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Monitoring indoor pollen over two years

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Monitoring indoor pollen over two years

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Abstract

The present study monitored a private apartment for pollen that had settled on the floor. Pollen was sampled and analysed from two rooms over a period of two years. The study showed that pollen accumulations indoors are dominated by wind-pollinated species reflecting the current flowering season. The adjacent vegetation and the cleaning activities influenced the pollen content. Pollen from zoophilous plants mostly originated from ornamental plants within the flat. Similarities and differences between the two rooms and the two years were observed. These findings have implications for forensic investigations as well as allergological research.

Keywords: *palynology, pollen distribution, seasonal changes, domestic environment, urban environment, forensic, χ -square-test*

Since people spend most of their time indoors and many people suffer from pollinosis, indoor pollen grains or their antigenic particles have been topic of various studies (Holmquist & Vesterberg 1999; Fahlbusch et al. 2000; Nayar & Jothish 2013). Such studies usually focus on only few pollen types that dominate the spectrum of indoor pollen (Tormo-Molina et al. 2009). Indoor pollen is highly dependent on the pollen concentration outdoors (Jantunen & Saarinen 2009). Especially vegetation in closest vicinity can influence the indoor pollen assemblages (Yankova 1991; Tormo-Molina et al. 2009). Forensic Palynology uses pollen and spores to answer questions concerning crimes (Bryant & Mildenhall 1998; Mildenhall 2004; Mildenhall et al. 2006). Pollen can be used to relate a suspect to a crime scene, reduce any list of suspects, verify or falsify an alibi and much more (Mildenhall 1990). However, little is known about indoor pollen from a forensic point of view. There are only a few studies, which focus on forensic implications. Morgan et al. (2008, 2014) investigated the distribution of pollen from flowers that were deliberately placed within a room. They report a correlation between the pollen findings and the proximity of

the sampling site to the flowers. Forensic investigations need to take all pollen types into account since sometimes even low numbers of a specific taxon can be of huge forensic importance (Mildenhall et al. 2006).

This paper is part of a survey dedicated to indoor pollen from a forensic point of view. In this study, a private apartment was monitored for pollen over a period of two years. The following questions should be answered: (1) Which pollen is found in the apartment and how similar are the pollen assemblages from two different rooms? (2) Does the pollen content change throughout the year and is there a correlation with the flowering season? (3) Is there an influence from the surrounding vegetation and to which extent? (4) How similar are the pollen assemblages from two consecutive years (2011 and 2012)? This work, however, did not focus on how pollen infiltrates a domestic dwelling.

Material and methods

From 1 April 2011 to 24 October 2012, two rooms of a private apartment (4th level, two occupants, no

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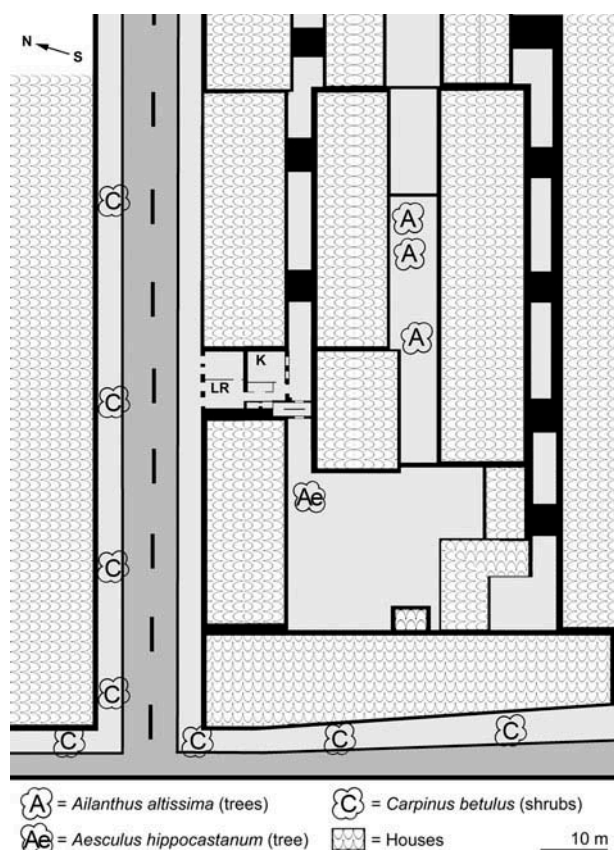


Figure 1. Location of the investigated flat and the surrounding area, including the vegetation in nearest vicinity. LR, living room; K, kitchen.

pets) in Vienna, Austria, were sampled for pollen deposited on the floor. The flat was chosen for the present study to simulate real life conditions, which include the opening of windows and doors at irregular intervals and usual cleaning activities. The two rooms were selected, because their windows are orientated in opposite directions (north-northwest and south-southeast). The outside vegetation nearest to the flat was documented (Figure 1).

Sampling was done approximately every ten days. Pollen was gathered by wiping the floor with duster sheets. Afterwards, the duster sheets were washed with distilled (pollen sterile) water mixed with a drop of Tween 20. By centrifugation (3000 rpm) of the washing water, solid and liquid phases were separated. The solid remains were processed using a modified acetolysis technique (Hesse 2009) according to the following protocol: (A) rinse with acetic acid (100%); (B) decant acetic acid and add acetolysis mixture: nine parts acetic acid anhydride (99%) and one part sulphuric acid (96%); (C) heat in a water bath (100 °C) for approximately eight minutes; (D) rinse with acetic acid (100%); (E) rinse with distilled water (several times); (F) rinse

with ethanol (99.8%) to dry the sample; (G) add glycerine to the sample and fill it into vials. Pristine duster sheets were processed as reference samples. During sample processing, the laboratory was monitored for pollen contamination.

The specimens were mounted on glass slides and examined with a light microscope (Olympus BX-53F). Pollen grains and spores were assigned to 98 different pollen types. It must be pointed out that these types are not completely identical with the associated taxonomic groups. For pollen grain identification standard literature (Reille 1992; Beug 2004; Hesse 2009) and the pollen database PalDat (Buchner & Weber 2000) was used. If crucial pollen grains remained unidentified, scanning electron microscopy was used (Ferguson et al. 2007).

At least 300 pollen grains per sample were counted. The percentages for the 20 most significant pollen types were calculated. For statistical analysis, χ -square-tests (Pearson 1900) were conducted. Large numbers of saccate pollen grains were floating in the washing water and subsequently lost during decantation. Therefore, this pollen type was excluded from analysis.

Results

A total of 138 samples were taken from the kitchen and the living room during the two years of study. Overall, 55 187 pollen grains were counted, of which 225 remained unidentified. The following 20 pollen types were classified as most important (Figure 2): Urticaceae, Poaceae, *Ambrosia*, *Betula*, *Quercus*, *Fraxinus*, *Populus*, *Plantago*, *Carpinus*, *Amaranthaceae*, *Ailanthus*, *Fagus*, *Cupressaceae*, *Alnus*, *Tilia*, *Sophora*, *Artemisia*, *Platanus*, *Corylus* and *Aesculus*. Additionally, 78 other types were distinguished summing up to 98 pollen types in total (Table I).

Living room (April 2011–October 2012)

Most samples from the living room were dominated by wind pollinated species, starting on 1 April 2011 with high counts of *Carpinus* and *Fraxinus* (Table II). On the 22 April, both *Carpinus* and *Fraxinus* were replaced by *Quercus* as the dominant type. This scheme is continued throughout the study as one dominant pollen type was replaced by another: *Quercus* (end April) followed by *Fagus* (May), Poaceae (June–July), *Ailanthus* (June), *Sophora* (July–August), Urticaceae (July–October) and *Ambrosia* (September–October). From November–March, no new important pollen types were found. Poaceae, *Ambrosia* and Urticaceae remained the highest scoring types in the living room until February 2012. From February, remarkable changes were again observed. A notable amount of *Corylus* (February–

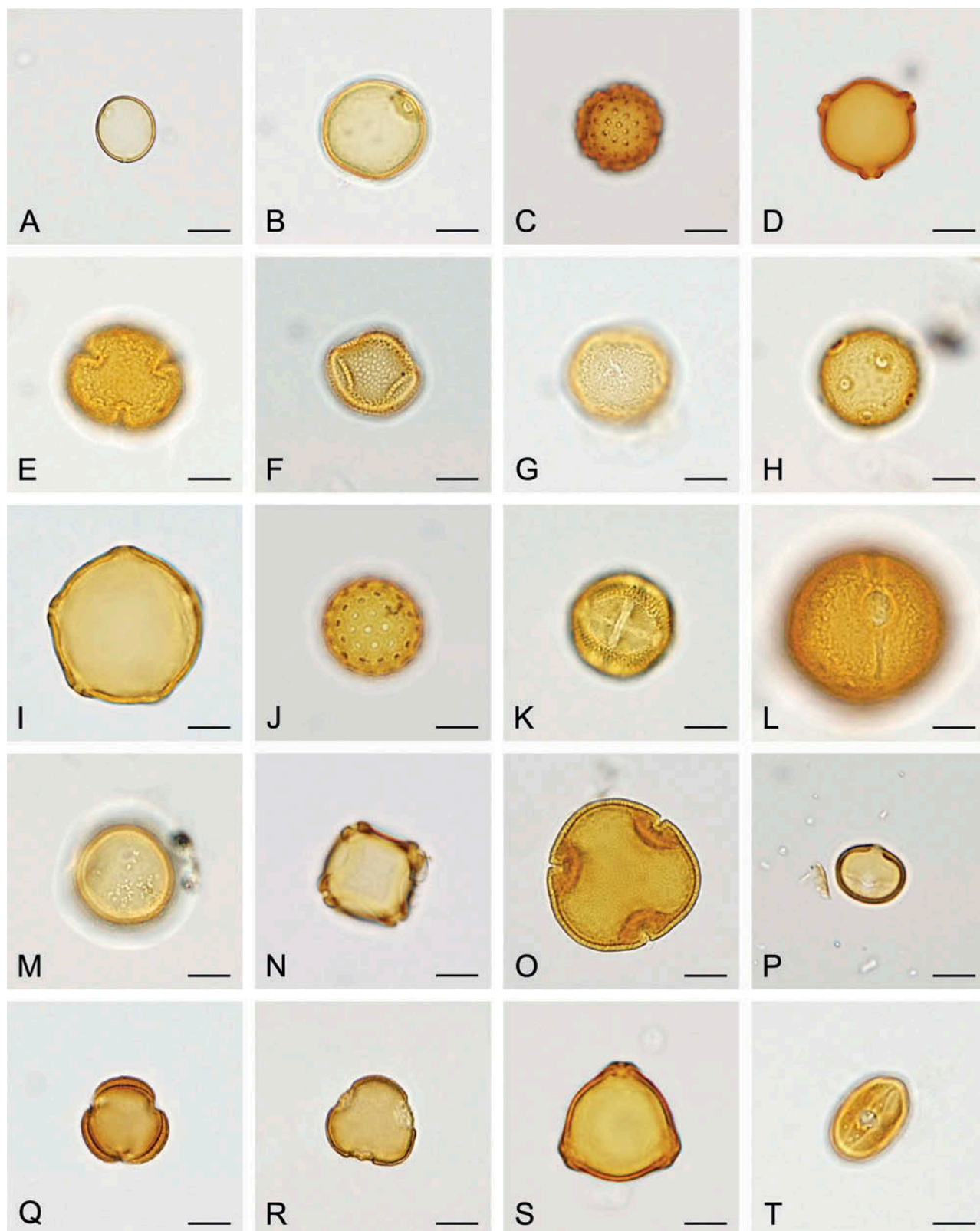


Figure 2. The 20 most important pollen types among the acetolysed pollen grains. **A.** Urticaceae. **B.** Poaceae. **C.** *Ambrosia*. **D.** *Betula*. **E.** *Quercus*. **F.** *Fraxinus*. **G.** *Populus*. **H.** *Plantago*. **I.** *Carpinus*. **J.** *Amaranthaceae*. **K.** *Ailanthus*. **L.** *Fagus*. **M.** *Cupressaceae*. **N.** *Alnus*. **O.** *Tilia*. **P.** *Sophora*. **Q.** *Artemisia*. **R.** *Platanus*. **S.** *Corylus*. **T.** *Aesculus*. Scale bars – 10 μ m.

March) and Caryophyllaceae (11%, 1 March) was followed by large amounts of *Alnus*, *Taxus* (both in March) and *Populus* (March–April). With the beginning of April, *Betula* dominated and overlapped *Populus*. In May, *Quercus* was the most common pollen type, succeeded by Poaceae, which dominated up to July. Large amounts of *Ailanthus* were found in June. Urticaceae dominated from July to August, followed by *Ambrosia* until the end of September. In October 2012, the last month observed, no type achieved a higher share than 17%. Most remarkably, *Betula* had the highest counts (16%) since May.

Kitchen (April 2011–October 2012)

In the kitchen, *Fraxinus*, *Populus* and *Alnus* were dominant in April 2011, while *Carpinus* had a small impact (Table III). *Quercus* had high counts in April and May, followed by *Fagus* (May), *Ailanthus* (June), Poaceae (June–July), Urticaceae (July–September) and *Ambrosia* (August–September). From October 2011 to the end of January 2012, no new important pollen types were found.

In February 2012, small amounts of *Corylus* and high amounts of Caryophyllaceae (28%, 26 February) were observed. These were followed by high counts for *Alnus* and *Taxus* (both March) as well as *Populus* and *Betula* (March–April). In May, *Quercus* was the most dominant pollen type and a notable amount of *Aesculus* was found. From June to September, *Ailanthus* (June), Poaceae (June–July), Urticaceae (July–August) and *Ambrosia* (August–September) were the most dominant pollen types. In October, all pollen types were below 15%. Again, *Betula* had the highest amount (12%) since May 2012.

Statistical analysis

For statistical analysis the samples were compared in their chronological order using χ -square-tests (Table IV). The first sample taken (1 April 2011) was compared to the second (10 April 2011), the second to the third (22 April 2011) and so on. In the living room, 52 out of 68 comparisons showed a significant difference ($p < 0.05$), whereas 16 out of 68 did not ($p > 0.05$). In the kitchen, 53 out of 68 comparisons showed differences, while 15 did not. Additionally, living room and kitchen samples from the same date were compared (Table V). According to these results, 59 out of 69 comparisons showed differences ($p < 0.05$), while ten did not ($p > 0.05$).

Discussion

The abundance of pollen in the environment has been investigated in numerous studies (Fischbach

1976; Jäger et al. 1991; Spieksma et al. 1995; Stuart et al. 2006). However, most of them focus on outdoor pollen (Romano et al. 1991; Horrocks et al. 1998, 1999) and only a few on indoor pollen (Yankova 1991; Tormo-Molina et al. 2009). Most of these indoor studies collected floating pollen from an elevated sampling site (Yankova 1991) and/or used pollen traps (Tormo-Molina et al. 2009). Only in a few cases, pollen was sampled directly from the floor. Enomoto et al. (2004) sampled pollen that had settled on the floor by using a vacuum cleaner and collected airborne pollen with petri dishes. They report, that much more pollen can be found on ground level, whereas nearly no pollen is found above a height of 50 cm.

Indoor pollen studies from a forensic point of view are very rare. Morgan et al. (2008, 2014) investigated the spatial distribution of pollen from cut flowers throughout a room. Sampling was done with adhesive tape mounted on scanning electron microscope stubs. This study mainly focused on pollen from the flowers, which they deliberately put there, but not on all the other pollen types within the room. They stated that pollen from cut flowers can stay in a room days and weeks after the flowers have been removed.

Seasonal changes

In the present study, all pollen types sampled from the floor were documented over two years. Results from the kitchen as well as the living room clearly show that most samples were dominated by pollen from anemophilous plants such as Poaceae, Urticaceae, *Ambrosia* or *Betula* (Tables II, III). The results also show that the pollen content of both rooms changed throughout the year. These changes correlate with the flowering season. Such a correlation is also reported by Caramiello et al. (1990) and Emberlin et al. (1990).

After the flowering season, much less pollen entered the flat and the pollen content did not change as significantly as before. This stagnation was statistically confirmed (Table IV). The χ -square-tests show that the pollen content in autumn/winter changed less often than during the flowering season. However, there are two remarkable observations: (1) The percentages of Urticaceae and *Ambrosia* decreased; (2) others like *Betula* (both rooms, October 2012) and Poaceae (mostly in the living room from November 2011) increased disproportionately. This leads to the following question: How can it happen that some pollen types (e.g. *Ambrosia*) are specifically disappearing while others (e.g. *Betula*) reappear in higher numbers long after their flowering season? This effect was probably generated by normal cleaning activities as

Table I. All pollen types assigned to their associated plant families.

Plant family	Pollen group
Adoxaceae (399)	<i>Sambucus</i> (358), <i>Viburnum</i> (41)
Alstroemeriaceae (3)	<i>Alstroemeria</i> (3)
Amaranthaceae (1434)	
Amaryllidaceae (11)	<i>Clivia</i> (11)
Apiaceae (56)	
Araceae (95)	<i>Spathiphyllum</i> (95)
Araliaceae (29)	<i>Hedera</i> (29)
Asteraceae (5273)	<i>Ambrosia</i> (3781), <i>Artemisia</i> (897), tubuliflorae Asteraceae (502) liguliflorae Asteraceae (89), <i>Centaurea</i> (4)
Berberidaceae (2)	
Betulaceae (6768)	<i>Betula</i> (3608), <i>Carpinus</i> (1450), <i>Alnus</i> (1063), <i>Corylus</i> (642) irregular shaped <i>Carpinus</i> (2), irregular shaped <i>Betula</i> (2), irregular shaped <i>Corylus</i> (1)
Bignoniaceae (1)	<i>Catalpa</i> (1)
Blasminaceae (38)	<i>Impatiens</i> (38)
Boraganiaceae (2)	
Brassicaceae (153)	
Buxaceae (4)	<i>Buxus</i> (4)
Caprifoliaceae (7)	<i>Lonicera</i> (6), other (1)
Caryophyllaceae (237)	
Cistaceae (1)	
Convolvulaceae (1)	<i>Convolvulus</i> (1)
Cornaceae (3)	<i>Cornus</i> (3)
Cupressaceae (1116)	
Cyperaceae (144)	
Ericaceae (31)	
Euphorbiaceae (401)	<i>Mercurialis</i> (395), other (6)
Fabaceae (1009)	<i>Sophora</i> (923), Caesalpinioideae (46), <i>Robinia</i> (26), other (14)
Fagaceae (4315)	<i>Quercus</i> (2972), <i>Fagus</i> (1183), <i>Castanea</i> (159), irregular shaped <i>Fagus</i> (1)
Fern spores (142)	<i>Dryopteris</i> (52), monolete spores (49), trilete spores (41)
Geraniaceae (1)	<i>Geranium</i> (1)
Ginkgoaceae (9)	<i>Ginkgo</i> (9)
Juglandaceae (451)	<i>Juglans</i> (451)
Lamiaceae (27)	
Liliaceae (96)	<i>Lilium</i> (84), <i>Tulipa</i> (12)
Malvaceae (976)	<i>Tilia</i> (959), irregular shaped <i>Tilia</i> (17)
Moraceae (432)	
Myrtaceae (3)	
Oleaceae (1983)	<i>Fraxinus</i> (1669), <i>Fraxinus ornus</i> (252), other (62)
Orobanchaceae (1)	
Pinaceae (9469)	<i>Pinus</i> (8826), <i>Picea</i> (599), <i>Abies</i> (37), irregular shaped <i>Pinus</i> (5), <i>Larix</i> (2)
Plantaginaceae (1479)	<i>Plantago</i> (1479)
Platanaceae (659)	<i>Platanus</i> (658), irregular shaped <i>Platanus</i> (1)
Poaceae (5476)	Cereals (647), irregular shaped Poaceae (1), other (4829)
Polygonaceae (280)	<i>Rumex</i> (209), <i>Fagopyrum</i> (1), other (70)
Ranunculaceae (73)	
Rosaceae (127)	
Rubiaceae (71)	<i>Galium</i> (71)
Salicaceae (1867)	<i>Populus</i> (1569), <i>Salix</i> (298)
Sapindaceae (829)	<i>Aesculus</i> (479), <i>Acer</i> (349), irregular shaped <i>Aesculus</i> (1)
Scrophulariaceae (2)	
Simaroubaceae (1390)	<i>Ailanthus</i> (1390)
Solanaceae (3)	
Taxaceae (641)	<i>Taxus</i> (641)
Typhaceae (12)	<i>Typha</i> (11), <i>Sparganium</i> (1)
Ulmaceae (154)	<i>Ulmus</i> (154)
Urticaceae/Cannabaceae (6523)	<i>Celtis</i> (2), other (6521)
Vitaceae (248)	<i>Vitis</i> (177), <i>Parthenocissus</i> (71)
Others (228)	unidentified (225), sulcate pollen (2), polyade (1)

Note: In brackets sum of counted pollen grains in all samples.

Table II. Pollen counts in the living room.

Date	Urticaceae	Paaceae	Ambrosia	Betula	Quercus	Fraxinus	Populus	Plantago	Carpinus	Amaranthaceae	Alnus	Tilia	Sophora	Artemisia	Platanus	Corylus	Asculus	Other pollen	Pollen grains counted
01.04.2011	0	1	0	5	0	29	11	0	31	0	0	0	0	0	0	1	0	9	308
10.04.2011	0	0	1	8	2	26	7	0	48	0	0	0	0	0	0	0	0	3	374
22.04.2011	1	0	0	8	23	13	3	0	14	0	0	0	0	0	14	1	0	11	296
02.05.2011	1	1	0	7	38	3	1	0	5	0	0	0	0	0	4	3	2	20	1795
12.05.2011	2	4	0	7	19	7	1	2	7	0	0	2	0	1	5	2	1	17	256
22.05.2011	1	9	0	7	22	11	0	2	9	0	0	1	0	0	4	1	2	21	311
01.06.2011	3	46	0	3	7	1	0	3	1	0	4	3	0	0	0	0	0	15	263
10.06.2011	18	22	0	1	1	1	0	1	3	1	1	5	1	0	0	1	0	15	303
21.06.2011	9	41	1	1	3	1	1	9	1	1	0	8	3	0	1	0	0	11	304
01.07.2011	24	46	0	1	1	1	0	10	1	0	1	2	1	0	1	0	0	6	349
11.07.2011	32	23	0	1	1	2	0	13	1	2	1	1	7	0	0	1	1	6	349
22.07.2011	19	21	0	1	2	2	0	10	1	3	4	0	18	0	0	1	1	9	1404
02.08.2011	28	14	0	2	4	2	0	3	3	2	3	2	14	1	0	0	0	8	299
10.08.2011	53	8	4	2	1	1	0	7	1	1	1	1	6	4	0	0	0	5	318
21.08.2011	64	5	7	0	0	0	0	3	0	3	0	0	2	10	0	0	0	4	306
29.08.2011	52	5	22	0	0	1	0	5	0	3	0	0	1	5	0	1	0	5	345
01.09.2011	53	7	21	0	0	0	0	3	1	3	0	0	1	3	0	0	0	6	353
09.09.2011	35	13	28	2	1	1	0	5	0	3	0	0	1	3	0	0	0	4	305
20.09.2011	25	10	22	1	0	1	0	10	1	9	1	0	2	4	0	0	1	8	299
28.09.2011	24	10	25	2	1	1	0	8	2	4	1	0	5	6	0	0	0	9	308
03.10.2011	13	8	26	3	2	1	1	5	1	10	2	2	2	5	1	1	0	12	300
11.10.2011	22	10	23	1	2	1	0	8	2	5	1	2	2	5	0	1	0	7	299
20.10.2011	22	9	18	2	4	3	0	6	2	4	1	0	2	6	1	0	1	11	298
01.11.2011	19	12	10	3	5	2	0	4	2	5	1	3	4	3	0	2	0	14	300
08.11.2011	19	16	16	5	4	3	0	4	3	4	1	1	2	1	0	1	0	10	296
18.11.2011	15	17	19	5	1	2	0	8	3	5	1	2	2	3	0	2	0	7	317
28.11.2011	18	12	9	6	5	4	0	7	6	3	1	2	1	3	1	1	0	13	304
01.12.2011	23	16	9	5	3	3	0	7	3	3	2	2	1	2	1	1	0	10	303
08.12.2011	26	17	7	4	4	5	1	3	5	2	1	3	1	1	1	0	0	11	304
19.12.2011	16	18	12	2	4	6	0	8	2	3	2	4	3	5	0	1	1	9	309
28.12.2011	17	17	9	6	2	3	1	5	3	3	3	1	9	2	2	1	0	8	303
08.01.2012	24	13	10	4	5	4	1	6	2	4	1	2	3	1	1	2	0	10	304
17.01.2012	24	15	9	6	3	3	3	4	5	2	3	3	3	3	1	1	0	10	309
28.01.2012	18	16	11	4	4	4	1	6	2	4	4	1	2	2	1	3	0	10	303
01.02.2012	12	16	12	5	3	7	1	5	4	2	1	3	1	2	3	2	0	15	295
07.02.2012	18	14	8	4	5	1	2	5	3	3	3	3	3	2	1	9	0	12	299
16.02.2012	25	15	7	4	5	4	1	3	3	2	1	1	3	1	1	6	0	10	300
26.02.2012	8	13	11	8	6	3	2	3	1	4	4	4	1	2	3	5	0	15	302
01.03.2012	10	16	11	6	5	2	0	4	2	5	2	2	1	3	1	3	1	19	301

(Continued)

Table II. (Continued).

Date	Urticaceae	Poaceae	Ambrosia	Betula	Quercus	Fraxinus	Populus	Plantago	Carpinus	Amaranthaceae	Alnus	Tilia	Sophora	Artemisia	Platanus	Corylus	Asculus	Other pollen	Pollen grains counted
07.03.2012	7	8	9	3	3	2	1	4	2	2	1	0	1	2	0	12	1	14	296
17.03.2012	5	6	3	2	2	0	3	1	0	2	26	1	1	0	0	5	0	39	304
28.03.2012	0	1	0	18	4	9	36	0	9	0	3	0	0	0	0	0	0	16	1191
02.04.2012	0	0	0	72	0	10	8	0	3	0	0	0	0	0	0	0	0	3	300
06.04.2012	0	0	0	57	1	9	14	0	6	0	4	1	0	0	0	0	0	8	305
16.04.2012	0	1	1	54	3	8	9	0	6	0	3	1	0	0	1	1	0	10	310
26.04.2012	2	2	1	39	3	4	15	0	5	1	4	3	0	0	5	1	0	16	305
03.05.2012	2	1	0	18	24	1	1	0	3	0	0	0	0	0	3	0	10	29	311
07.05.2012	6	4	0	14	25	3	3	1	3	0	0	0	0	0	4	1	7	24	293
16.05.2012	3	6	1	22	17	2	3	1	3	0	1	0	1	0	4	0	4	31	297
26.05.2012	0	32	0	14	4	1	1	0	0	0	3	1	4	0	1	0	1	36	310
01.06.2012	1	31	0	10	5	1	1	1	1	1	1	0	13	0	1	0	2	12	308
05.06.2012	2	34	0	7	5	1	2	0	1	0	3	10	1	0	1	0	1	11	303
15.06.2012	5	32	1	6	4	2	1	2	1	1	2	13	0	0	1	0	1	14	301
25.06.2012	21	32	0	5	1	0	1	4	2	2	1	14	1	0	0	0	1	10	305
02.07.2012	14	36	0	4	2	0	1	5	2	5	1	9	0	0	1	0	0	14	309
07.07.2012	26	24	0	4	2	1	1	10	1	2	2	6	1	0	0	1	1	13	315
15.07.2012	35	16	1	8	1	0	1	4	2	3	2	4	7	0	0	0	0	10	305
25.07.2012	49	11	0	3	2	1	1	5	1	3	1	3	4	0	0	0	0	10	312
01.08.2012	49	15	1	2	1	2	0	7	1	7	1	1	2	1	1	0	0	8	307
16.08.2012	13	11	0	1	1	1	0	9	1	12	0	0	4	37	0	0	0	6	304
24.08.2012	10	6	6	4	1	2	1	9	2	15	0	1	2	27	0	0	1	9	315
31.08.2012	36	12	15	3	1	1	1	3	0	9	1	1	1	2	0	2	0	7	315
03.09.2012	14	14	26	3	1	3	1	2	1	14	0	2	1	5	0	0	0	8	304
13.09.2012	12	9	32	3	2	1	0	4	1	14	2	2	1	3	0	1	0	8	309
23.09.2012	20	11	23	4	2	1	0	4	1	10	0	2	1	4	2	0	0	10	304
01.10.2012	4	13	14	7	6	1	2	4	3	6	1	2	1	3	1	2	2	19	295
04.10.2012	8	20	7	8	5	3	2	2	2	8	1	3	1	4	3	1	1	12	298
13.10.2012	7	10	13	16	5	2	1	3	1	6	0	5	1	2	1	1	1	15	303
24.10.2012	17	11	12	14	3	2	1	4	1	7	1	3	1	4	1	1	2	11	303

Note: Percentages of pollen types (rounded to integer) ordered by date. 'Pollen grains counted' gives the total number of pollen grains counted in this sample (excluding saccate pollen and unidentifiable palynomorphs). Within each pollen type, the ten highest values are bold.

well as cleaning in the course of the sampling procedure. Through these cleaning activities, many pollen grains were removed. It is assumed that cleaning is not selective for specific pollen types. Therefore, the same pollen types remained in the same ratios. Since inaccessible areas (e.g. under furniture) were cleaned less frequently than open areas (like the sampling areas), more pollen had accumulated there. Thus, pollen entering the flat would account for a larger share in open areas than at inaccessible areas. This resulted in a heterogeneous pollen accumulation within the apartment at the end of the flowering season. As pollen was gradually removed through cleaning at open areas, pollen from the latest flowering plants dominated there. In contrast, inaccessible areas contained pollen from the whole year, since they were not cleaned as intensively.

After the end of the flowering season, the normal cleaning activities removed most pollen grains from the open (sampling) areas. Also recognisable in the amount of pollen found in the samples was that spring/summer samples had remarkable more pollen than winter samples. Thus, most pollen grains in the apartment remain at inaccessible areas. It is further assumed that different vectors (e.g. wind, the two inhabitants, visitors) relocated pollen from the inaccessible areas to the open areas. This might explain the high amounts of Poaceae (November 2011–February 2012) and *Betula* (September–October 2012) that were recorded towards the end of the year up to spring. Poaceae and *Betula* pollen grains that still remained at inaccessible areas were moved to the sampling areas and, therefore, reappeared in the samples. Since so little pollen remained at the open areas, even a few pollen grains of *Betula* or Poaceae were able to generate a measurable effect. Such movements happen throughout the year, but cannot be recognised when there is still a lot of pollen at the open areas, which superimposes the pollen grains moved there. It seems unlikely that pollen from outside caused this effect, as the amount of pollen in the air decreases dramatically after the flowering season.

The persistence of allergenic pollen in indoor dust beyond the flowering season can be of high importance for allergological studies and could be important for people suffering from pollinosis (Yli-Panula & Ahlholm 1998).

Inter-room differences

The pollen content in kitchen and living room differ on most sampling days (Table V). As mentioned earlier, pollen from wind-pollinated taxa like Poaceae, Urticaceae or *Ambrosia* were dominant. These taxa grow throughout Vienna. Since wind

pollinated pollen can be found far away from the flowering plant and in almost all pollen samples (Bryant et al. 1990), it is almost impossible to pinpoint the individual plants these pollen grains originate from. However, some types are worth taking a closer look at because the amounts of pollen differ significantly between the two rooms.

Carpinus was very dominant in the living room in April 2011 (up to 48%), but not in the kitchen (maximum of 7%). This gradient might be explained by the position of the windows within the apartment and the adjacent vegetation. The windows of the living room open to a street on the north side where several large (up to 9 m) *Carpinus betulus* L. shrubs grow (Figure 1). However, *Ailanthus* was found in much higher numbers in the kitchen (up to 48%) than in the living room (up to 24%). The explanation is similar: the kitchen windows open to the south, where three *Ailanthus altissima* (Mill.) Swingle trees grow (Figure 1). These findings are in accordance with the study of Yankova (1991), who studied private dwellings in Sofia. The author also reports a significant influence of the local vegetation on the assemblage and quantity of indoor pollen.

Furthermore, in September 2011 and 2012, *Ambrosia* was very dominant in both rooms but always more abundant in the kitchen. Similar differences can be observed for *Betula*, *Quercus* and *Populus*, while Urticaceae, Poaceae, *Plantago*, *Tilia* and *Sophora* had higher amounts in the living room. The reason for these differences cannot be explained so far, as such plants are not growing in close vicinity (radius of 200 to 300 m) predominantly to the north or south side of the flat.

Pollen grains are affected by a variety of processes between dehiscence and recovery. With the increasing distance from the plant to a room, factors like wind speed and direction, objects that act as a physical barrier, interaction through animals, temperature, and sinking speed of pollen grains (Bryant et al. 1990; Wiltshire 2006) might have an even greater impact. Thus, for plants further away it is even more difficult to retrace how their pollen reached and entered the rooms. Nevertheless, the sporadic opening of the windows throughout the flat shall be mentioned as one possible reason, since Yankova (1991) reported that the pollen types and their abundances indoors were dependent on the duration of airing.

Annual differences

The years 2011 and 2012 showed similar results regarding the pollen types. The most important pollen types were found in both years at approximately

Table III. Pollen counts in the kitchen.

Date	Urticaceae	Poaceae	Ambrosia	Betula	Quercus	Fraxinus	Populus	Plantago	Carpinus	Amaranthaceae	Alnus	Tilia	Sophora	Artemisia	Platanus	Corylus	Asculus	Other pollen	Pollen grains counted
01.04.2011	2	1	0	3	0	17	23	0	5	0	18	0	0	0	0	10	0	17	319
10.04.2011	1	1	0	9	0	42	23	0	7	0	2	0	0	0	0	1	0	11	313
22.04.2011	2	1	0	4	40	3	2	0	3	0	0	0	0	0	11	1	1	24	317
02.05.2011	0	1	1	5	29	3	3	0	5	0	1	0	0	0	7	2	3	28	303
12.05.2011	3	4	0	10	15	9	3	1	5	0	1	0	0	0	5	4	4	25	278
22.05.2011	2	9	1	7	20	11	3	0	4	0	0	0	0	1	2	0	2	27	301
01.06.2011	4	22	4	5	13	3	1	0	4	2	3	4	0	0	2	2	2	15	323
10.06.2011	6	20	0	1	3	1	1	1	1	0	1	4	0	0	0	0	0	9	306
21.06.2011	13	26	1	5	3	2	0	5	2	0	2	7	0	0	0	0	1	13	302
01.07.2011	25	36	1	2	3	1	1	5	1	2	1	5	0	0	0	0	0	6	309
11.07.2011	19	25	0	3	4	2	2	8	2	3	1	4	1	0	1	0	2	12	310
22.07.2011	27	16	1	4	3	1	0	6	1	5	0	1	19	0	1	0	1	11	307
02.08.2011	33	10	0	2	2	1	1	4	1	3	1	1	17	1	2	1	1	9	307
10.08.2011	43	9	5	1	2	1	0	6	1	7	0	1	9	4	0	0	0	3	301
21.08.2011	54	3	7	1	0	0	0	4	0	4	1	0	1	15	1	1	1	6	303
29.08.2011	25	6	42	1	1	0	0	4	0	6	0	1	1	6	0	0	0	7	306
01.09.2011	27	7	40	1	1	0	0	2	1	7	1	0	1	3	0	0	0	6	307
09.09.2011	15	13	53	1	2	0	0	3	0	1	1	0	1	2	0	0	0	7	303
20.09.2011	17	8	38	0	1	0	0	4	0	5	1	1	2	2	0	0	1	16	305
28.09.2011	18	10	29	3	1	1	1	3	2	7	1	0	1	2	0	1	0	16	302
03.10.2011	9	11	22	4	3	2	1	4	1	7	3	2	2	3	0	0	0	21	309
11.10.2011	12	9	25	3	2	2	0	4	2	7	2	0	0	3	0	1	1	20	301
20.10.2011	11	8	25	2	8	3	1	4	2	4	2	2	1	3	2	1	0	13	306
01.11.2011	9	13	15	4	4	2	2	3	2	4	2	2	3	1	4	2	0	15	300
08.11.2011	12	10	14	4	5	3	1	2	2	4	1	2	2	4	1	1	0	16	301
18.11.2011	13	10	13	4	4	3	1	2	1	3	2	2	2	4	1	2	1	22	305
28.11.2011	10	13	13	5	3	3	1	5	3	7	2	1	1	3	1	2	2	18	303
01.12.2011	8	12	18	6	7	3	1	3	2	4	1	2	2	3	2	1	0	18	299
08.12.2011	9	12	13	5	4	2	1	4	2	3	1	2	1	2	2	1	0	28	312
19.12.2011	11	13	11	7	4	5	2	3	4	2	3	3	1	3	1	1	2	15	310
28.12.2011	7	11	10	6	3	2	2	2	3	4	3	2	2	2	1	1	1	26	307
08.01.2012	8	9	16	7	6	2	1	3	4	5	3	3	1	1	2	1	1	15	305
17.01.2012	15	9	11	11	4	4	3	3	4	1	3	2	1	3	0	2	1	12	305
28.01.2012	6	11	14	6	8	2	2	4	2	5	3	3	1	1	0	3	1	17	300
01.02.2012	7	16	17	6	7	4	2	3	2	4	1	2	1	2	1	1	1	13	305
07.02.2012	4	13	8	6	8	4	2	3	5	3	6	1	0	3	1	10	1	14	305
16.02.2012	8	11	10	11	3	7	3	2	2	3	5	2	1	4	1	5	1	13	304

(Continued)

Table III. (*Continued*).

Date	Urticaceae	Poaceae	Ambrosia	Betula	Quercus	Fraxinus	Populus	Plantago	Carpinus	Amaranthaceae	Alnus	Tilia	Sophora	Artemisia	Platanus	Corylus	Asculus	Other pollen	Pollengrains counted
26.02.2012	4	8	6	3	3	3	2	1	4	3	8	2	0	3	1	4	1	36	306
01.03.2012	4	8	11	7	4	1	1	2	2	1	3	3	1	3	2	5	1	34	305
07.03.2012	3	8	9	7	5	5	1	2	3	2	15	3	0	1	1	8	0	17	307
17.03.2012	3	2	1	1	1	1	11	0	1	0	27	1	0	1	0	8	0	36	311
28.03.2012	0	0	0	25	3	14	29	0	6	0	4	0	0	0	0	0	0	12	318
02.04.2012	1	1	0	39	0	12	18	0	7	0	3	0	0	0	0	2	0	12	309
06.04.2012	0	0	0	29	0	13	26	0	7	0	3	0	0	0	0	0	0	14	304
16.04.2012	0	0	0	46	2	11	10	0	5	0	3	0	0	0	5	1	0	11	309
26.04.2012	1	2	1	32	10	5	17	1	5	0	2	0	0	0	3	1	1	12	304
03.05.2012	0	1	0	9	30	1	0	0	5	0	0	0	0	0	3	0	8	37	304
07.05.2012	4	3	1	13	30	1	2	0	2	0	1	0	0	0	3	0	11	23	306
16.05.2012	4	7	0	12	25	4	4	0	2	0	1	0	1	0	5	0	8	25	303
26.05.2012	1	19	0	16	11	2	4	1	1	0	1	0	0	0	3	0	5	29	310
01.06.2012	1	31	1	5	5	1	8	1	2	0	1	0	0	0	2	0	3	15	306
05.06.2012	1	19	0	7	8	1	2	1	0	1	1	6	0	0	2	0	3	14	308
15.06.2012	3	16	1	5	5	1	1	1	2	0	4	5	0	0	0	0	1	15	300
25.06.2012	12	27	1	4	3	1	2	3	1	3	3	11	0	0	2	1	2	17	313
02.07.2012	7	26	0	7	8	3	4	2	1	4	2	5	1	0	1	0	2	19	307
07.07.2012	14	30	1	6	4	1	2	2	2	4	1	5	1	0	1	1	2	18	296
15.07.2012	18	14	2	6	7	2	3	5	2	2	1	4	2	1	2	1	3	21	305
25.07.2012	24	14	0	9	2	2	2	7	1	5	1	2	4	0	4	0	2	15	301
01.08.2012	30	14	2	6	3	1	1	5	2	5	1	3	2	2	2	1	2	16	307
16.08.2012	49	6	1	5	2	0	0	2	1	6	1	1	2	17	1	0	0	5	311
24.08.2012	39	4	10	3	4	1	1	4	1	11	1	1	2	8	0	0	1	7	311
31.08.2012	15	10	23	2	1	8	2	3	1	9	1	2	0	5	3	1	1	9	316
03.09.2012	14	7	30	3	3	1	1	3	1	13	3	1	3	4	2	1	1	10	305
13.09.2012	6	13	50	1	1	1	0	1	0	11	1	1	1	2	1	1	1	8	307
23.09.2012	5	8	25	9	8	2	1	3	2	8	2	3	1	3	1	2	1	12	302
01.10.2012	6	11	9	9	6	3	2	3	4	7	1	2	2	5	3	1	2	16	307
04.10.2012	7	8	14	12	7	2	1	3	2	10	2	4	4	2	0	4	0	11	293
13.10.2012	6	7	13	12	7	2	3	3	3	6	1	2	1	2	2	3	1	17	307
24.10.2012	7	7	9	6	11	3	3	2	3	5	4	2	0	1	2	3	1	23	302

Note: Percentages of pollen types (rounded to integer) ordered by date. 'Pollen grains counted' gives the total number of pollen grains counted in this sample (excluding saccate pollen and unidentifiable palynomorphs). Within each pollen type, the ten highest values are bold.

Table IV. χ^2 -Square-tests for chronologically adjacent samples.

Date 1	Date 2	d.f.	Living room		d.f.	Kitchen	
			χ^2	<i>p</i> -Value		χ^2	<i>p</i> -Value
01.04.2011	10.04.2011	6	44.04	0.000	9	135.92	0.000
10.04.2011	22.04.2011	8	231.58	0.000	10	406.18	0.000
22.04.2011	02.05.2011	12	186.69	0.000	10	17.07	0.073
02.05.2011	12.05.2011	10	348.87	0.000	13	54.96	0.000
12.05.2011	22.05.2011	10	28.36	0.002	14	43.76	0.000
22.05.2011	01.06.2011	11	162.37	0.000	16	100.30	0.000
01.06.2011	10.06.2011	7	123.52	0.000	12	154.07	0.000
10.06.2011	21.06.2011	9	85.03	0.000	7	67.42	0.000
21.06.2011	01.07.2011	7	57.11	0.000	7	37.03	0.000
01.07.2011	11.07.2011	6	46.81	0.000	8	13.49	0.096
11.07.2011	22.07.2011	11	50.42	0.000	9	82.53	0.000
22.07.2011	02.08.2011	9	61.84	0.000	8	11.96	0.153
02.08.2011	10.08.2011	10	87.42	0.000	11	38.05	0.000
10.08.2011	21.08.2011	6	22.86	0.001	8	65.66	0.000
21.08.2011	29.08.2011	6	34.73	0.000	5	126.09	0.000
29.08.2011	01.09.2011	6	3.23	0.779	6	7.69	0.262
01.09.2011	09.09.2011	6	22.97	0.001	6	34.99	0.000
09.09.2011	20.09.2011	6	21.93	0.001	6	29.34	0.000
20.09.2011	28.09.2011	7	12.58	0.083	6	4.96	0.548
28.09.2011	03.10.2011	8	27.21	0.001	12	34.65	0.001
03.10.2011	11.10.2011	10	16.44	0.088	12	17.28	0.139
11.10.2011	20.10.2011	12	12.12	0.436	15	32.71	0.005
20.10.2011	01.11.2011	14	30.02	0.008	18	36.19	0.007
01.11.2011	08.11.2011	15	17.62	0.283	17	22.40	0.170
08.11.2011	18.11.2011	14	22.80	0.064	16	21.10	0.175
18.11.2011	28.11.2011	14	37.49	0.001	14	17.35	0.238
28.11.2011	01.12.2011	14	13.33	0.500	12	19.00	0.088
01.12.2011	08.12.2011	12	14.30	0.282	15	30.26	0.011
08.12.2011	19.12.2011	13	37.27	0.000	17	64.46	0.000
19.12.2011	28.12.2011	15	37.63	0.001	16	31.38	0.012
28.12.2011	08.01.2012	13	25.86	0.018	14	38.44	0.000
08.01.2012	17.01.2012	14	17.99	0.207	17	33.81	0.009
17.01.2012	28.01.2012	15	21.27	0.128	16	43.29	0.000
28.01.2012	01.02.2012	13	11.64	0.557	16	24.64	0.076
01.02.2012	07.02.2012	14	41.62	0.000	16	49.62	0.000
07.02.2012	16.02.2012	14	21.84	0.082	14	32.36	0.004
16.02.2012	26.02.2012	15	57.98	0.000	14	124.38	0.000
26.02.2012	01.03.2012	15	42.75	0.000	14	28.89	0.011
01.03.2012	07.03.2012	15	122.01	0.000	15	62.23	0.000
07.03.2012	17.03.2012	12	131.65	0.000	14	195.28	0.000
17.03.2012	28.03.2012	7	510.69	0.000	9	268.22	0.000
28.03.2012	02.04.2012	8	371.11	0.000	7	24.49	0.001
02.04.2012	06.04.2012	5	37.25	0.000	7	12.63	0.082
06.04.2012	16.04.2012	7	15.85	0.026	9	54.52	0.000
16.04.2012	26.04.2012	8	29.82	0.000	9	38.38	0.000
26.04.2012	03.05.2012	12	237.79	0.000	11	217.21	0.000
03.05.2012	07.05.2012	13	37.95	0.000	11	109.63	0.000
07.05.2012	16.05.2012	14	30.03	0.008	13	33.51	0.001
16.05.2012	26.05.2012	15	191.51	0.000	12	66.43	0.000
26.05.2012	01.06.2012	8	114.05	0.000	9	105.93	0.000
01.06.2012	05.06.2012	5	5.24	0.387	7	37.38	0.000
05.06.2012	15.06.2012	7	7.84	0.347	9	25.87	0.002
15.06.2012	25.06.2012	7	64.03	0.000	10	118.42	0.000
25.06.2012	02.07.2012	8	20.58	0.008	12	28.21	0.005
02.07.2012	07.07.2012	7	28.90	0.000	11	17.46	0.095
07.07.2012	15.07.2012	9	39.89	0.000	13	30.98	0.003
15.07.2012	25.07.2012	8	22.32	0.004	14	29.65	0.009
25.07.2012	01.08.2012	7	14.46	0.044	11	13.00	0.293
01.08.2012	16.08.2012	5	62.37	0.000	8	64.92	0.000
16.08.2012	24.08.2012	4	2.77	0.597	9	55.64	0.000

(Continued)

Table IV. (Continued).

Date 1	Date 2	d.f.	Living room		d.f.	Kitchen	
			χ^2	<i>p</i> -Value		χ^2	<i>p</i> -Value
24.08.2012	31.08.2012	7	88.44	0.000	11	83.29	0.000
31.08.2012	03.09.2012	9	51.67	0.000	12	33.20	0.001
03.09.2012	13.09.2012	10	17.93	0.056	8	43.93	0.000
13.09.2012	23.09.2012	10	18.57	0.046	7	63.59	0.000
23.09.2012	01.10.2012	10	43.86	0.000	13	36.10	0.001
01.10.2012	04.10.2012	15	25.73	0.041	15	28.62	0.018
04.10.2012	13.10.2012	12	33.23	0.001	16	34.09	0.005
13.10.2012	24.10.2012	13	24.14	0.030	18	26.74	0.084

Note: In the living room, 52 out of 68 comparisons showed a significant difference ($p < 0.05$) in the pollen assemblages from one sampling date to the next, 16 out of 68 did not ($p > 0.05$; marked in bold). In the kitchen, 53 out of 68 comparisons showed differences while 15 did not. *p*-Values are rounded to three decimal places.

Table V. χ -Square-tests for living room and kitchen from the same sampling date.

Date	2011			Date	2012		
	d.f.	χ^2	<i>p</i> -Value		d.f.	χ^2	<i>p</i> -Value
01.04.2011	9	157.78	0.000	08.01.2012	13	51.45	0.000
10.04.2011	5	154.20	0.000	17.01.2012	14	22.73	0.065
22.04.2011	10	89.26	0.000	28.01.2012	15	44.71	0.000
02.05.2011	12	63.57	0.000	01.02.2012	13	25.47	0.020
12.05.2011	12	23.69	0.022	07.02.2012	15	51.55	0.000
22.05.2011	11	15.90	0.145	16.02.2012	15	68.67	0.000
01.06.2011	12	69.64	0.000	26.02.2012	17	127.70	0.000
10.06.2011	7	49.41	0.000	01.03.2012	14	40.37	0.000
21.06.2011	7	42.89	0.000	07.03.2012	14	53.24	0.000
01.07.2011	7	44.45	0.000	17.03.2012	11	57.43	0.000
11.07.2011	11	46.70	0.000	28.03.2012	8	37.90	0.000
22.07.2011	9	39.80	0.000	02.04.2012	5	66.41	0.000
02.08.2011	11	18.62	0.068	06.04.2012	6	61.31	0.000
10.08.2011	8	15.10	0.057	16.04.2012	9	20.83	0.013
21.08.2011	5	7.99	0.157	26.04.2012	10	32.27	0.000
29.08.2011	6	57.80	0.000	03.05.2012	8	30.16	0.000
01.09.2011	6	51.32	0.000	07.05.2012	12	15.82	0.200
09.09.2011	5	50.52	0.000	16.05.2012	15	44.44	0.000
20.09.2011	9	37.95	0.000	26.05.2012	10	73.47	0.000
28.09.2011	10	28.45	0.002	01.06.2012	7	32.28	0.000
03.10.2011	13	28.98	0.007	05.06.2012	6	37.25	0.000
11.10.2011	11	28.65	0.003	15.06.2012	7	63.91	0.000
20.10.2011	14	31.47	0.005	25.06.2012	9	15.97	0.067
01.11.2011	17	40.29	0.001	02.07.2012	10	48.34	0.000
08.11.2011	14	31.72	0.004	07.07.2012	9	36.54	0.000
18.11.2011	16	44.72	0.000	15.07.2012	11	47.95	0.000
28.11.2011	13	27.27	0.011	25.07.2012	10	49.71	0.000
01.12.2011	15	67.65	0.000	01.08.2012	6	18.26	0.006
08.12.2011	14	106.63	0.000	16.08.2012	6	13.36	0.038
19.12.2011	15	37.24	0.001	24.08.2012	8	34.93	0.000
28.12.2011	14	73.51	0.000	31.08.2012	9	56.99	0.000
				03.09.2012	10	20.98	0.021
				13.09.2012	8	37.16	0.000
				23.09.2012	12	56.38	0.000
				01.10.2012	17	16.44	0.493
				04.10.2012	14	48.64	0.000
				13.10.2012	17	26.81	0.061
				24.10.2012	13	58.76	0.000

Note: 59 out of 69 comparisons showed a significant difference ($p < 0.05$) in the pollen assemblages while 10 out of 69 did not ($p > 0.05$; marked in bold). *p*-Values are rounded to three decimal places.

the same time of the year and more or less in the same amount (Tables II, III). However, some important differences between the two years were documented. In April 2011, a lot of *Carpinus* was found in the living room (up to 48%), while in 2012, *Carpinus* only reached 9% at the end of March. This might be explained by the fact that in 2012, the *Carpinus betulus* shrubs, at least the ones adjacent to the flat, dropped their inflorescences before anthesis. Thus, their impact was less than in 2011.

In 2012, *Betula* achieved much higher counts (maximum 72%, 2 April, living room) than in 2011 (maximum 10%, 12 May, kitchen). Annual variations in the amount of *Betula* pollen were also reported by Emberlin et al. (1993). In several other studies, a two-year-rhythm of high and low *Betula* pollen concentrations was reported (Andersen 1980; Jäger et al. 1991; Spieksma et al. 1995).

Other findings

Pollen from zoophilous (animal-pollinated) plants was found in much smaller amounts than pollen from anemophilous (wind-pollinated) and ambophilous (wind- and animal-pollinated) plants. This might be explained by the fact that pollen from animal-pollinated plants is usually not airborne, and can only be expected close to the flowering plant (Klaus 1987; Fægri et al. 1992; Eyring 1996). They were possibly transferred into the apartment by garments, as it is well known, that shoes (Horrocks et al. 1999; Riding et al. 2007) and clothing (Bull et al. 2006; Zavada et al. 2007; Takahashi et al. 2008; Jantunen & Saarinen 2011) can be vectors for pollen.

Additionally, pollen from Caryophyllaceae (March 2012), *Lilium* (May 2012) and *Spathiphyllum* (throughout both years) were found in higher amounts. The source for these pollen grains were bunches of flowers and pot plants. Cut flowers of Caryophyllaceae and *Lilium* were present in the kitchen at the time in question. A *Spathiphyllum* pot plant was also growing in the kitchen of the flat, but this specimen did not flower after December 2010. With 8% on the 28 December 2011 (kitchen), *Spathiphyllum* had remarkable high counts, even a year after the plant flowered for the last time. Yet, after two years, traces of *Spathiphyllum* were still present. A pollen transfer from cut flowers to a room was also reported by Morgan et al. (2014). Such rare pollen types can act as a marker that makes an otherwise common pollen spectrum unique, which can be of high importance for forensic investigations.

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