i>	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (0.8)
Driver total	159 (31.4)	200 (39.5)	23 (11.5)	41 (8.1)	72 (14.2)	13 (2.6)	21 (4.2)	506
Child car seats								
<i>D. pteronyssinus</i>	125 (34.2)	134 (36.6)	22 (15.0)	41 (11.2)	38 (10.4)	10 (2.7)	18 (5)	366 (91)
<i>E. maynei</i>	13 (39.4)	13 (39.4)	0 (0)	1 (3)	2 (6.1)	4 (12.1)	0 (0)	33 (8.2)
<i>D. farinae</i>	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.7)
Child total	141 (35.1)	147 (36.6)	22 (15.0)	42 (10.4)	40 (10)	14 (3.5)	18 (4.5)	402
Overall	300 (33)	347 (38.2)	45 (13.0)	83 (9.1)	112 (12.3)	27 (3)	39 (4.3)	908

80.2% of 106 driver seats and 77.4% of 106 child car seats contained dust mites (78.8% of car seats overall). The median / mean \pm SD mites/g of dust for driver ($28.34 / 53.03 \pm 74.49$) and child car seats ($23.34 / 53.10 \pm 80.34$) were similar, although the variation between samples overall was large (Fig. 1). Of the driver and child car seats sampled, 12.3% and 15.1% respectively (13.7% overall) exceeded the accepted lower threshold for sensitisation of 100 mites/g of dust (Platts-Mills *et al.*, 1989). The maximum number of mites found in a driver seat was 61 (equivalent to 406.67 mites/g of dust) while the maximum found in a child car seat was 23 (equivalent to 426.72 mites/g of dust); the samples containing these mite numbers were taken from separate cars.

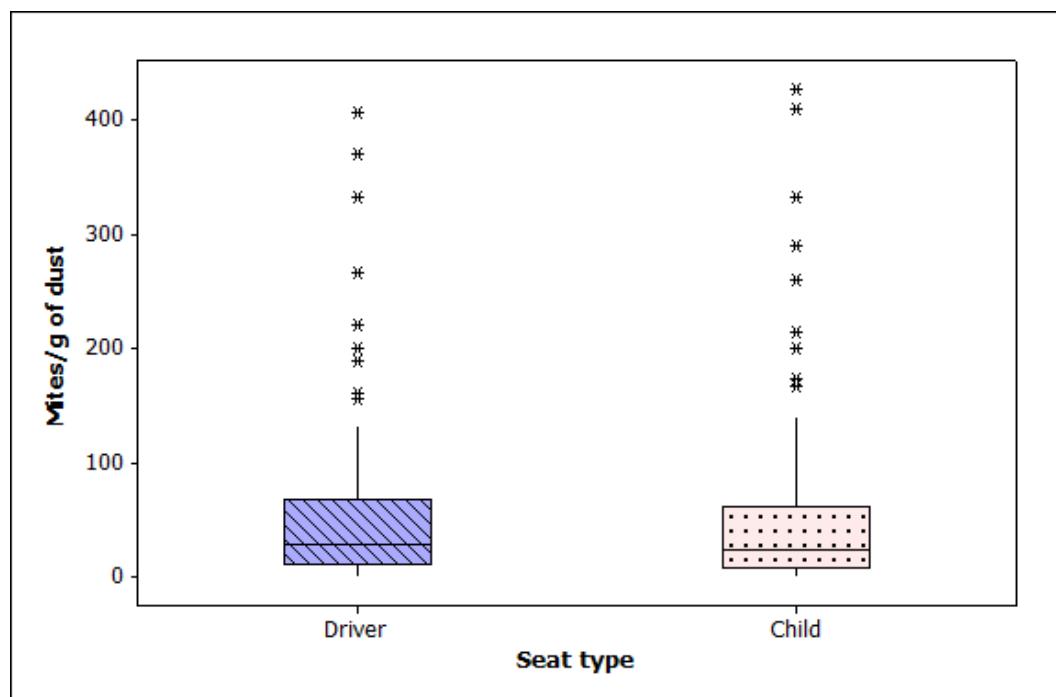


Fig 1. Mites/g of dust found in driver seats (median = 28.5, mean = 53.05) and child car seats (median = 23.5, mean = 53.11).

3.4.2 Der p 1 analysis

Of the 37 driver seats tested for Der p 1 concentrations, 34 (91.9%) had detectable levels of Der p 1, with a median / mean \pm SD concentration of $0.53 / 1.08 \pm 1.57 \mu\text{g/g}$ and a maximum concentration of $8.3 \mu\text{g/g}$. Similar concentrations were found in 34 child car seats tested, with 29 (85.3%) bearing detectable levels of Der p 1 with a median / mean \pm S.D. concentration of $0.43 / 1.12 \pm 1.87 \mu\text{g/g}$ and a maximum of $10.03 \mu\text{g/g}$. Of the driver seats tested, six (16.2%) were above the lower sensitisation threshold of $2 \mu\text{g/g}$ (Platts-Mills *et al.*, 1997). Five (14.7%) of the child car seats tested contained Der p 1 levels in excess of the lower sensitisation threshold, with one child seat exceeding the upper sensitisation threshold of $10 \mu\text{g/g}$. There was a significant correlation (Pearson correlation, $P = 0.001$) between the mites/g of dust recovered and the corresponding concentration of Der p 1 detected in each car seat tested (Fig. 2).

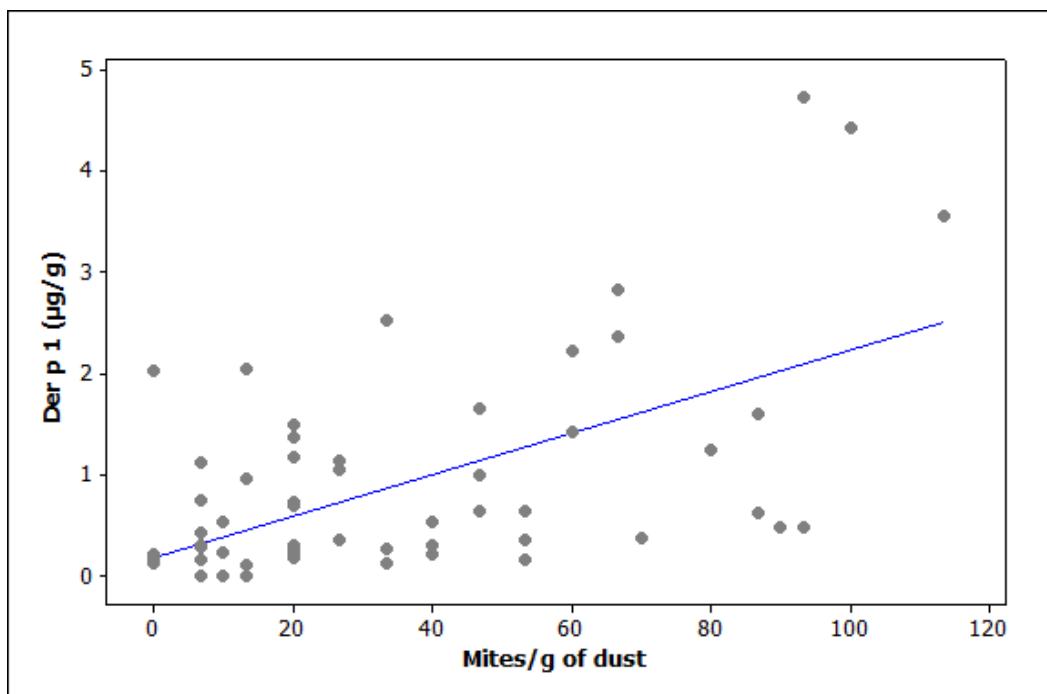


Fig 2. Correlation of house dust mites (mites/g of dust) with concentrations of Der p 1 allergen ($\mu\text{g/g}$) measured from 71 car seats (Pearson correlation, $P = 0.001$).

3.4.3 Inferential Analysis

A GLM was fitted to the driver and child car seat data using ‘square root of mites/g of dust’ as the response variable in each case. For the driver seat data, input variables 1 – 4 were used in the analysis i.e. 1) ‘time spent by driver in the car per week’; 2) ‘age of the driver seat’; 3) ‘time since the car was last vacuumed;’ and 4) ‘whether the participant was atopic or not’. The resulting p-values obtained from these analyses are displayed in Table 3. Input variable 1 ‘time spent by driver in the car per week’ showed a significant ($P = 0.010$) effect on the response variable, with more mites/g of dust recovered from seats where drivers spent 6-<10 hours per week in their car compared to 1-<3 hours (Tukey’s test $P = 0.017$). Also for driver seats, input variable 3 ’time since the car was last vacuumed’ showed a significant effect ($P = 0.049$, while input variable 4 ‘whether the participant was atopic or not’ showed a significant effect ($P = 0.042$) on the response variable for the child car seat data (Table 3 and Fig. 3). Although the GLM did not show any evidence of an effect of the age of child car seats on the response variable ($P = 0.181$) there is a slight upward trend, indicating that more mites were present with increasing age of the child car seats (Fig. 4). A significant positive correlation (Pearson correlation, $P = 0.000+$) existed between the mites/g of dust found in driver seats and child seats of the same car (Fig. 5), illustrating that, in general, if densities of mites were high in one seat in the car they were also high in the other seat. Based on this information, it was unsurprising that there was no significant difference (paired-samples t-test) between the mean mites/g in driver seats compared to that of child car seats of the same car ($P = 0.749$).

Table 3. General Linear Model effects of input variables (with p-values) on the square root of mites/g of dust recovered from driver seats and child car seats.

	Driver seats	Child car seats
Atopic person present	$P = 0.460$	$P = 0.042^*$
Seat age	$P = 0.242$	$P = 0.181$
Seat vacuumed	$P = 0.049^*$	$P = 0.513$
Time spent in car	$P = 0.010^*$	$P = 0.713$

* indicates a statistically significant p-value

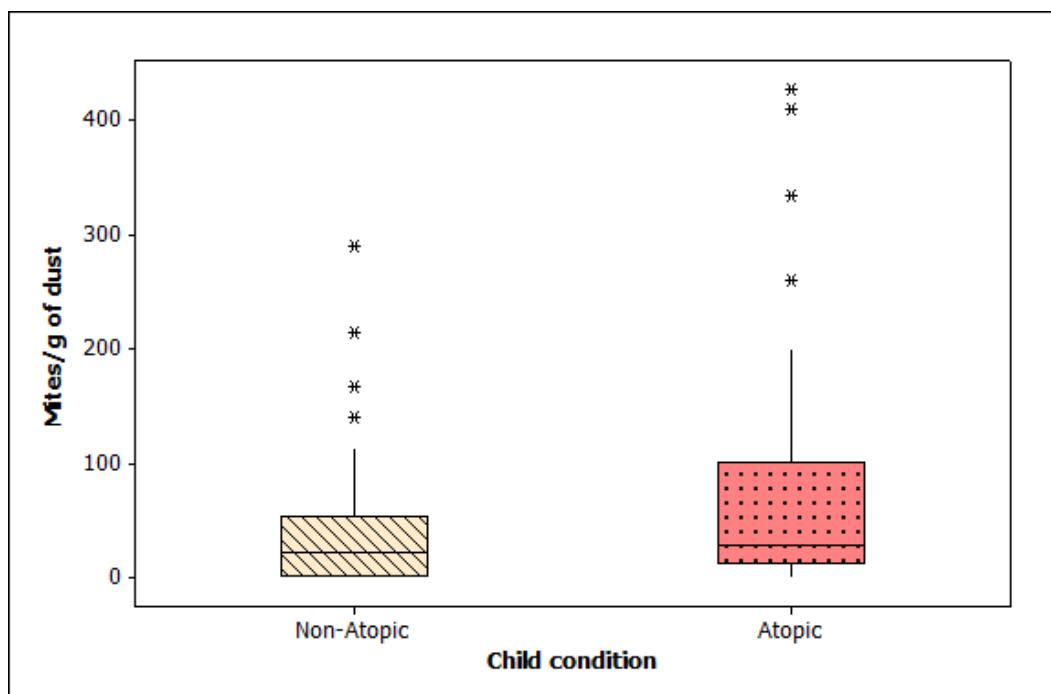


Fig 3. Mites/g of dust found in child car seats where the child was non-atopic (median = 21.10, mean = 40.25) and atopic (median = 28.34, mean = 80.30). Child condition showed a statistically significant effect on the square root of mites/g of dust in the General Linear Model analysis ($P = 0.042$).

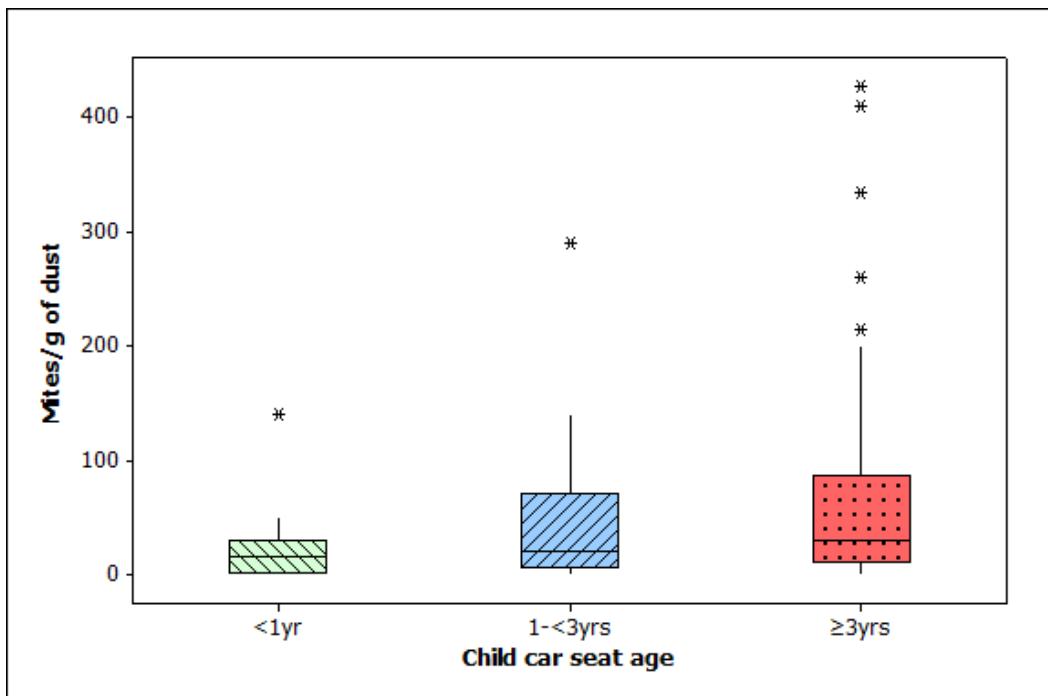


Fig 4. Square root of mites/g of dust for three child car seat age categories: <1 year (median = 15.12, mean = 26.05); 1-<3 years (median = 20, mean = 41.42); and ≥ 3 years (median = 30, mean = 67.28).

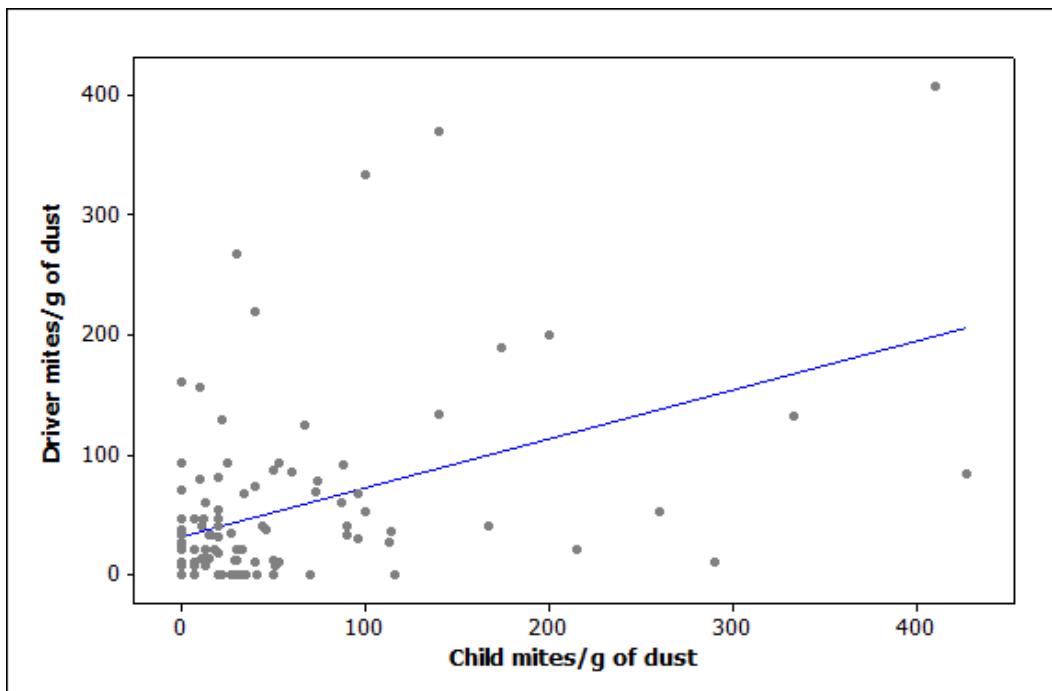


Fig 5. Correlation of square root of mites/g of dust with driver seats and child car seats of the same car (Pearson correlation, $P = 0.000+$).

3.5 Discussion

The presented study examined the presence of house dust mites in cars and to the authors' knowledge, is the first to make particular reference to child car seats as a reservoir for mites and their associated allergens. The presence of pyroglyphid larva, protonymph and tritonymph life stages in addition to gravid female specimens indicate that the mites recovered were breeding, resident populations living in the car seats as opposed to dead or desiccated specimens transferred from an indoor location via clothing or some other mechanism. Twelve species of mites were identified, of which nine are known to produce allergens associated with inducing sensitisation (Müsken *et al.*, 2003; Colloff, 2009). This highlights that while pyroglyphid mites such as *D. pteronyssinus*, *D. farinae* and *E. maynei* are probably the most important and widely studied mites from an epidemiological point of view, there are other allergen producing mites which could also play a role in inducing or aggravating atopic conditions. Recent discoveries of cross-reactivity allergens in *Tarsonemus* mites, which were found in this study, suggest that they may be important in triggering IgE antibodies in the human body (Xue-Ying *et al.*, 2011) while other species found such as *Acarus farris*, *Glycyphagus domesticus*, *Lepidoglyphus destructor* and *Cheyletus eruditus* have all been linked to inducing sensitisation (Müsken *et al.*, 2003). While these results show that there is a varied acarofauna present in car seats, the species composition is reflective of that found in typical homes (Solarz *et al.*, 1998; Colloff, 2009).

A mean of 53.03 mites/g of dust from 106 driver seats in this study fits within the range of 42.5 to 81.3 mites/g of dust found in 139 automobile driver seats in the USA (Neal *et al.*, 2002). Although mean Der p 1 levels were slightly lower (1.1 µg/g) than mean Der 1 levels in Ohio (1.3 µg/g), they exceed mean levels from 60 private cars in Brazil (0.24 µg/g) (Justino *et al.*, 1995). A significant correlation ($P = 0.001$) between mites/g recovered and Der p 1 allergen content was observed, a result similar to ones previously recorded in samples from sofas and bedding (Tera *et al.*, 2004). In addition, more mites were found in older child car seats than in newer ones, an observation analogous to similar studies conducted on mattresses (Mihrshahi *et al.*, 2002), which have found that in general older

mattresses contain more dust and bear higher concentrations of house dust mite allergen.

It is important to note that direct comparisons between this and other studies needs to be treated with caution given that: a) all studies consist of different sample sizes; b) the climates and geographical locations of each study site are different; and c) no other studies have previously sampled child car seats for house dust mite content. House dust mites have been reported to be present in higher densities in damp humid climates in contrast to dry, arid ones (Veale *et al.*, 1996) with populations known to fluctuate seasonally (Solarz *et al.*, 1998; Arlian *et al.*, 2001; Ćustović *et al.*, 1995; Platts-Mills *et al.*, 1997). While it must be noted that the majority of studies deal specifically with dust samples collected from homes and not from car seats, it is likely that the same basic requirements for survival such as suitable temperature and relative humidity apply. Sampling in this study took place in the summer months of May, June and July which are some of the warmer months of the year in Ireland and are likely to coincide with optimum growth conditions for mite proliferation (Marks *et al.*, 1995). It is probable that resident mite populations in car seats fluctuate seasonally also, with associated implications for sensitisation risks.

The input variables ‘time spent in the car per week’ and ‘time since the car was last vacuumed’ showed a significant effect on mite densities in driver seats. Cars where the drivers spent longer amounts of time in their cars resulted in more mites recovered ($P = 0.010$) whereas driver seats that were vacuumed within the previous two months had lower mite densities ($P = 0.049$) than those that were not vacuumed during this time. Although the input variables ‘time spent in the car per week’ and ‘time since the car was last vacuumed’ showed a significant effect on the response variable in driver seats, the same was not true for child car seats. The latter could be explained by the fact that in reality, the frequency of vacuuming for child car seats could indeed be higher than reported in questionnaires, as these seats are often subjected to vacuuming or washing after a wetting episode or spillages of food or drinks. The disruption caused by such events, in addition to the fact that child seats (especially infant carriers) are often removed temporarily

from cars after journeys or during vacuuming / washing, may also have had a bearing on the house dust mite densities of these seats.

There was a significant difference ($P = 0.042$) between the mean mites/g of dust recovered from seats of atopic children compared to that found in seats of non-atopic children, with higher mean mites/g recovered from the former (Fig. 3). Previous investigations have reported that mattresses of atopic dermatitis sufferers tend to have denser house dust mite populations compared to those of healthy non-atopics (Colloff, 2009), which also seems to be reflected in the results from this study, for children at least. A statistically significant correlation ($P = 0.000+$) was observed between the mites/g of dust found in child car seats and driver seats of the same car, suggesting that the conditions that influenced mite propagation within the car seats were common to both seat types. It may be possible that other variables not measured in this study, such as relative humidity and temperature, may play an important role in determining house dust mite proliferation in the microhabitat of the car seat fabrics.

3.6 Conclusions

This study confirms that child car seats are home to a range of species of house dust mites, which can be present in concentrations high enough to induce or aggravate allergen associated conditions diseases or symptoms, especially in children who are genetically predisposed. Under EU law, children under the age of 12 are required to use the appropriate child restraint in cars, an environment which until now had previously been unexplored with regard to house dust mite populations. In order to minimise exposure to house dust mites and their allergens, it is recommended that car seats are subjected to regular vacuuming, a process which has been shown to be effective in removing mites and allergens from carpets (Arlian *et al.*, 2001). Older child car seats had higher mean house dust mite densities than newer seats, which suggests that frequent replacement or washing of child car seat covers may be required in cases where a child is genetically predisposed towards hypersensitivity due to the inhalation of house dust mite allergens. While washing clothes in hot water at temperatures $> 55^{\circ}\text{C}$ has been found to be effective at removing mites and allergens (Platts-Mills *et al.*,

1997), it is not yet clear whether this is effective for car seat covers. Recent research involving the irradiation of house dust mite cultures with UV-C light has shown effective mortality of adults and reduction in hatchability of eggs (Lah *et al.*, 2012), though this has not been tested on resident mite populations in their natural environments. The findings of this research should instigate future studies in an attempt to understand further the environmental conditions which play a role in house dust mite propagation in child car seats, with a view to developing mitigation measures to abate or eradicate populations to help reduce instances of allergen associated conditions.

3.7 Acknowledgements

The authors would like to express gratitude to Owen Doherty, Erica Dix and Kevin McCaffrey for their help in the collection of dust samples and to all of the willing parents who made this study possible. Also a special thanks to Airmid Health Group Ltd. who performed the allergen analysis on dust samples and to the NUI Galway College of Science Fellowship for providing the funding to carry out this research.

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Chapter 4:

Dynamics of house dust mite transfer in modern clothing fabrics

This paper has been accepted for publication in the journal *Annals of Allergy, Asthma and Immunology* (January 2015)

4. Dynamics of house dust mite transfer in modern clothing fabrics

4.1 Abstract

House dust mites are one of the principal causes of allergic sensitisation in people globally. Clothing is largely presumed as being the mechanism by which house dust mites are distributed between locations in a home, yet little research to date has investigated the capacity with which various clothing fabric types serve as vectors for their accumulation and dispersal. While it has been demonstrated that car seats provide habitats for house dust mite populations, the dynamics involved in the transfer of mites to clothing via car seat material is still unknown. To investigate this, car seat material was seeded with both live and dead house dust mites and subjected to contact with plain woven cotton, denim and fleece. Contact forces equivalent to the mass of a typical adult and child were administered for different periods of time. Fabric type, mite condition (live or dead) and the force applied all had a significant effect on the transfer efficiency of house dust mites from car seat material to receiving fabrics, while duration of contact was found to have no effect. In particular, mean numbers of mites transferred to fleece (compared to denim and woven cotton) were greater for each treatment. These findings demonstrate that clothing type can have important implications for the colonisation of other biotopes by house dust mites, with potential for affecting an individuals' personal exposure to house dust mite allergens.

Key words: house dust mites, clothing, fabrics, contact, transfer, applied force.

4.2 Introduction

Allergic sensitisation to house dust mites is a worldwide problem (Platts-Mills *et al.*, 1997; Portnoy *et al.*, 2013; Arroyave *et al.*, 2014). Mattresses, carpets and upholstery serve as the primary habitats for house dust mites – areas where their main dietary component i.e. human skin scales accumulate easily. To date, a wealth of research examining the presence and density of house dust mites in such habitats has been conducted (Solarz, 1998; Halken *et al.*, 2003; de Oliveira *et al.*, 2003; Sercombe *et al.*, 2007). Presence and quantities of house dust mites have also been examined in private vehicles (Justino *et al.*, 1995; Neal *et al.*, 2002; Clarke *et al.*, 2014) while public transport vehicles such as buses (Ćustović *et al.*, 1996), trains (Uehara *et al.*, 2000), aeroplanes (Wickens *et al.*, 1997) and taxis (Taketomi *et al.*, 2006) have all been identified as important sources of house dust mite allergen exposure.

House dust mites are also present in clothing, which has been identified as the most likely mechanism by which mites colonise other locations and are dispersed throughout the home (Colloff, 1987; Mollet and Robinson, 1996). The potential of clothing to harbour large populations of house dust mites was demonstrated by Bischoff and Fischer (1990) who recovered over 30,000 live specimens from a single knitted jacket using the heat escape sampling method. In Australia, a mean density of 8.6 live mites per 100 cm² was recovered from eight out of 15 clothing items also using the heat escape sampling method, while a mean quantity of 15.9 ug/g of Der 1 allergen was reported from 35 clothing items (Tovey *et al.*, 1995). Teplitsky *et al.* (2008) reported mites from all samples taken from clothing and bedding of 19 children with atopic dermatitis in Israel, listing a range of species present.

Increased personal house dust mite allergen exposure through clothing has been observed previously (De Lucca *et al.*, 2000), with woollen garments shown to contain higher levels of Der p 1 than other fabrics (Siebers *et al.*, 1996; De Lucca *et al.*, 2000). In contrast, a US study recovered low densities of house dust mites and Der 1 allergen content from clothing (Neal *et al.*, 2002), but noted that 70% of

the individuals whose clothing samples contained mites also had mites in their automobiles and homes. Trials using stained house dust mites to examine their dispersal in a home revealed that specimens released on a couch appeared on children's clothing via contact with the couch after three hours, while also dispersing to the family vehicle within 24 hours (Mollet and Robinson, 1996).

A recent investigation found that over 80% of child car seats (Clarke *et al.*, 2014) sampled in a survey contained mites, while over 15% contained mite densities above the lower threshold level of 100 mites/g of dust (Platts-Mills *et al.*, 1989), highlighting the importance of a previously neglected focus for house dust mite populations. Although it is likely that these populations were originally established from indoor biotopes via clothing, it is also possible that the reverse is true i.e. that car seats serve as important biotopes for contaminating clothing with house dust mites. Contact between clothing and car seats, either in an instantaneous capacity or over longer time periods, may have the propensity to transport mites between biotopes within the home or elsewhere, as well as contributing to an individual's personal allergen exposure. Recent research has suggested that the traditional view of greater house dust mite allergen exposure at night when resting in bed may need to be reviewed, as greater levels of personal allergen exposure were detected in association with domestic activity and proximity to other people during the day rather than in bed (Tovey *et al.*, 2013). Personal allergen exposure is also likely to be influenced by the type of clothing worn by individuals, as everyday clothing materials often vary considerably with regard to fabric type. In addition, it is not known whether properties of specific clothing fabrics including weave pattern and pile structure affect the capacity of a material to transfer house dust mites.

The capacity at which different fabric types serve as vectors in the dispersal of mites has, to the best of the authors' knowledge, not yet been examined. McDonagh *et al.* (2012) investigated particle-borne contamination by surface-to-surface contact between soft and hard surfaces including cotton, synthetic fleece, plastic laminate and brass. They reported contact transfer efficiencies ranging from 2 to 45% depending on the type of the surfaces in contact, observing an

increase in the mass of particles transferred with increased surface roughness. Transfer efficiencies of particles were also affected by the force applied using weights (0.15 kg - 10.56 kg) between the two surfaces, with contact time having little or no effect.

In an attempt to explore the transfer of house dust mites through contact with fabrics in a practical setting, car seat material (100% polyester) was seeded with a known quantity of house dust mites. These were then subjected to contact with three uncontaminated or ‘clean’ fabric types used commonly in everyday clothing, consisting of distinctly different weave properties and pile structures. Experiments were carried out under controlled laboratory conditions. The combined effects of fabric type, the contact force applied and the duration of contact were observed to determine the effects of these variables on the transfer efficiencies of house dust mites between a typical car seat environment and modern clothing fabrics.

4.3 Materials and Methods

For each experimental treatment car seat cover material (Fig. 1a) made of 100% polyester (dimensions 15cm x 15cm) was marked with a grid of 36 (4cm²) squares. A single house dust mite specimen (*Dermatophagoides pteronyssinus*) was placed at the centre of each square using a fine dissection needle. Each contaminated piece of car seat fabric was subjected to contact with a receiving uncontaminated fabric, examined prior to contact to ensure the absence of mites. Receiving fabrics consisted of the following three materials, which are common constituents of modern day clothing and also vary quite considerably with regard to their microtopography: a) plain woven cotton (Fig. 1b) – high quality Egyptian cotton (thread count of 400) with a smooth surface topography; b) denim (Fig. 1c) – a twill weave with thicker threads, more durable than plain weave and consisting of a more undulating surface topography; c) fleece (Fig. 1d) – a dense, synthetic fibrous material with a deep pile length of 3-4 mm.

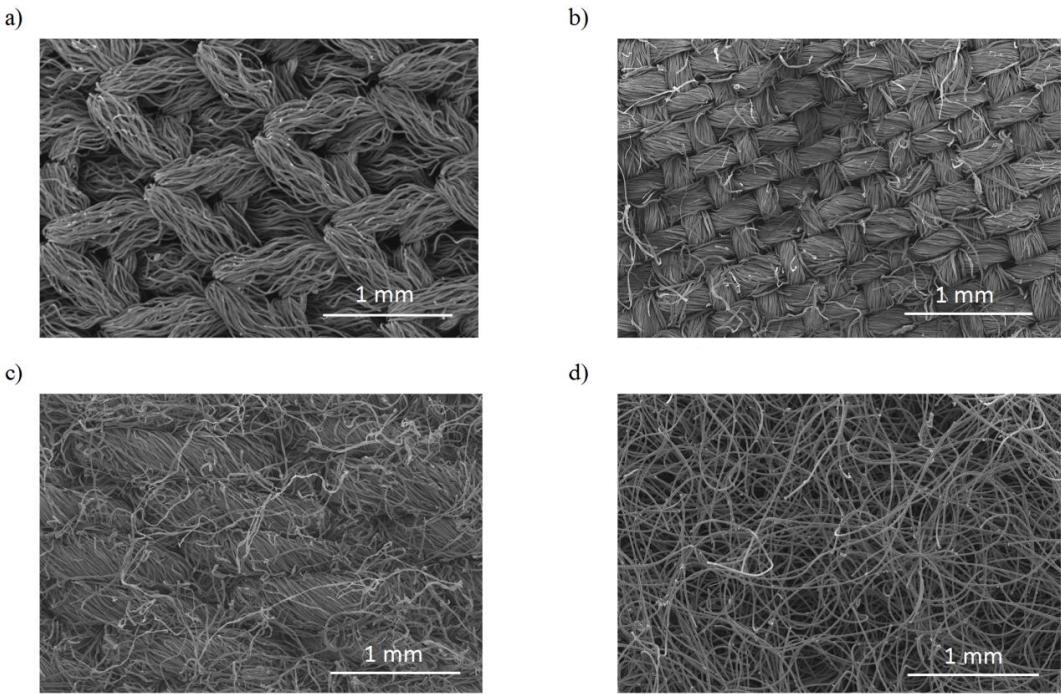


Fig. 1. Scanning electron micrographs of the various fabric types used in the experiment. **a)** car seat cover material (100% polyester) **b)** Plain woven cotton (100% Egyptian cotton) **c)** Denim (100% cotton) **d)** Fleece (100% polyester).

Each of the three receiving fabrics was subjected to contact with the contaminated car seat material under two different forces and for two different time periods. The forces applied were chosen to replicate: (i) the approximate force exerted by a child (aged 3 years) sitting in a child car seat (i.e. 50N); (ii) the approximate force exerted by an adult sitting in a car seat (i.e. 75N). These values were deduced from average age-weight percentile charts (National Center for Health Statistics, 2000), with the values modified to account for the ratio of standing - to - sitting masses for both adults and children. These ratios were established in the current study from convenience sampling of both adults and children. Each applied force scenario was performed for two different time durations: (i) an instant contact period of 3 seconds approx.; and (ii) a sustained contact period of 20 minutes. The first time period was chosen to replicate the act of a person sitting in a seat and quickly getting up again, while the second sustained contact period was chosen with reference to the average daily commuting time of citizens in Europe (National Travel Survey 2012, UK). Experiments were undertaken separately

with: (i) dead mites and (ii) live mites, as both are encountered in a natural setting (Arlian *et al.*, 1982; Neal *et al.*, 2002; Clarke *et al.*, 2014). For treatments using live mites, the perimeter of the experimental area was painted with a barrier fluid known as *Insect-a-slip* (BioQuip Products, Inc.) which, on the basis of preliminary trials, prevented the mites escaping from the experimental area. Each treatment (each combination of mite condition, fabric type, applied force and duration of contact) was replicated 20 times, resulting in 480 observations in total. Fabrics were attached to a polystyrene foam sheet which was attached on its lower surface to metal plate (Fig 2), the function of the foam being to replicate a typical car seat as closely as possible, while also allowing for compression of the plates to achieve the desired contact forces. When aligned correctly, the fabrics were then subjected to contact with each other using an axial torsion tester (Instron 8874, Fig. 2), with which the desired force and duration of contact could be programmed for each treatment in the experiment. Mites were subsequently counted on: (i) the receiving fabric and (ii) the original contaminated fabric to ensure account was taken of all seeded mites.

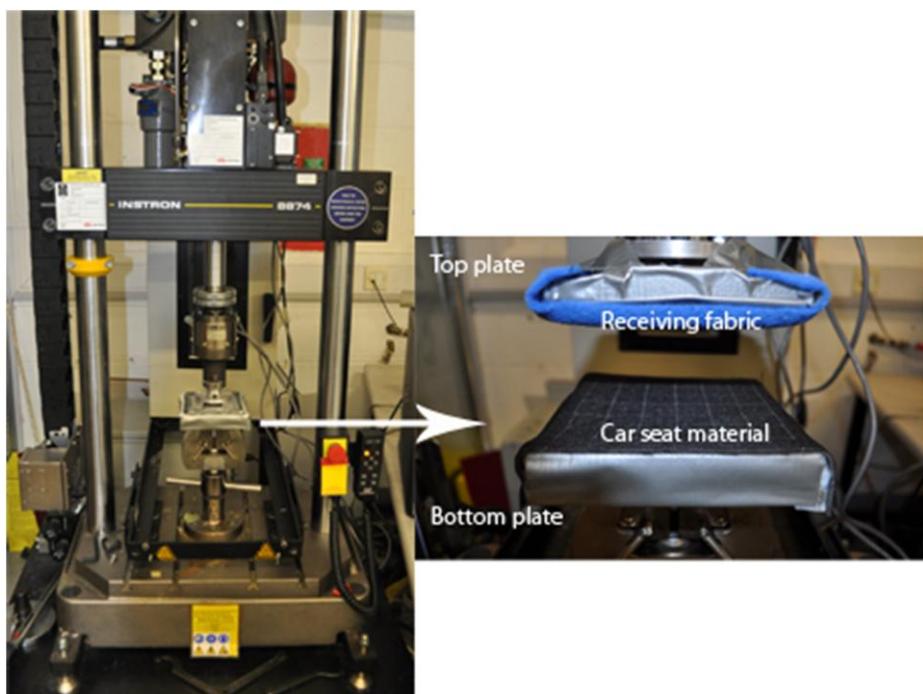


Fig. 2. Axial torsion tester (Instron 8874) consisting of two contact plates to which was attached the contaminated car seat fabric (bottom plate) and the receiving fabrics plain woven cotton, denim and fleece (top plate).

4.4 Results

4.4.1 Descriptive analysis

Mean transfer efficiencies for various treatments (Table 1) ranged from 7.2 % (live mites transferred to denim under a force of 50 N for 3 seconds) to 19.1% (dead mites transferred to fleece under a force of 75N for 20 minutes). With the exception of three treatments using plain woven cotton, the mean number of dead mites transferred to fabrics in each treatment was greater than those for live mites (Table 1). For both dead and live mites, mean numbers of mites transferred to fleece were greater than those transferred to plain woven cotton and denim (Fig. 3).The mean number of mites transferred under an applied force of 75N was greater than the number transferred under 50N for both dead and live mites (Fig.4). Finally, mean dead mites transferred after 20 minutes of contact were greater than for 3 seconds but for live mites, slightly greater mean numbers were transferred after 3 seconds than after 20 minutes (Fig. 5).

Table 1. Mean \pm SD (percentage transfer efficiencies) of house dust mites transferred to each fabric type under each treatment parameter tested. Force applied; 50N (F1); 75 N (F2) Duration of contact = 3 seconds (T1); 20 minutes (T2).

		Plain woven cotton	Denim	Fleece
Condition	Treatment	Mean \pm SD (% transferred)	Mean \pm SD (% transferred)	Mean \pm SD (% transferred)
Dead	F1, T1	3.0 \pm 1.2 (8.3)	3.4 \pm 1.0 (9.4)	6.4 \pm 1.7 (17.8)
	F1, T2	3.3 \pm 1.6 (9.2)	3.9 \pm 1.3 (10.8)	6.3 \pm 1.8 (17.5)
	F2, T1	3.4 \pm 1.0 (9.4)	4.2 \pm 1.6 (11.7)	6.1 \pm 1.9 (16.9)
	F2, T2	4.1 \pm 1.3 (11.4)	5.2 \pm 1.5 (14.4)	6.9 \pm 2.0 (19.1)
Total		3.4 \pm 1.3 (9.4)	4.2 \pm 1.5 (11.7)	6.4 \pm 1.8 (17.8)
Live	F1, T1	3.0 \pm 1.4 (8.3)	2.6 \pm 1.1 (7.2)	4.3 \pm 1.5 (11.9)
	F1, T2	3.4 \pm 1.3 (9.4)	2.8 \pm 1.3 (7.8)	4.2 \pm 1.4 (11.7)
	F2, T1	3.6 \pm 1.6 (10.0)	3.1 \pm 1.6 (8.6)	5.6 \pm 1.8 (15.6)
	F2, T2	3.2 \pm 1.4 (8.9)	3.1 \pm 1.5 (8.6)	4.9 \pm 2.0 (13.6)
Total		3.3 \pm 1.3 (9.2)	2.9 \pm 1.4 (8.1)	4.7 \pm 1.7 (13.1)

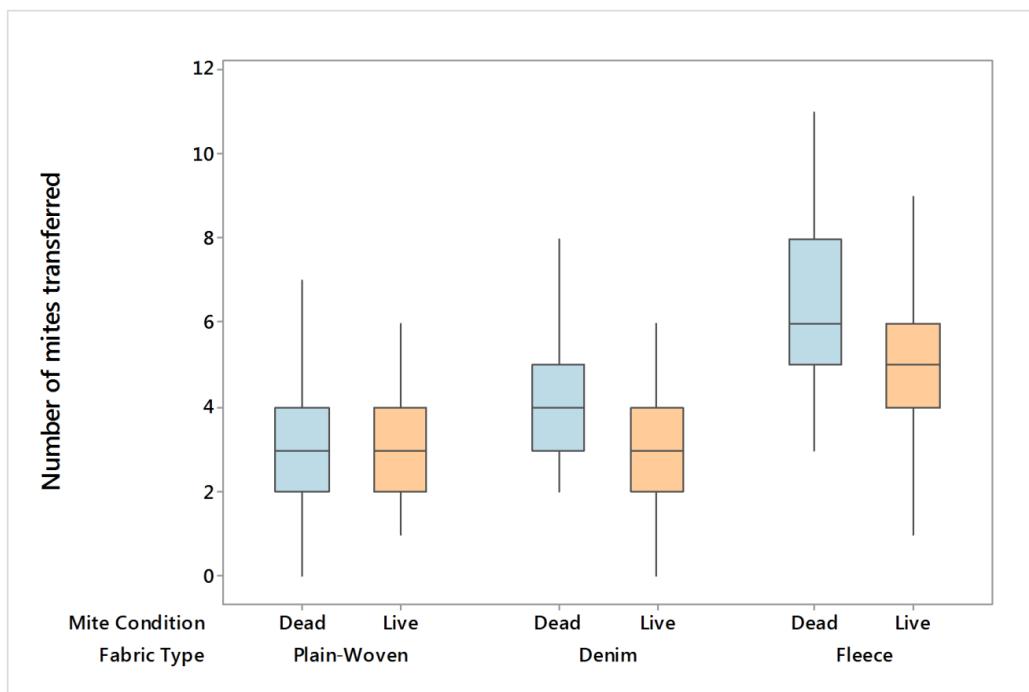


Fig.3 Dead and live mites transferred to each fabric type. Dead mites: Plain woven cotton (mean = 3.4 ± 1.3 S.D.); Denim (mean = 4.2 ± 1.5 S.D.); Fleece (mean = 6.4 ± 1.8 S.D.) Live mites: Plain woven cotton (mean = 3.3 ± 1.3 S.D.); Denim (mean = 2.9 ± 1.4 S.D.); Fleece (mean = 4.7 ± 1.7 S.D.).

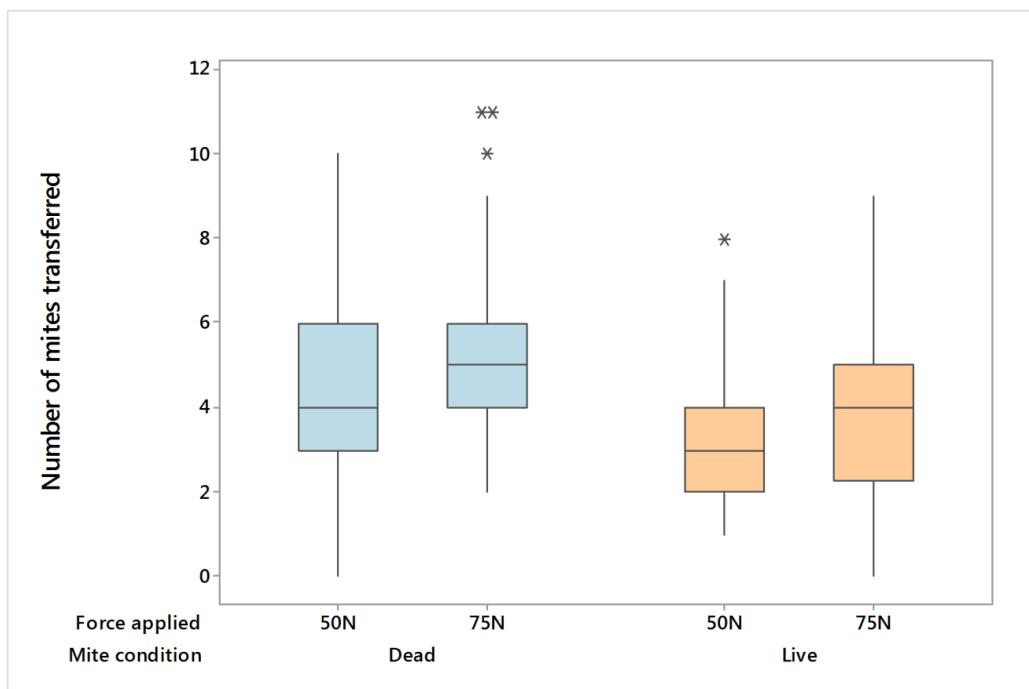


Fig. 4. Dead and live mites transferred to receiving fabrics according to each force applied. Dead mites: 50 N (mean = 4.4 ± 2.0 S.D.); 75 N (mean = 5.0 ± 2.0 S.D.) Live mites: 50 N (mean = 3.4 ± 1.5 S.D.); 75 N (mean = 3.9 ± 1.8 S.D.)

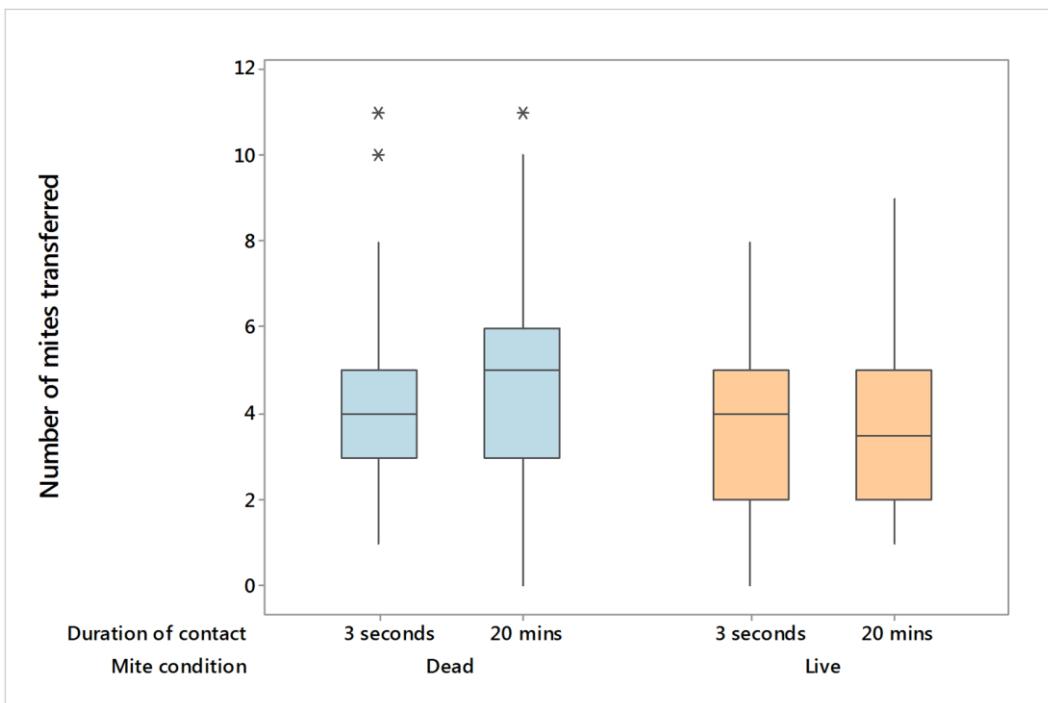


Fig. 5. Dead and live mites transferred to receiving fabrics according to duration of contact. Dead mites: 3 seconds ($\text{mean} = 4.4 \pm 2.0 \text{ S.D.}$); 20 minutes ($\text{mean} = 4.9 \pm 2.1 \text{ S.D.}$) Live mites: 3 seconds ($\text{mean} = 3.7 \pm 1.7 \text{ S.D.}$); 20 minutes ($\text{mean} = 3.6 \pm 1.6 \text{ S.D.}$).

4.4.2 Inferential analysis

Data were analysed using the statistical package Minitab 16 Statistical Software (2010). General Linear Model (GLM) analyses were used to postulate a model for the number of mites transferred as a function of a number of input variables. As the validity of this model requires homogeneity of variances between the means for the different treatments, the response variable ‘number of mites transferred’ was first transformed to the ‘square root of the number of mites transferred’, then modelled using following input variables: ‘Mite Condition’(i.e. dead and live), ‘Fabric Type’(i.e. plain woven cotton, denim and fleece), ‘Force Applied’(50N or 75N) and ‘Duration of Contact’ (3 seconds and 20 minutes). This model examined the effect of every possible combination of factors on the response variable i.e. the square root of the number of mites transferred, to determine which of the factors tested had a significant overall effect on the response variable. Additionally, this model was used to test for the presence of any interactions between the factors used.

The resulting main effects output from the GLM analysis is shown in Table 2. The input variables ‘Mite condition’, ‘Fabric type’ and ‘Force applied’ all showed significant effects on the response variable. There was, however, no observed effect of ‘Duration of contact’ on the response variable. Further post-hoc analyses (Tukey pairwise comparisons) were conducted on the three levels of the factor ‘Fabric type’, which showed a significant difference between the overall mean number of mites transferred to plain woven cotton and to fleece and between the mean number of mites transferred to denim and to fleece (Table 2).

Table 2. Portion of the general linear model output showing the P-values associated with each main effect and the 95% confidence intervals (CI) for pairwise comparisons (difference in population means) of the levels of each factor.

Variables	Factor levels	P-value ^a	95% (simultaneous) CI
Mite condition			
Dead - Live	2	P = 0.000	0.26 ± 0.07
Fabric type			
	3	P = 0.000	
Pairwise comparisons for fabric			
Plain woven cotton - Denim		P = 0.708	0.03 ± 0.10
Plain woven cotton - Fleece		P = 0.000	0.53 ± 0.10
Denim - Fleece		P = 0.000	0.50 ± 0.10
Force applied			
75N - 50N	2	P = 0.000	0.14 ± 0.07
Time of contact			
20 minutes – 3 seconds	2	P = 0.197	0.05 ± 0.07

^a Significant P-values are given in bold.

The GLM also concluded that there was an interaction between the factors ‘Mite Condition’ and ‘Fabric Type’ (Table 3). It is important to note the presence of interactions between factors in this type of a model, as it may be the case that the change in the expected number of mites transferred when we move from one level of a factor to another may depend on the level of another factor in the analysis. Hence, presence of an interaction between factors can potentially lead to an incorrect conclusion in the main effects output. In this case, the rate of change between the number of dead mites and the number of live mites transferred to plain-woven cotton was significantly different from that of denim, concluding a significant interaction was present. However, on examining the main effects plots, the veracity of the relevant p-values of the main effects in Table 2 could be confirmed, concluding that presence of this interaction did not have an overall influence on the factors involved.

4.5 Discussion

This study reveals that the quantity of house dust mites transferred from car seat material was determined by a number of variables. Among the three receiving fabrics used, fleece exhibited the highest mean transfer efficiency with both dead mites (17.8%) and live mites (13.1%), compared to 11.7% / 8.1% respectively for denim and 9.4% / 9.2% respectively for plain woven cotton (Table 1). The differences in mean transfer efficiencies between fabrics are most likely explained by the contrasting micro-topographies of each fabric. Fleece consists of a relatively complex network of polyester fibres (Fig. 1d), providing a greater capacity to ‘pick up’ and transfer house dust mites when placed in contact with an area of contaminated car seat material. A similar phenomenon was observed by McDonagh *et al.* (2012) who reported fleece as having a greater capacity to accumulate particulate contaminants than other materials such as cotton and plastic laminate, most likely due to the higher fibre pile present. Denim, a hard wearing fabric, consists of a twill weave pattern of diagonal parallel ribs of cotton threads resulting in an undulating, exposed topography. When viewed through SEM imaging (Fig. 1c), it is evident that many individual strands of cotton appear to become undone, creating an almost ‘fuzzy’ like appearance. Similar to fleece, the presence of these ‘loose’ fibres may enhance the ability of bodies of house

dust mites to adhere to denim, though evidently not to the same degree as fleece. The plain woven cotton presents a very tight weave consisting of very few loose strands (Fig. 1b) resulting in a smooth, even topography which is likely to be the reason why less mites were transferred to this fabric.

With the exception of plain woven cotton, a greater mean number of dead mites were transferred than live mites for each treatment investigated. This may be attributed to instinctive capabilities that living mites possess to survive in their environment. *D. pteronyssinus* has sucker-like appendages or pulvilli at the ends of its legs (Fig. 6), possibly aiding it to avoid transference by gaining a strong foothold on the substrate (Colloff, 2009). Since dead mites would not possess this ability, the lack of resistance to transfer may explain this result. It was observed on a number of occasions that after seeding, individual mites would move about on the fabric surface until finding a depression in the fabric, sometimes even managing to burrow underneath an individual thread. Since this did not happen with dead mites, it may also help explain why mean transfer efficiencies were higher with dead than with live mites. Although the movement of live mites within the test area could not be accounted for once they were seeded, this is nonetheless representative of normal population dynamics in a natural setting. House dust mites behave like any other arthropod in the animal kingdom, with priorities which include searching for food particles and potential mating opportunities. Temperature and humidity are vital to the survival of house dust mites (Spieksma, 1997; Hart, 1998) and house dust mites in mattresses are known to burrow to prevent water loss (Colloff, 2009). It is likely that the same principles for survival also apply in car seats, which may explain the burrowing behaviour of some live mites in this study.

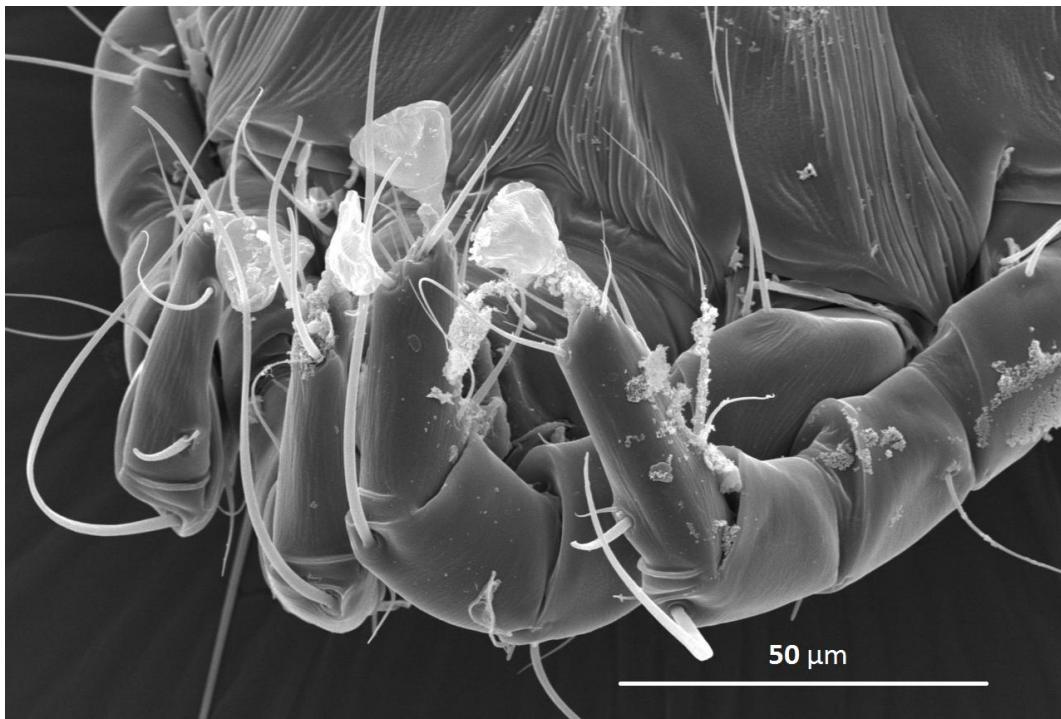


Fig. 6. Scanning electron micrograph of *D. pteronyssinus* showing pulvilli at ends of legs. These appendages enable the species to gain a stronger foothold in the substrate.

As reported in the GLM output, there was a significant difference between the number of mites transferred between an applied force of 50N and 75N, with greater mean abundances transferred at the latter factor level. This suggests that small changes in force can affect the transference of house dust mites between car seats and clothing materials. Similar observations in relation to the impact of applied forces on transference of deposited aerosol particles have been reported previously (McDonagh *et al.*, 2012), while also noting that applied forces had different effects on transference of these particles depending on the type of contact fabric / material involved. The inferential analysis in this study concluded that there was no overall effect of the duration of contact on the number of mites transferred from car seat material to the receiving fabrics. This result is synonymous with other studies including the examination of the transfer of the bacteria *Salmonella typhimurium* from tiles, wood and carpet to food (Dawson *et al.*, 2006) and the transfer of particulate matter between different surfaces (McDonagh *et al.*, 2012), both of which reported contact times to have no effect on the transfer efficiencies of their respective experimental subjects.

Although the aim of this study was to replicate, as closely as possible, a natural setting by which the transference of house dust mites to modern clothing fabrics could be assessed, some limitations of the study should be mentioned. In natural settings, mite populations can vary substantially and are governed by a host of external variables including substrate type (Colloff, 1998), temperature and relative humidity (Spieksma, 1997; Hart, 1998; Arlian *et al.*, 1999), food availability (Colloff, 1998) and species-species interactions (Colloff, 2009). Additionally, populations in nature consist of a mixture of both live and dead mites (Arlian *et al.*, 1982; Neal *et al.*, 2002). As an attempt to accurately represent a natural house dust mite population would invariably be inaccurate, it was decided that using dead mites and live mites in separate treatments ensured reproducibility of the method. While there were significant effects on transfer efficiencies between the applied contact forces of 50N and 75N, it remains unclear whether this trend is likely to continue with increasing or decreasing applied forces. Similarly, although this experiment showed no effect of ‘Duration of contact’ on transfer efficiencies, these focussed on two factor levels only, making no inferences for contact durations between three seconds and 20 minutes or more than 20 minutes. Although three common constituents of modern clothing were examined, other synthetic and natural clothing fabrics such as nylon and wool were not, which leaves scope for investigation in future studies.

4.6 Conclusions

Despite the above, this research sheds new light on understanding the dynamics involved regarding the transfer of house dust mites via clothing, which may have the propensity to transport and establish mite populations in uncontaminated textiles and furnishings of human environments. Subject to temperature and relative humidity optima, Matsumoto *et al.* (1986) revealed population doubling times of 24.3 days for *D. pteronyssinus*. Transfer efficiencies for live mites of up to 11.9% were observed in this study, which implies that mite populations could be established relatively quickly in clothing subjected to contact with surfaces bearing mite populations, though the rate of population growth would likely depend on the initial population size transferred and other governing factors such as food availability, temperature and relative humidity.

From the point of view of sensitisation, the presence of dead mites in car seats and their subsequent transference to clothing may be just as important as live mites. Allergens produced by dust mites are known to persist in the environment for up to several months until they are eventually broken down, presumably by microbial decomposition (Colloff, 2009). The allergen pool created by the mites when living may still remain in the mite's bodies and their immediate microenvironment for some time after death, hence presenting a sensitisation risk. Recent research by Tovey *et al.* (2013) identified clothing as a probable source of significant daily allergen exposure, although a re-evaluation of quantities of both allergen and mite bodies as risk factors for sensitisation in this context is required. Nonetheless, this research reveals that house dust mites are transferred to fleece more readily than to plain woven cotton or denim, a point which sensitised persons may need to be aware of in the context of potentially increased allergen exposure associated with this fabric type.

In conclusion, it is recommended that future investigations should determine the effects of fabric type, applied force and duration of contact at more factor levels than covered in the scope of this study. Different mite loadings and other contaminated material types such as those derived from house furniture, carpets and bedding should be investigated with regard to mite transference to uncontaminated fabric types. Finally, the factors involved in the transportation of house dust mite allergens as well as the mites bodies themselves should be assessed.

4.7 Acknowledgements

The authors would especially like to thank Liam Brennan at the National Centre for Biomedical Engineering Science, NUI Galway for the use of the Instron machine, and Kerry Thompson and all the staff at the Centre for Microscopy and Imaging, NUI Galway for assisting in the capture of the SEM images used in this article. Also a special thanks to the National University of Ireland Galway College of Science Fellowship, for providing the funding to undertake this research.

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Chapter 4: Transfer of dust mites

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Chapter 5:

General Discussion

5. General Discussion

While dust mites have been studied extensively by numerous researchers throughout the world over the last fifty years or so, some key gaps in the knowledge have persisted. Until now, there had been no comprehensive research on dust mites in Ireland, while many aspects of dust mite research in relation to cars, domestic pets and clothing have been understudied. The purpose of this study was to finally address some of the unknowns in this field, while it is hoped that the results obtained will form a substantial foundation for future research. This section first discusses some of the limitations of the overall study, in addition to outlining future research recommendations concerning dust mites and their associated allergens. Finally, the key findings from the research as a whole are presented along with some concluding reflections.

5.1 Limitations of the study

From the outset of this research, every effort was made to ensure that crucial aspects such as study design, collection of data and the interpretation of these data were executed to the highest standard possible. Despite this, certain limitations were experienced and they will now be addressed.

1. Questionnaire interpretation

In addition to gathering mite and allergen data from dust samples described in Chapters 2 and 3 of this thesis, questionnaires were also implemented in an attempt to gain as much information as possible about various environmental, structural and behavioural factors which may have to some degree, had a bearing on dust mite quantities or species composition (see Appendix I and II). In both studies, over 30 questions were included in the questionnaire covering a broad spectrum of topics in relation to the aforementioned factors. Although the initial intention was to use GLM analyses on the questionnaire data gathered in Chapter

2, this was eventually decided against due to concerns over an insufficient sample size. Therefore, a chi-squared analyses was used instead, while some questionnaire data were used for descriptive statistics in the results of that chapter.

In the case of the study in Chapter 3, there was a sufficient sample size to use GLM analyses, although the compilation of these data for use in the analyses did create challenges. Most of the sampling for this research study took place outside schools at drop off and pick up times. In the interest of time management, questionnaires were issued to participants while sampling was carried out simultaneously. Although the participant always had an assistant to aid in completing the questionnaire, there were instances where participants either did not complete the form fully due to time restraints, or deliberately left certain answers blank for various reasons. Where some data were omitted, these variables had to be discarded entirely due to an uneven number of dust samples and relevant questionnaire data pertaining to those samples. Similarly, responses for certain questions where ranges were used e.g. a range of times spent sitting in a car per week , were in some cases either not selected at all by any participants or had contained too few observations to be included in the statistical models. Therefore, these were in some instances amalgamated into wider categories so that they could be used in the analyses.

2. Collection of samples

Collection of dust samples were dependant on various factors including availability of sampling sites and the time restraints during which the sampling had to be completed. In Chapter 2, samples were taken in homes during the months of October – February for two consecutive years to accommodate two final year BSc projects concerning dust mites during these times. In Chapter 3, child car seat sampling took place from May – July as: a) schools were open which increased the possibility of obtaining as large a sample size as possible; b) the weather was relatively dry which enhanced co-operation by potential participants. Although it is well documented that peak dust mite populations and allergen levels in most regions typically occur in late summer or early autumn (Arlian *et*

al., 1982; Van der Heide, 1994; Solarz, 1997; Crisafulli *et al.*, 2007; Farmaki *et al.*, 2010), sampling during these times was not possible within the scope of these studies.

Although as many samples as possible were collected (in the cases of Chapters 2 and 3) and processed (in the case of Chapter 4) in the research studies conducted in this thesis, time windows in which the collection and the processing of samples could take place were relatively short. For Chapters 2 and 3, the kindness and co-operation of participants was absolutely essential in obtaining samples, whereas in Chapter 4 samples had to be processed within a two month time period. Considering this, realistic goals for obtaining sufficient samples had to be set and achieved within allocated timeframes. From a statistical viewpoint this is not an ideal scenario, as underpowered studies can sometimes lead to false conclusions (Suresh and Chandrashekara, 2012). However, as expert statistical advice was obtained prior to commencing any statistical analyses in this thesis, the best statistical approach was used to analyse the data in each case.

At the outset of a study, it is recommended that certain factors are determined which may help provide the researcher with an adequate sample size estimate for their proposed research (Kadam and Bhalerao, 2010). Generally, these include such factors as an acceptable level of significance (i.e. the p-value), the power of the study (usually at least 80%), the expected effect size between two treatment variables in question, the underlying event rate in the population and the standard deviation of the population (the last three of factors of which can be estimated from previous similar studies). Using this information, it is possible to calculate a sufficient sample size (n) using the following formula:

$$n = \frac{2(Z_\alpha + Z_{1-\beta})^2 \sigma^2}{\Delta^2}$$

In this formula, For Z_α , Z is a constant, set by convention according to the accepted α error and whether it is a one-sided or two-sided effect. In the case of $Z_{1-\beta}$, Z is a constant set by convention according to the desired power of the study. Finally, σ is the estimated standard deviation, while Δ is the difference in effect of

two interventions which is required (the estimated effect size) (Kadam and Bhalerao, 2010).

3. Allergen analysis

A research grant from the College of Science Thomas Crawford Hayes Trust Fund was awarded to perform allergen analysis on dust samples in Chapters 2 and 3. However, as this covered the fee for the quantification of one allergen type only, it was decided that this should be prioritised for Der p 1 allergen since: a) *D. pteronyssinus* was found to be the most prevalent mite species found; and b) Der p 1 is widely regarded as being one of the main dust mite allergens that are capable of inducing sensitisation (Platts-Mills *et al.*, 1997). Although quantification of a range of mite allergens would be preferred, this was not possible due to funding restrictions.

4. Temperature and humidity measurements

Temperature and relative humidity readings were taken at all biotopes sampled in Chapter 2. However, since each sampling site was visited only once, these data were omitted from the final analysis since it is known that processes such as cooking, bathing and ventilation can cause relative humidity and temperature to rise and fall intermittently throughout the course of a day (Arlian *et al.*, 1999). While temperature and relative humidity measurements averaged over a longer period of time would have been more reflective of the true values, the narrow time windows during which samples were collected did not permit continuous measurements.

5. Fabric types, duration of contact and applied forces of contact

The study described in Chapter 4 examined the dynamics of dust mite transfer in one practical scenario i.e. the transfer of live and dead dust mites from car seat material to three modern fabric types, subjected to two different applied forces for

two durations of contact. If time had permitted, a number of other fabric types (both contaminated and receiving fabrics), applied forces and durations of contact could have been investigated. Due to time restraints and availability of the axial torsion tester, the experiments had to be completed within a two month time frame.

5.2 Future recommendations

- As highlighted in this thesis, the role of non-pyroglyphid species in inducing sensitisation should not be ignored. It is important that when reporting the mite fauna of dust samples in any future research, every attempt should be made to include the full suite of species present. As researchers have only relatively recently begun to understand the significance of these species and the allergens that they produce, it is essential that all species should be recorded in the event that somewhere down the line, research data could be re-evaluated in the light of subsequent findings with regard to these species' role in epidemiology. Where assays for other mite allergens are available, these should be examined in conjunction with mite counts in order to provide researchers with a robust dataset.
- Although Chapter 2 describes the most comprehensive published study focussing on the mite fauna in the microenvironment of domestic cats and dogs to date, the number of observations for each animal type were not even, with the bedding of seven cats examined compared to twenty seven dogs. Considering this, it was interesting to find that some mite species found were specific to bedding of cats (*O. cynotis* and Eriophyidae sp.) and dogs (*A. siro* and *C. eruditus*). A larger sample size with an even number of dust samples from the bedding of each animal type would provide a more comprehensive dataset, thus allowing a more thorough assessment of the mite species that could have stronger associations with one animal type over another.
- Child car seats have now been identified as a biotope of significant importance with regard to housing dust mite densities and their allergens. Although

vacuuming is the predominant method by which people eradicate dust in car environments, the results obtained in this research showed no effect of recent vacuuming on dust mite densities. Washing clothes in hot water $> 55^{\circ}\text{C}$ has been described as an effective method of removing mites and allergens (Platts-Mills *et al.*, 1997), while success washing clothes with chemicals such as benzyl benzoate and eucalyptus oil has also been described (Tovey and McDonald, 1997; Bischoff *et al.*, 1998), yet to the best of the authors knowledge, the efficacy of these methods has not been tested on car seats or car seat covers. The irradiation of dust mite cultures with UV-C light has recently been investigated (Lau *et al.*, 2012) and showed effective mortality of adults and reduction in hatchability of eggs, yet this has not been tested on dust mites outside of laboratory environments. With reference to mite and allergen mitigation in child car seats, these are all viable means of eradication yet must be tested in future investigations to determine their effectiveness.

- The transfer of dust mites from car seat material to three commonly worn receiving fabrics was examined in the study described in Chapter 4, but future work should examine different contaminated fabrics. These could include natural clothing fabrics such as wool and synthetic fabrics such as nylon and lycra, which may exhibit different properties with regard to microtopography and to forces such as electrostatic charge which may affect their ability to transfer mites. Different loading densities of mites, receiving fabrics, compression forces, rotary forces and durations of contact should all be examined with regard to both picking up mites from a contaminated source and releasing mites from a contaminated source to an uncontaminated surface. This would enhance the research already achieved in this study and bridge the gap in understanding how dust mites are distributed and come to colonise other suitable biotopes. These variables could also be examined with regard to allergens, which likely have important implications for personal allergen exposure.

5.3 Key findings

1. A large proportion of Irish homes sampled contained dust mite densities exceeding the risk factors for sensitisation, while supporting a range of other allergenic mite species.

A survey of 60 homes revealed that 65% and 55% of mattresses and furniture items respectively sampled exceeded the lower threshold for sensitisation (100 mites/g of dust) while 21% and 14% respectively exceeded the upper sensitisation threshold (500 mites/g of dust). The frequencies of these sensitising densities are higher than reported in numerous other studies which have sampled similar biotopes (Moyer *et al.*, 1985; Racewicz, 2001; Randall *et al.*, 2003; Motoki *et al.*, 2007; Farmaki *et al.*, 2010; Soltani *et al.*, 2011). In addition, the presence of at least 19 species of mites from ten different genera is reported. Of these species, eleven are known producers of allergens (Solarz *et al.*, 2004; Colloff, 2009; Beroiz *et al.*, 2014). As reflected in studies of similar climates and latitudes (Raffan *et al.*, 2005; Zock *et al.*, 2006), *D. pteronyssinus* was the most prevalent species found, consisting of 59% of the total mite fauna identified in this study. Despite the dominance of this species, the presence of a range of other species including pyroglyphid, storage mite and other mite species reveal a rich diversity present in Irish homes. The fact that many of these bear allergenic properties, a point echoed by numerous studies (Hughes, 1976; Cusack *et al.*, 1976; Armentia *et al.*, 1997; Fernandez-Caldas, 1997; Chambers *et al.*, 1999; Radon *et al.*, 2000; Solarz, 2004; Vidal *et al.*, 2004; Baker and Swan, 2013; Celebioglu *et al.*, 2013) is important in the context of sensitisation and should not be underestimated.

2. Household pets can facilitate in the introduction of a variety of mite species into the home, but their presence does not result in overall increased resident mite quantities or Der p 1 allergen levels.

A comparison of mite species richness in two home categories, homes with pets and homes without pets, revealed that the former contained a more diverse fauna of mites (17 species) compared to the latter (13 species). This can be attributed to the high diversity of mites found in pet beds from which a total of 16 species were

identified. Specimens from genera associated with a multitude of habitats including soil dwellers (order Oribatida), bird and mammal ectoparasites (families Laelapidae and Psoroptidae), phytophagous mites (family Eriophyidae), storage mites (families Acaridae, Glycyphagidae and Tarsonemidae), in addition to predatory dust dwelling mites (family Cheyletidae) and house dust mites (Pyroglyphidae) were all found in pet beds. The range of habitats encountered by a typical dog or cat during their daily activities in a rural landscape, added to the fact that they are close to the ground and possess hair coats that are ideal for picking up mites from various substrates, most likely explains why such a diversity of mite species was observed in their bedding and resting places.

Mite densities and Der p 1 allergen levels from both mattresses and furniture items from homes with pets were lower than homes without pets, but not significantly so. This is possibly explained by the presence of a higher diversity of species found in former category, which may have resulted in higher competition between species, thus preventing the dominance of any one species and hence resulting in lower allergen levels. The factors determining mite densities are complex and variables such as relative humidity, temperature, food availability and species-species interactions most likely play important roles. Nonetheless, the presence of pets clearly introduce a variety of mite species into the home and considering whatever dynamics are involved, may be inadvertently responsible for lowering overall dust mite levels with prospects for improving human health.

3. Child car seats can contain sensitising levels of dust mites and their associated allergens.

While some studies have examined the presence of dust mites and allergen levels in cars (Justino *et al.*, 1995; Neal *et al.*, 2002; Taketomi *et al.*, 2006) none have previously attempted to quantify mite numbers or mite allergen levels in child car seats. This study examined both child car seats and driver seats, of which over 77% and 80% respectively contained mites, while Der p 1 was detected in 85% and 92% of child car seats and driver seats tested respectively. Overall, there was a significant correlation ($P = 0.001$) between mites/g of dust recovered and the levels of Der p 1 present in the seats sampled, while the mites/g of dust recovered

from child car seats were correlated with driver seats of the same car ($P = 0.000$). This suggests that the conditions governing mite propagation within cars were common to both seat types, which may be of importance when considering mite eradication and mitigation strategies.

In total, 12 species of mites were identified in the survey of which nine are known to produce allergens (Chambers, 1999; Müsken *et al.*, 2000; Xue-Ying *et al.*, 2011). Although pyroglyphid species including *D.pteronyssinus*, *D.farinae* and *E.maynei* were most predominant, mites from several families / orders were present including members of the Acaridae, Glycyphagidae, Cheyletidae, Tarsonemidae, Mesostigmata and Oribatida. The presence of larva, protonymph and tritonymph stages as well as gravid female pyroglyphid specimens suggests that mite populations were living and breeding in the car seats at the time of sampling. Of the child car seats examined, over 15% contained mite quantities above the lower threshold of sensitisation of 100 mites/g of dust, while almost 15% of the child car seats tested for Der p 1 also exceeded the equivalent lower sensitisation threshold for allergen exposure (2 µg/g of dust). For driver seats, mite quantities exceeded 100 mites/g of dust in over 12% of seats, while sensitising levels of Der p 1 were found in over 16% of the driver seats tested.

Of the input variables examined in the inferential analysis for both seat types, contrasting results were observed. For child car seats, the presence of an atopic child was shown to have a significant effect on mite densities ($P = 0.042$), with higher densities observed from the seats of atopic children compared to non-atopics. Although this effect was not shown for driver seats, time since the driver seat was last vacuumed ($P = 0.049$) and the time spent by the occupant in the seat per week ($P = 0.010$) was shown to have a significant effect on mite densities. Lower quantities of mites were recovered from seats that had been vacuumed within the previous two months compared to those that had not been vacuumed during this time, while more mites were recovered from seats where drivers spent between 6 and 10 hours per week in their car compared to between 1 and 3 hours.

4. Transfer of mites through contact with clothing is dependent on multiple factors including fabric type, the active state of the mites present and the force applied during contact.

While clothing as a source of mite populations and their allergens has been explored previously (Bischoff and Fischer, 1990; Tovey *et al.*, 1995; Teplitsky *et al.* 2008) and has been hypothesised as one of the main means for dispersal of mites and allergens (Colloff, 1987; Mollet and Robinson, 1996; Neal *et al.*, 2002), the transfer capabilities of different clothing types, subject to various external variables had not previously been investigated. The experiment carried out in this study found that among three fabrics tested, fleece had the highest transfer efficiency for both live and dead mites (over 13% and 17% respectively) compared to denim (over 8% and 11% respectively) and plain woven cotton (both over 9%). The differences in transfer efficiencies between the fabrics are likely determined by the contrasting microtopography of each. Fleece consists of a complex network of polyester strands which appeared to facilitate the collection of mites regardless of whether the mites were dead or alive. At the micro level, denim exhibits an undulating surface topography consisting of multiple diagonal weaves of cotton threads, with many loose individual cotton strands which possibly also enabled the collection of mites. In contrast, the surface of the plain woven cotton is much more smooth and exposed, consisting of a very tight weave and high thread count which evidently does not accumulate mites to the same capacity as fleece or denim.

Transfer efficiencies of mites were also influenced by other variables examined including the applied force of contact and the active state of the mites. More mites were transferred when subjected to an applied force of 75N compared to 50N, an observation synonymous with other studies examining the effect of force on the transfer of particulate matter (McDonagh *et al.*, 2012), while more dead mites were transferred than live mites. This is most likely due to the fact that live mites can engage with the substrate by burrowing or clinging on using their specially adapted feet when subjected to disturbance, thereby preventing or limiting transference. The propensity for woollen garments to contain higher levels of dust

mite allergen than other fabrics has been highlighted previously (Siebers *et al.*, 2003; De Lucca *et al.*, 2000), and with this study it is now evident that the same can be said for mite bodies in fleece. These results could have important implications for dust mite distribution, colonisation and epidemiology in relation to allergen exposure.

5.4 Conclusions

The research conducted as part of this thesis set out to uncover some previously unknown aspects of dust mite research, while attempting to bridge the knowledge gap in understudied areas. As revealed in Chapters 2 and 3, densities of mites and Der p 1 allergen levels capable of inducing sensitisation were frequently found in all biotopes tested, with the frequency of these sensitising levels exceeding those from comparable biotopes in studies from various locations worldwide (Moyer *et al.*, 1985; Racewicz, 2001; Randall *et al.*, 2003; Motoki *et al.*, 2007; Farmaki *et al.*, 2010; Soltani *et al.*, 2011). These levels were most likely influenced by a combination of factors including physical and environmental variables, behavioural regimes of the occupants and optimum climatic conditions present in Ireland. Since prevalence of allergen related illnesses such as asthma are particularly high in Ireland (estimated as the fourth highest in the world at 9.8% of the population – Asthma Society of Ireland), it is important that mitigation measures are used as much as possible to alleviate symptoms that may be caused directly by the presence of dust mites and their allergens. Although it is difficult to determine accurately how many cases of asthma are directly related to dust mite allergen exposure in Ireland, it is estimated that between one third and a half of worldwide cases can be attributed to dust mite allergen (Colloff, 2009). Hence, from the point of view of reducing instances of mite associated conditions, allergen avoidance is of paramount importance. Many methods to reduce or remove mite populations in to avoid or minimise allergen exposure have been employed by countless researchers over the years with mixed results.

Vacuuming is by far the most convenient and readily available means of removing dust from bedding, carpets and upholstered materials in the home, yet

investigations examining the efficacy of vacuuming in removing dust mite populations and allergen concentrations have yielded mixed results. Arlian *et al.* (1982) reported that successive vacuuming did not significantly reduce mite abundance in fabric-upholstered furniture or carpeted floors. In addition, Sercombe *et al.* (2007) found that vacuuming changed the distribution of Der p 1 in the vertical pile structure of a carpet but did not reduce the overall allergen content, while other studies have reported significant reductions in allergen concentrations after vacuuming (Couper *et al.*, 1998; Zock *et al.*, 2006). Nadchatram (2005) reported that while there are multiple benefits to vacuuming including the removal of mites, allergens, skin scales and other detritus on which mites feed, only the surface material is successfully removed, with mite populations persisting deep into the substrate. Some evidence of an effect of vacuuming was apparent in Chapter 2 of this study, where 43% of participants in homes with pets had vacuumed their mattresses within the previous two months, coinciding with lower dust mite and Der p 1 densities, in contrast to higher mite and allergen levels in mattresses from homes without pets, where only 18% of mattresses had been vacuumed during this time. Although regular vacuuming likely has some beneficial value in removing dust mites and allergens, it is probably most effective when used in conjunction with other methods of eradication and avoidance.

Treatment of materials with a chemical acaricide such as benzyl benzoate is one method of killing mites and reducing allergens, although it is generally reported as being effective on a short term basis only (Lau-Schadendorf *et al.*, 1991; Woodfolk *et al.*, 1995). The chemical permethrin has been found to be very effective at killing mites (Cameron, 1997). Experiments testing the efficacy of permethrin-impregnated mattress liners (Cameron and Hill, 2002) reported a significant reduction in dust mites for up to 27 months after treatment, while significant reductions in allergen concentrations were observed for 15 months. Washing clothes at temperatures above 55°C reportedly kills mites and removes allergens (Platts-Mills *et al.*, 1997; Tovey and McDonald, 1997) while laundry additives such as eucalyptus oil and tee-tree oil have also been shown to be effective (Tovey and McDonald, 1997; Bischoff *et al.*, 1998). Tovey and

Woolcock (1994) demonstrated that exposing carpets to direct sunlight proved lethal to resident mites, most likely due to the dramatic effect of lowered humidity and raised temperature. As mite populations and allergen levels rise and fall seasonally, it is likely that mitigation methods will be more effective at certain times of the year, depending on the overall densities of mites or allergen levels present in a place in time and the efficacy of mitigation or removal method used. However, as the literature reports varying levels of success employing different eradication measures, it is suggested that a combination of the described methods is probably most effective at reducing mite densities and allergen levels. Although bedding will almost always be a focal point for mites due to the accumulation of skin scales and optimum environmental conditions being created, simple domestic design procedures like removing carpets and limiting upholstered furnishings reduces the availability of suitable habitat for mites and will limit, to some extent, the production of allergens.

Although not examined in Chapter 3, it is likely that similar methods of mite eradication and avoidance employed in homes would be effective in cars, though the efficacy of these requires investigation. As the study found a significantly higher density of mites associated with atopic children compared to non-atopics, extra care may be needed when choosing cleaning regimes. Disturbance of reservoir allergen during processes such as vacuuming can cause the allergen to become airborne and more likely to be inhaled (Colloff, 2009) while chemical acaricides such as benzyl benzoate can be toxic and in some cases cause allergic reactions (Cameron and Hill, 2002). Therefore, while a combination of the described methods is probably most effective, methods such as hot washing and exposure to direct sunlight may be the most risk free mite eradication procedures for child car seats at least, due to the ease at which they can be removed from cars. In relation to regular car seats, hot washing may have to be carried out in situ, and while steam cleaning has been shown as an effective method for killing mites and reducing Der p 1 levels in carpets (Colloff *et al.*, 1995) this has not, to the best of the author's knowledge, been tested on car seats.

The sensitisation thresholds set out at the First International Workshop on Indoor Allergens and Asthma, which were based on levels of Der p 1 and Der f 1 allergen concentrations (Platts-Mills *et al.*, 1989), have been used by the majority of studies published thereafter, enabling researchers to gauge the significance of their results from an epidemiological perspective. Ćustović and Chapman (1998) revisited this debate by conducting trials to determine whether measurement of airborne allergen would be a more accurate means of assessing exposure than the established thresholds derived from reservoir dust allergen concentrations. They concluded that at that time, airborne sampling was insufficiently sensitive to produce reliable and repeatable results and that the traditional approach of allergen measurement in reservoir dust would still provide the best index for exposure, provided that the quantification of allergen per unit weight of dust and per unit area is reported.

Since this time however, airborne allergen sampling has been re-examined. Personal allergen exposure through the use of an air sampling pump attached to participants' shoulders was investigated by Tovey *et al.* (2013). They reported that higher allergen concentrations were detected during the day while participants carried out daily activities in contrast to concentrations detected in bed at night. While the authors did not suggest that a new sensitisation threshold needs to be established, they did indicate that in light of their findings, the main sources of dust mite allergen exposure probably needs to be re-evaluated. This may imply that researchers need to adopt a fresh approach with regard to the significance of individual mite counts from a sensitisation point of view. As also suggested by Tovey *et al.* (2013), clothing could be an important source of daily allergen exposure, while certain fabric types like woollen garments have been identified as bearing higher levels of allergen than others (Siebers *et al.*, 2003; De Lucca *et al.*, 2000). Results obtained in Chapter 4 of this thesis have bridged the knowledge gap with regard to the dynamics involved in the transfer of mites through different clothing fabrics, while also revealing that fleece has a greater capacity to accumulate mite bodies themselves. Although it is recommended that future experiments should investigate various other fabric types and external variables

regarding the transfer of mites, this result is something that atopic people should be aware of when choosing clothing garments and bed covers.

As stressed at various stages throughout this thesis, storage mites have been proven to be an important source of allergens. The thresholds for sensitisation currently used in most dust mite studies however, are based on levels of group 1 allergens (allergens mainly derived from digestive enzymes and faecal pellets of pyroglyphid mites) and to date, there is still no separate established threshold for allergens derived from storage mites (Chambers *et al.*, 1999). Since occupational and domestic exposure to these mites is now well known, it is time that the establishment of sensitisation thresholds in relation to all allergen producing mites should be prioritised. Advancements in this area will undoubtedly contribute to our understanding of this aspect of epidemiology, hopefully leading to reductions in cases of mite associated conditions in future generations.

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Appendix I

Chapter 2: Questionnaire

Section A – General Home Questions

1. Are you

Male

Female

2. Had you ever heard of dust mites before today?

Yes

No

a. If Yes to above, where did you hear about them?

3. How many people live in your home?

Please specify number _____

4. How many children live in this home and what are their ages?

5. What type of home do you live in?

- | | | | | | | |
|--------------------------|---------------|---|--------------------------|------------|--------------------------|------------|
| <input type="checkbox"/> | Terraced | → | <input type="checkbox"/> | One Storey | <input type="checkbox"/> | Two Storey |
| <input type="checkbox"/> | Semi-detached | → | <input type="checkbox"/> | One Storey | <input type="checkbox"/> | Two Storey |
| <input type="checkbox"/> | Detached | → | <input type="checkbox"/> | Bungalow | <input type="checkbox"/> | Two Storey |
| <input type="checkbox"/> | Apartment | | | | | |

6. How old is your home?

(Please state the approximate age of your home in years)

7. What type of heating do you use in your home?

(Please tick any which are appropriate)

- | | | | |
|--------------------------|---------------------|--------------------------|------------------------------|
| <input type="checkbox"/> | Radiators | <input type="checkbox"/> | Storage heaters |
| <input type="checkbox"/> | Under floor heating | <input type="checkbox"/> | Electric heaters |
| <input type="checkbox"/> | Fan heaters | <input type="checkbox"/> | Solid Fuels (Please specify) |
| <input type="checkbox"/> | Other | | |
-

8. Are the windows in your home;

- | | |
|--------------------------|---------------|
| <input type="checkbox"/> | Single glazed |
| <input type="checkbox"/> | Double glazed |
| <input type="checkbox"/> | Triple glazed |

9. Have you had any sprayed foam or pumped cavity insulation service carried out in the walls of your home?

Yes

No

If Yes, what type of service was it and when was it carried out?

10. Do you use any electric air conditioning or dehumidifiers in your home?

Air Conditioning

Dehumidifiers

11. How do you dry your clothes?

Tumble dryer

Washing line

Indoors (e.g clothes horse beside a heat source or on radiator)

In a hot press

12. How many bedrooms are in your home?

Please specify number _____

13. Please specify the type of flooring in each of the following rooms in your home:

Room	Flooring Type		
	Carpet	Wood	Other
Bedroom 1.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bedroom 2.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bedroom 3.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bedroom 4.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bedroom 5.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Living room	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

- a. If **Other** to above, please specify what type of flooring you have and what room it is in
-

14. How often is the living room vacuumed?

- | | |
|---|--|
| <input type="checkbox"/> Daily | <input type="checkbox"/> Every 2 – 4 months |
| <input type="checkbox"/> Weekly | <input type="checkbox"/> Every 4 – 6 months |
| <input type="checkbox"/> Fortnightly | <input type="checkbox"/> Every 6 – 8 months |
| <input type="checkbox"/> Monthly | <input type="checkbox"/> Every 8 – 12 months |
| <input type="checkbox"/> Every 2 months | <input type="checkbox"/> Longer than 12 months |

- 15. Have any beds or carpets in your home been treated with any pesticide or anti-dust mite chemicals?**

Yes
 No

If Yes , when was this treatment carried out? _____

Section B – Childs Bedroom

- 1. How many children sleep in this room?**

Please specify number of children

- 2. Please specify the age(s) of the child or children who sleep in this room**
-

- 3. Has your child (children) been diagnosed with asthma?**

Yes
 No

a. **If Yes, is their asthma worse in**

- Spring
- Summer
- Autumn
- Winter
- Always the same

b. **Do you know what causes their asthma or what makes their symptoms worse**

Please give a reason to the best of your knowledge

4. **Has your child been diagnosed with Eczema, Dermatitis or Allergic Rhinitis?**

- Dermatitis
- Eczema
- Allergic Rhinitis
- None

a. **If Yes, is their eczema / dermatitis / allergic rhinitis worse in**

- Spring
- Summer
- Autumn
- Winter
- Always the same

b. Do you know what causes your eczema / dermatitis / allergic rhinitis or what makes your symptoms worse?

Please give a reason to the best of your knowledge

5. Are any pets allowed into the room?

- Yes
- No

If Yes, are they allowed in the room:

- Rarely
- Often
- All of the time

6. How old is the mattress in this room?

- Less than 1 year
- 1-2 years
- 2-3 years
- More than 3 years

If more than 3 years, please specify age of the mattress being sampled

7. Has the mattress ever been turned?

Yes

No

a. If Yes, please specify when the mattress was last turned

8. Is the mattress protected with a mattress cover?

Yes

No

a. If Yes , is it:

Plastic style

Netted style

Quilted style

Don't Know

9. Has the mattress itself ever been vacuumed or cleaned in any way?

Yes

No

a. If Yes, when was this last carried out?

10. Please indicate the floor type in this bedroom

- Carpet
- Wood
- Other

11. How often is this bedroom swept or vacuumed?

- | | |
|---|--|
| <input type="checkbox"/> Daily | <input type="checkbox"/> Every 2 – 4 months |
| <input type="checkbox"/> Weekly | <input type="checkbox"/> Every 4 – 6 months |
| <input type="checkbox"/> Fortnightly | <input type="checkbox"/> Every 6 – 8 months |
| <input type="checkbox"/> Monthly | <input type="checkbox"/> Every 8 – 12 months |
| <input type="checkbox"/> Every 2 months | <input type="checkbox"/> Longer than 12 months |

a. Please indicate when the floor was last swept or vacuumed

12. If the floor is carpet, has it been washed with special carpet cleaning products (i.e shampoos or steamers)?

- Yes
- No

If Yes, please specify which product and when this cleaning was last carried out?

13. How old is the pillow in this bed?

- Less than 1 year
- 1-2 years
- 2-3 years
- More than 3 years

If more than 3 years, please specify age of the pillow in the bed being sampled

14. How old is the duvet / blanket / quilt?

- Less than 1 year
- 1-2 years
- 2-3 years
- More than 3 years

If more than 3 years, please specify age of the duvet / blanket / quilt in the bed being sampled

15. How often is the pillow case / duvet cover washed or replaced with another case/ cover?

- | | |
|---|--|
| <input type="checkbox"/> Daily | <input type="checkbox"/> Every 2 – 4 months |
| <input type="checkbox"/> Weekly | <input type="checkbox"/> Every 4 – 6 months |
| <input type="checkbox"/> Fortnightly | <input type="checkbox"/> Every 6 – 8 months |
| <input type="checkbox"/> Monthly | <input type="checkbox"/> Every 8 – 12 months |
| <input type="checkbox"/> Every 2 months | <input type="checkbox"/> Longer than 12 months |

a. **Please indicate when the pillow case / duvet cover was last washed or replaced?**

Has the duvet ever been dry cleaned?

Yes

No

If Yes, when was this last carried out?

16. Do you allow food and drinks to be consumed in your child's bedroom?

Yes

No

a. **If Yes, do you allow this:**

Rarely

Often

All of the time

17. Are the windows in the room opened;

Daily

Weekly

Fortnightly

Monthly

Never

Section C – Adults Bedroom

1. How many people sleep in this room?

Please specify number _____

2. Do any children sleep in this room?

Yes

No

If yes, please specify number _____

3. Have you been diagnosed with asthma?

Yes

No

a. If Yes, is your asthma worse in:

Spring

Summer

Autumn

Winter

Always the same

- b. **Do you know what causes your asthma or what makes your symptoms worse?**

Please give a reason to the best of your knowledge

4. **Have you been diagnosed with Eczema, Dermatitis or Allergic Rhinitis?**

- Dermatitis
- Eczema
- Allergic Rhinitis
- Neither

- a. If Yes, is your eczema / dermatitis / allergic rhinitis worse in:

- Spring
- Summer
- Autumn
- Winter
- Always the same

- b. **Do you know what causes your eczema / dermatitis / allergic rhinitis or what makes your symptoms worse?**

Please give a reason to the best of your knowledge

5. Are any pets allowed into the room?

Yes

No

If **Yes**, are they allowed in the room:

Rarely

Often

All of the time

6. How old is the mattress in this room?

Less than 1 year

1-2 years

2-3 years

More than 3 years

If more than 3 years, please specify age of the mattress being sampled

7. Has the mattress ever been turned?

Yes

No

a. If **Yes, please specify when the mattress was last turned**

8. Is the mattress protected with a mattress cover?

Yes

No

a. If Yes , is it:

Plastic style

Netted style

Quilted style

Don't Know

9. Has the mattress itself ever been vacuumed or cleaned in any way?

Yes

No

a. If Yes, when was this last carried out?

10. Please indicate the floor type in this bedroom

- Carpet
- Wood
- Other

11. How often is this bedroom swept or vacuumed?

- | | |
|---|--|
| <input type="checkbox"/> Daily | <input type="checkbox"/> Every 2 – 4 months |
| <input type="checkbox"/> Weekly | <input type="checkbox"/> Every 4 – 6 months |
| <input type="checkbox"/> Fortnightly | <input type="checkbox"/> Every 6 – 8 months |
| <input type="checkbox"/> Monthly | <input type="checkbox"/> Every 8 – 12 months |
| <input type="checkbox"/> Every 2 months | <input type="checkbox"/> Longer than 12 months |

a. Please indicate when the floor was last swept or vacuumed

12. If the floor is carpet, has it been washed with special carpet cleaning products (i.e shampoos or steamers)?

- Yes
- No

If Yes, please specify which product and when this cleaning was last carried out?

13. How old is the pillow in this bed?

- Less than 1 year
- 1-2 years
- 2-3 years
- More than 3 years

a. If more than 3 years, please specify age of the pillow in the bed being sampled

14. How old is the duvet / blanket / quilt?

- Less than 1 year
- 1-2 years
- 2-3 years
- More than 3 years

If more than 3 years, please specify age of the duvet / blanket / quilt in the bed being sampled

15. How often is the pillow case / duvet cover washed or replaced with another case/ cover?

- | | |
|---|--|
| <input type="checkbox"/> Daily | <input type="checkbox"/> Every 2 – 4 months |
| <input type="checkbox"/> Weekly | <input type="checkbox"/> Every 4 – 6 months |
| <input type="checkbox"/> Fortnightly | <input type="checkbox"/> Every 6 – 8 months |
| <input type="checkbox"/> Monthly | <input type="checkbox"/> Every 8 – 12 months |
| <input type="checkbox"/> Every 2 months | <input type="checkbox"/> Longer than 12 months |

- a. **Please indicate when the pillow case / duvet cover was last washed or replaced?**
-

Has the duvet ever been dry cleaned?

- Yes
 No

If Yes, when was this last carried out?

- 16. Do you consume food and drinks in the bedroom?**

- Yes
 No

- a. **If Yes, do you do this:**

- Rarely
 Often
 All of the time

17. Are the windows in the room opened:

- Daily
- Weekly
- Fortnightly
- Monthly
- Never

Section D – Pets

1. Please specify any pets you have. List the type of pet (e.g Cat or Dog), their age and their breed (if known).

Pet 1: _____ Age: _____ Breed: _____

Pet 2: _____ Age: _____ Breed: _____

Pet 3: _____ Age: _____ Breed: _____

Pet 4: _____ Age: _____ Breed: _____

Pet 5: _____ Age: _____ Breed: _____

Pet 6: _____ Age: _____ Breed: _____

Pet 7: _____ Age: _____ Breed: _____

2. If you have a dog (s) please indicate (roughly) what size he / she is by ticking the appropriate box below

- | | | | | | | |
|--------|-------|--------------------------|--------|--------------------------|-------|--------------------------|
| Dog 1: | Small | <input type="checkbox"/> | Medium | <input type="checkbox"/> | Large | <input type="checkbox"/> |
| Dog 2: | Small | <input type="checkbox"/> | Medium | <input type="checkbox"/> | Large | <input type="checkbox"/> |
| Dog 3: | Small | <input type="checkbox"/> | Medium | <input type="checkbox"/> | Large | <input type="checkbox"/> |
| Dog 4: | Small | <input type="checkbox"/> | Medium | <input type="checkbox"/> | Large | <input type="checkbox"/> |
| Dog 5: | Small | <input type="checkbox"/> | Medium | <input type="checkbox"/> | Large | <input type="checkbox"/> |
| Dog 6: | Small | <input type="checkbox"/> | Medium | <input type="checkbox"/> | Large | <input type="checkbox"/> |

3. Where does your pet spend most of his / her time?

- Inside the house
- Outside the house
- Both

4. Does your pet sleep inside the house?

- Yes
- No

5. How long do your pets spend indoors each day?

Please specify _____

6. If your pet sleeps outside of the house, does he / she sleep under a sheltered structure of any kind? Please specify.

7. Approximately how long does your pet (s) spend in their bed / resting place

Please specify _____

8. How often is the pet bedding washed or changed?

- More than once every week
- Once every week
- Once every 2 weeks
- Once every month
- Once every 6 months
- Once every year
- Never

9. When was the last time the bedding was washed or vacuumed?

10. What method do you use to wash the pet bedding (e.g. washing machine/dryer, hand washing, vacuuming etc.)

11. Do you use any insecticides or pesticides, or any other chemicals on the bedding?

12. Does your pet have any known skin conditions or parasites that you are aware of (e.g. dermatitis, ticks, fleas etc.)

Bedroom Sampling Checklist

Name of participant: _____ Date: _____

Room Type: Child's Adults

1. Flooring type Carpet Wood Other: _____

2. Carpet pile length 1. _____

2. _____

3. _____

3. Rugs present? Pile length: _____ Type: _____

Pile length: _____ Type: _____

Pile length: _____ Type: _____

4. Mattress cover
 Plastic Netted Quilted

6. Mattress vacuumed If so, when last vacuumed _____

7. Window glazing Single Double Trip

8. Under floor heating?

9. Ventilation duct?

10. Radiators present? Other heat source: _____

11. Temperature: _____ Humidity: _____

11. Other comments:

Furniture Sampling Checklist

1. Name of participant: _____ Date: _____
- Room Type: Sitting room Dining room
 Kitchen Other _____
2. Flooring type Carpet Wood Other _____
3. Carpet pile length 1. _____
2. _____
3. _____
4. Rugs present? Pile length: _____ Type: _____
 Pile length: _____ Type: _____
 Pile length: _____ Type: _____
5. Window glazing Single Double Triple
6. Under floor heating?
7. Ventilation duct?
8. Radiators present? Other heat source: _____
9. Temperature: _____ Humidity: _____
10. Furniture type sampled: Armchair Couch
 Other _____
11. Material _____

12. Used by pet? Yes No

13. Furniture vacuumed Yes No

If Yes, when last vacuumed _____

Other comments:

Appendix II

Chapter 3: Questionnaire

Appendix II

Questionnaire (Chapter 3)

Section A

1. Are you

Male

Female

2. Had you ever heard of dust mites before today?

Yes

No

a. If Yes to above, where did you hear about them?

Section B

1. How many people live in your home?

Please specify number _____

2. How many children do you have and what are their ages?

3. Please write the registration and make of your car below (so that we can track the sample back to you, should you wish to know the result)

4. How long have you had this car?

5. How much time do you spend in this car per week?

- | | |
|---|---|
| <input type="checkbox"/> 1 hour or less | <input type="checkbox"/> 2 hours |
| <input type="checkbox"/> 3 hours | <input type="checkbox"/> 4 hours |
| <input type="checkbox"/> 5 hours | <input type="checkbox"/> 6 hours |
| <input type="checkbox"/> 7 hours | <input type="checkbox"/> 8 hours |
| <input type="checkbox"/> 9 hours | <input type="checkbox"/> 10 hours or more |

6. How much time does the child / children you collect spend in this car per week?

- | | |
|---|---|
| <input type="checkbox"/> 1 hour or less | <input type="checkbox"/> 2 hours |
| <input type="checkbox"/> 3 hours | <input type="checkbox"/> 4 hours |
| <input type="checkbox"/> 5 hours | <input type="checkbox"/> 6 hours |
| <input type="checkbox"/> 7 hours | <input type="checkbox"/> 8 hours |
| <input type="checkbox"/> 9 hours | <input type="checkbox"/> 10 hours or more |

7. Do you have any pets?

- Yes
 No

a. If Yes, please state what type of pet(s) you have and how many

b. Do you allow your pets in this car?

- Yes
 No

c. If Yes, where do you allow them to sit in this car?

(Please tick any appropriate areas)

- Back passenger seats Floor of back passenger seat
 Front passenger seats Floor of front passenger seat
 Boot

8. Do you allow food and drinks to be consumed in this car?

Yes

No

9. When was the last time you vacuumed the inside of this car?

Less than 2 months ago

More than 2 months ago

Never

10. Have you ever had this car professionally cleaned or valeted?

Yes

No

a. If Yes, when was this service **last** carried out? _____

11. Do you have anti-dust mite seat covers in your car, or has it ever been treated with a pesticide of any kind?

Yes

No

12. How old is the child car seat in this vehicle?

Less than 1 year

1-2 years

2-3 years

More than 3 years

13. Has the child car seat ever been vacuumed?

Yes

No

a. If Yes, when was the child car seat last vacuumed?

Less than 2 months ago

More than 2 months ago

Never

14. Does your car have air conditioning?

Yes

No

a. If Yes, would you use it:

All the time

Regularly

Less often

Never

Section C

1. What type of home do you live in?

- | | | | | | | |
|--------------------------|---------------|---|--------------------------|------------|--------------------------|------------|
| <input type="checkbox"/> | Terraced | → | <input type="checkbox"/> | One Storey | <input type="checkbox"/> | Two Storey |
| <input type="checkbox"/> | Semi-detached | → | <input type="checkbox"/> | One Storey | <input type="checkbox"/> | Two Storey |
| <input type="checkbox"/> | Detached | → | <input type="checkbox"/> | Bungalow | <input type="checkbox"/> | Two Storey |
| <input type="checkbox"/> | Apartment | | | | | |

2. How old is your home?

(Please write the approximate age of your home in years)

3. What type of heating do you use in your home?

(Please tick any which are appropriate)

- Central Heating
- Solid fuel (Stove, range or open fire)

4. Are the windows in your home:

- Single glazed
- Double glazed
- Triple glazed

5. Do you use any electric air conditioning or dehumidifiers in your home?

- Air Conditioning
- Dehumidifiers

6. Please specify the type of flooring in each of the following rooms in your home:

Room	Flooring Type		
	Carpet	Wood	Other
Bedroom	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Living room	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

- a. If **Other** to above, please specify what type of flooring you have and what room it is in
-

7. How often are the bedrooms swept or vacuumed?

- Daily Every 2 – 4 months
- Weekly Every 4 – 6 months
- Fortnightly Every 6 – 8 months
- Monthly Every 8 – 12 months
- Every 2 months Longer than 12 months

8. How often is the living room swept or vacuumed?

- | | |
|---|--|
| <input type="checkbox"/> Daily | <input type="checkbox"/> Every 2 – 4 months |
| <input type="checkbox"/> Weekly | <input type="checkbox"/> Every 4 – 6 months |
| <input type="checkbox"/> Fortnightly | <input type="checkbox"/> Every 6 – 8 months |
| <input type="checkbox"/> Monthly | <input type="checkbox"/> Every 8 – 12 months |
| <input type="checkbox"/> Every 2 months | <input type="checkbox"/> Longer than 12 months |

9. If you have pets, do you allow pets into your home:

- | |
|---------------------------------------|
| <input type="checkbox"/> Sometimes |
| <input type="checkbox"/> Often |
| <input type="checkbox"/> All the time |
| <input type="checkbox"/> Never |

10. Do you use mattress covers in your home?

- | |
|------------------------------|
| <input type="checkbox"/> Yes |
| <input type="checkbox"/> No |

a. If Yes , are they:

- Plastic style
- Netted style
- Quilted style
- Don't Know

11. Have any beds or carpets in your home been treated with any pesticide or anti-dust mite chemicals?

- Yes
- No

a. If Yes , when was this treatment carried out? _____

Section D

1. Are you a smoker?

- Yes
- No

a. If Yes, do you smoke in this car?

- Yes
- No

2. Does anyone else smoke in your house?

Yes

No

a. If Yes , how many people smoke? _____

b. Does anyone else smoke in this car?

Yes

No

3. Have you been diagnosed with asthma?

Yes

No

a. If Yes, is your asthma worse in:

Spring

Summer

Autumn

Winter

Always the same

- b. **Do you know what causes your asthma or what makes your symptoms worse?**

Please give a reason to the best of your knowledge

4. **Have any of your children been diagnosed with asthma?**

Yes

No

- a. If Yes, please state how many _____

and

What age they are _____

- b. **Is their asthma worse in:**

Spring

Summer

Autumn

Winter

Always the same

- c. **Do you know what causes their asthma or what makes their symptoms worse?**

Please give a reason to the best of your knowledge

5. **Have you been diagnosed with Eczema, Dermatitis or Allergic Rhinitis?**

- Dermatitis
- Eczema
- Allergic Rhinitis
- Neither

- a. If Yes, is your eczema / dermatitis / allergic rhinitis worse in

- Spring
- Summer
- Autumn
- Winter
- Always the same

- b. **Do you know what causes your eczema / dermatitis / allergic rhinitis or what makes your symptoms worse?**

Please give a reason to the best of your knowledge

6. Do any of your children suffer from eczema, dermatitis or allergic rhinitis?

Yes

No

a. If Yes, please state how many _____

and

What age they are _____

b. Is their eczema / dermatitis / allergic rhinitis worse in

Spring

Summer

Autumn

Winter

Always the same

c. Do you know what causes their eczema / dermatitis / allergic rhinitis or what makes their symptoms worse?

Please give a reason to the best of your knowledge