

Cross-Reactivity Between Pollen Extracts from Six *Artemisia* Species

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Abstract

Pollen extracts of six different *Artemisia* species, *A. vulgaris*, *A. scoparia*, *A. princeps*, *A. tridentata*, *A. annua*, and *A. campestris* were compared using SDS-PAGE, IEF, immunoblotting, and immunoelectrophoretic methods. The band patterns obtained after SDS-PAGE and IEF showed a large degree of similarity between the extracts. Immunoblotting of these gels using a pool of sera from patients allergic to *A. vulgaris* gave essentially the same IgE-binding band pattern with all the extracts, demonstrating an extensive degree of cross-reactivity between *A. vulgaris* and the other *Artemisia* species. FRIE using a polyspecific antiserum against *A. vulgaris* showed that all the extracts contained several antigens that were immunologically identical to antigens in *A. vulgaris* extract. Antigens showing immunological identity to the important *A. vulgaris* allergens Ag 12 and Art v II were present in all the extracts. The cross-reactivity between *A. vulgaris* and *A. princeps* was further verified by screening of ten Korean and nine Norwegian individual patient sera against extracts of both species in SDS-PAGE or IEF immunoblotting. Both groups of patients had essentially the same pattern of reactivity towards both pollen extracts.

Key words

Artemisia spp., pollen, allergens, cross-reactivity.

Abbreviations and Symbols

CIE:	crossed immunoelectrophoresis
CRIE:	crossed radioimmunoelectrophoresis
FRIE:	fused rocket immunoelectrophoresis
Ag:	antigen
IgE:	immunoglobulin E
SDS:	sodium dodecyl sulphate
PAGE:	polyacrylamide gel electrophoresis
IEF:	isoelectric focusing
NCM:	nitrocellulose membrane
CBB:	Coomassie brilliant blue
PAS:	periodic acid Schiff
ELISA:	enzyme-linked immunosorbent assay
MW:	molecular weight
pI:	isoelectric point
RAST:	radioallergosorbent test

Introduction

Pollen from mugwort, *Artemisia vulgaris*, is a common cause of allergy in Europe in late summer (late July – early September) (1–3). The antigenic and allergenic composition of mugwort pollen extracts have been investigated by several groups using CIE/CRIE (4–7). Up to 43 antigens have been recorded, of which 31 were found to migrate towards the anode. 8–11 different allergens were identified in these studies and 1–2 of these allergens were classified as major allergens (5, 6). The MW and pI distribution of the mugwort pollen allergens was recently investigated by Nilsen et al. (8) who identified at least 15 IgE-binding bands after SDS-PAGE/immunoblotting and at least 16 IgE-binding bands after IEF/immunoblotting.

Two mugwort pollen allergens Art v I (9) and Art v II (10) have been isolated and further characterized.

Pollen samples from two other *Artemisia* spp., *A. scoparia* and *A. princeps*, have been shown to be important causes of allergic reactions in India (11) and Korea (12), respectively. Extracts of *A. scoparia* have been investigated (11) and 37 anodic antigens were identified by CIE, and isolated; one allergen, denoted Art VIb was partly characterized. Park et al. (13) detected 16 IgE-binding components in *A. princeps* extracts by SDS-PAGE/immunoblotting.

Few studies have investigated the allergenic cross-reactivity between different *Artemisia* spp. Lin et al. (14) observed extensive cross-reactivity between pollen extracts from nine different *Artemisia* spp. by ELISA-inhibition assays. Extracts of *A. scoparia* and *A. princeps* pollen were not included in this study, and the individual allergens responsible for the cross-reactivity were not identified. The reports on the allergen composition of *A. scoparia* and *A. princeps* pollen extracts (11, 13) suggested similarities between allergens from these species and allergens from *A. vulgaris*. It was therefore of interest to compare these extracts with regard to allergenic cross-reactivity using methods that identified individual allergens. Three other *Artemisia* species were also included in the study, *A. tridentata* which is an evergreen bush growing in the western part of U.S.A. and *A. campestris* and *A. annua* which are Eurasian plants.

Materials and Methods

Pollen

Pollen samples from *Artemisia vulgaris*, *A. annua*, *A. campestris*, and *A. tridentata* were delivered from Allergen AB (Engelholm, Sweden, batch nos. 010182121, 031984201, 015085901, and 052584902, respectively). Pollen from *A. princeps* (defatted) was obtained from Hae Sim Park, Seoul, Korea, and those from *A. scoparia* from S. V. Gangal, New Delhi, India.

Scanning electron microscopy

The electron micrographs were recorded in a Jeol JSM-35C scanning electron microscope and photographed on Ilford film type FP4. The samples were prepared by placing the pollen on stubs and coating them with gold/palladium (60% Au + 40% Pd) by means of a sputter coater unit type Polaron E 5000.

Rabbit antibodies

Rabbit antibodies against Art v II (Anti-Art v II) were prepared by immunization with immunoprecipitates of the allergen obtained by CIE essentially as described by Harboe and Closs (15). Rabbit antibodies against Ag12⁴ (anti-Ag 12) and a polyspecific antiserum against mugwort (a Av 0979, 120190 A6), anti-mugwort, were kindly provided by Dr. H. Ipsen, ALK-Research, Hørsholm, Denmark.

Human sera

A serum pool was prepared from equal volumes of sera from 64 individuals having RAST-class 3 against *A. vulgaris* pollen. Nine of the individual sera from Norwegian *A. vulgaris* allergic patients described by Nilsen et al. (8) were used in this study. Ten sera from Korean allergic patients showing positive skin prick test against a mixture of different *Artemisia* species were also used.

Pollen extracts

All pollen extracts were prepared essentially as described earlier (16).

Protein content

The modified Lowry method (17) was used for the determination of the total protein content of the extracts using bovine serum albumin as standard.

Carbohydrate content

Total carbohydrate content and the monosaccharide composition of the extracts was determined by GLC after methanolysis and TMSi-derivatization as previously described (10).

SDS-PAGE and analytical IEF

Both SDS-PAGE (15% T, 2.7% C) and IEF (pH 3.5–10) were performed according to the procedures previously described (8). Both gels were stained for protein with CBB and carbohydrate by the PAS method (18).

Immunoblotting

Immunoblotting both of SDS-PAGE and IEF gels were performed essentially as described by Nilsen et al. (8) using the Mini-Trans-Blot[®] or the Trans-Blot[®] cell (Bio-Rad). After blotting and blocking of the NCM, the membrane was incubated with patient serum (1–3), followed by ¹²⁵I anti-IgE (3,000,000 cpm/ml). IgE-binding bands were detected by autoradiography.

Immunoelectrophoretic methods

FRIE and tandem-CIE were performed essentially as described by Harboe and Just-Svendsen (19) and Krøll (20), respectively. The FRIE experiments were performed using anti-Art v II (45 µl/cm²), anti-Ag-12 (5.5 µl/cm² in the anodic gel, 10.0 µl/cm² in the cathodic gel), or anti-mugwort antiserum (10 µl/cm²) in the antibody-containing gel. The gels were stained with CBB. Tandem-CIE was performed using anti-mugwort antiserum (12.5 µl/cm²) in the antibody-containing gels.

Results and Discussion

Pollen morphology

Pollen from the six *Artemisia* species had different colours, varying between white, yellow, and green. The difference in colour may be due to different degree of maturity, but may also be explained by the fact that the degree of purity of the pollen preparations differed. For example, the green pollen preparation of *A. scoparia* was found to contain significant amounts of non-pollen plant material when examined by scanning electron microscopy.

Scanning electron microscopy of the pollen showed a great degree of morphological similarity (Fig. 1). We observed that the pollen from *A. scoparia* appeared to be smaller than those from the other plants. This is consistent with what was found both by Singh and Joshi (21) and Lodari et al. (22), but the differences are so small that they cannot be used for differentiation between *A. scoparia* and the other *Artemisia* species.

⁴ The antigen numbers used in this presentation refer to the designations used by Smetstad Paulsen et al. (1985). Ag 12, Ag 9, and Ag 13, correspond to the allergens denoted Ag 20, Ag 4, and Ag 11, respectively, by Ipsen et al. (1985) (Ipsen, personal communication).

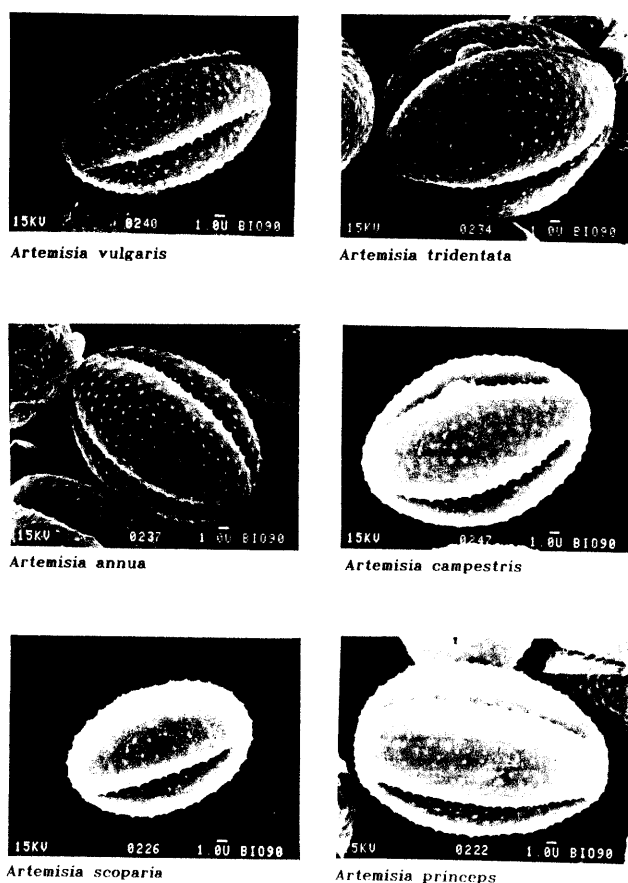


Fig. 1 Scanning electron microscopy of pollen from the six *Artemisia* spp. studied. The pictures are taken equatorial using a Jeol JSM-35C electron microscope after preparation of the pollen with gold/palladium (60 % Au/40 % Pd). The marker on the pictures is 1.0 μ m.

Chemical composition of the pollen extracts

The pollen extracts were prepared as described previously (16) without defatting, except for the pollen from *A. princeps*, which had been defatted by H. S. Park prior to sending the material. Defatting of pollen will normally not influence on the composition of the pollen extracts (23).

The extracts were characterized with respect to the content of total protein, total carbohydrate, and the monosaccharide composition (Table 1). Extracts of *A. scoparia* pollen had the highest apparent protein content and the lowest carbohydrate content. This result should, however, be viewed in the light of the observation that *A. scoparia* pollen preparations contained significant

amounts of non-pollen plant material. The results obtained for the chemical composition of *A. scoparia* extract may thus not be directly comparable with the results obtained for the other pollen extracts.

SDS-PAGE, transfer to NCM, and detection of IgE-binding components

SDS-PAGE was performed with extracts which had been dialysed with a cut off of 3,500. After staining of the gel for protein (with CBB) or for carbohydrate (with PAS) (Figs. 2 A and B), similar band patterns were obtained for all the extracts, especially in the MW region 25,000–90,000. The similarity in the band patterns was also evident after transfer to NCM and detection of IgE-binding components using a pool of sera from *A. vulgaris* allergic patients (Fig. 2 C). All the extracts seemed to

Pollen from	Protein ^a content	Carbohydr. ^b content	Ara	Monosaccharide composition ^{b,c}				
				Rha	Xyl	Gal	Glc	GalA
<i>A. vulgaris</i>	11.4	31.6	21.2	1.2	1.2	4.4	2.3	1.3
<i>A. tridentata</i>	14.1	52.5	39.1	2.9	0.8	7.6	1.9	0.1
<i>A. annua</i>	13.0	44.7	36.4	0.9	0.8	5.0	1.9	0.7
<i>A. campestris</i>	9.5	45.6	36.9	0.8	0.8	4.1	2.3	0.7
<i>A. scoparia</i>	17.9	26.2	19.1	0.9	0.8	2.9	1.9	0.6
<i>A. princeps</i>	14.2	52.4	41.6	0.8	0.9	6.6	1.9	0.7

^a Determined by the modified Lowry method.^b Determined by gas chromatography as the TMS-derivatives of the methylglycosides.^c N-Acetylglucosamine was present in trace amounts in all extracts.**Table 1** Total protein and carbohydrate content of the six different pollen extract preparations.**Fig. 2** SDS-PAGE (15% T, 2.7% C) and immunoblotting of the six *Artemisia* spp. pollen extracts. **A)** Gel stained for protein using CBB, **B)** gel stained with PAS to detect carbohydrate-containing components, and **C)** binding of human IgE to the different components detected by in-cubation of the NCM with a pool of sera from *A. vulgaris* allergic patients, followed by (¹²⁵I)-anti-IgE and autoradiography. The lanes marked v, t, a, c, s and p correspond to extracts from *A. vulgaris*, *A. tridentata*, *A. annua*, *A. campestris*, *A. scoparia*, and *A. princeps*.

contain IgE-binding bands corresponding to most of the IgE-binding bands in the *A. vulgaris* extract, although the staining intensity of the different bands differed between the extracts. Five IgE-binding components previously identified as important in *A. vulgaris* (8), i.e. Ag 12 (diffuse band with MW about 28,000), Ag 13 (MW 61,000), and three bands with MWs about 12,000, 39,000 and 43,000 seemed to be present in all the extracts. The allergen *Art v* II (MW 20,000) (16) does not bind IgE after SDS-PAGE (8), but a weak band corresponding to this allergen seemed to be present in the CBB-stained gel of all the extracts (with the possible exception of *A. scoparia*).

In general, the greatest differences between the different allergen extracts were found in the low molecular weight region (<25,000). *A. scoparia* extract which differed most from the other allergen extracts, gave only one band (MW about 13,000) in this region. One reason for the observed differences between the allergen extracts may be variations in the maturity of the pollen from the different species and the purity of the pollen samples.

IEF, transfer to NCM, and detection of IgE binding components

IEF of the six different *Artemisia* extracts also gave very similar band patterns, especially in the pI region between 4.15 and 6.20 (Fig. 3). In the CBB-stained gel (Fig. 3A) at least 21 protein bands could be observed in most of the extracts. About 12 of these bands contained carbohydrate as demonstrated by PAS-staining (results not shown). The extract of *A. tridentata* differed from the other extracts by containing a strong protein band with pI 8.70.

Detection of IgE-binding components using a pool of sera from *A. vulgaris* allergic patients indicated that all the extracts contained more or less of the same IgE-binding components in the anodic region (Fig. 3B). Larger differences in the band patterns were observed in the cathodic region where especially the extracts of *A. tridentata* and *A. campestris* gave a larger number of more intensely stained IgE-binding bands than *A. vulgaris*.

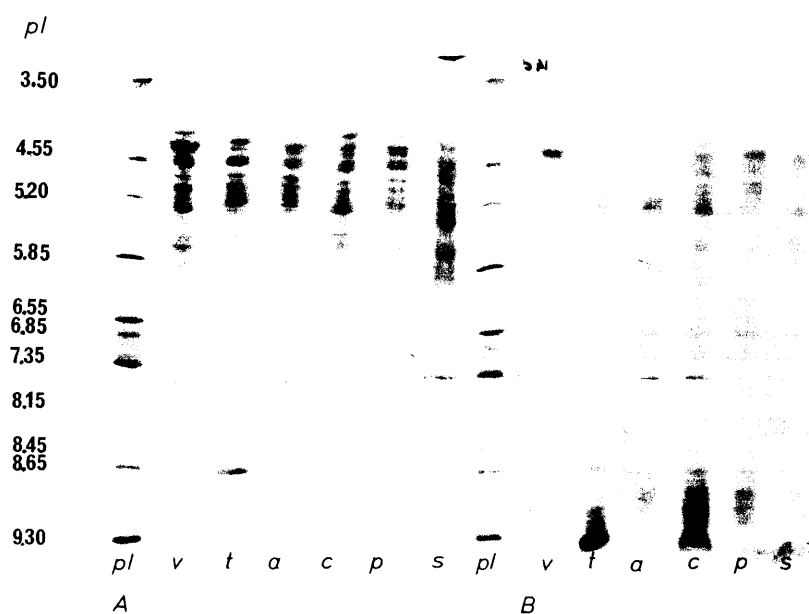


Fig. 3 IEF (pH gradient 3.5–10) and immunoblotting of the *Artemisia* spp. pollen extracts. **A**) Gel stained with CBB, **B**) Detection of IgE-binding

components as described in the legend of Fig. 2. The lanes are marked as described in Fig. 2.

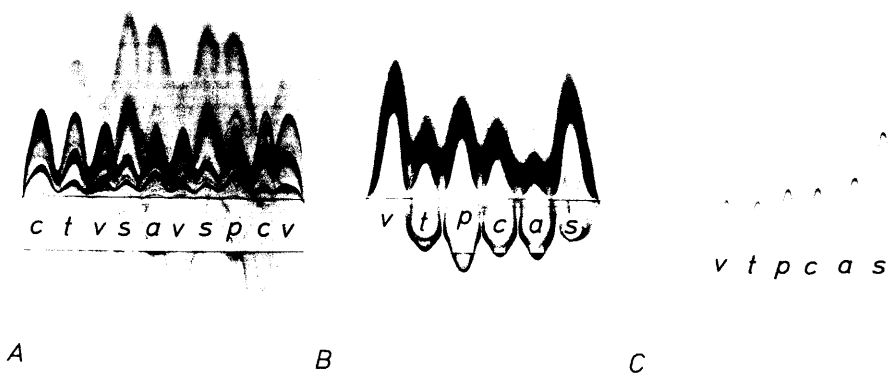


Fig. 4 FRIE of the six *Artemisia* spp. pollen extracts using the following rabbit antisera in the antiserum-containing gels: **A**) anti-mugwort (10 µl/cm²), **B**) anti-Art v II (10 µl/cm²) in anodic gel, 10 µl/cm² in

cathodic gel), and **C**) anti-Art v II (45 µl/cm²). The gels are stained with CBB.

Comparison by immunoelectrophoresis

In order to investigate the antigenic relationship between the components of the different *Artemisia* extracts, FRIE was performed using a polyspecific antiserum against *A. vulgaris* extract (Fig. 4A), an antiserum against the major *A. vulgaris* pollen allergen Ag 12 (Fig. 4B), or an antiserum against the important *A. vulgaris* allergen Art v II (Fig. 4C). The results demonstrated an extensive degree of immunological identity between components of the different extracts. Fig. 4A shows that all the extracts seem to contain antigens that are immunologically identical to all or most of the antigens in the *A. vulgaris* extract. Most of the anodic antigens seemed to be present in all the extracts, but some of the cathodic antigens were present in small amounts, if at all, in the extracts of

A. vulgaris and *A. scoparia*. This is consistent with the IEF-results. All extracts seemed to contain components that were immunologically identical to the two *A. vulgaris* allergens Ag 12 and Art v II (Figs. 4A–C).

Tandem-CIE (Fig. 5) of extracts from *A. vulgaris* and *A. princeps* using polyspecific antiserum against *A. vulgaris* extract in the second dimension gel showed the presence of at least 4 antigens with either complete or partial immunological identity (Art v II, Ag 9, Ag 13, and a fourth weak antigen).

Screening of sera from Korean and Norwegian patients against extracts from *A. vulgaris* and *A. princeps* pollen

Sera from ten individual Korean patients showing a positive skin prick test towards an extract of mixed *Artemisia* species, were tested in SDS-PAGE/immunoblotting with extracts from *A. vulgaris* and *A. princeps*. As seen in Fig. 6 and Table 2, the individual Korean patient sera gave very similar reaction patterns with the two different extracts.

Similar results were obtained when the same two extracts were separated by IEF, transferred to NCM, and incubated with individual sera from Norwegian patients (Fig. 7). Also the Norwegian patient sera seemed to detect similar (or identical) components in the two different extracts.

These results are in agreement with those obtained above and also show that people from different parts of the world may get allergic reactions after exposure to plants of a related species from another part of the world.

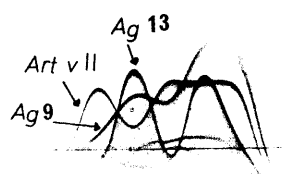


Fig. 5 Tandem-CIE of pollen extracts from *A. vulgaris* and *A. princeps* using anti-mugwort rabbit antiserum in the antibody-containing gels ($12.5 \mu\text{l}/\text{cm}^2$).

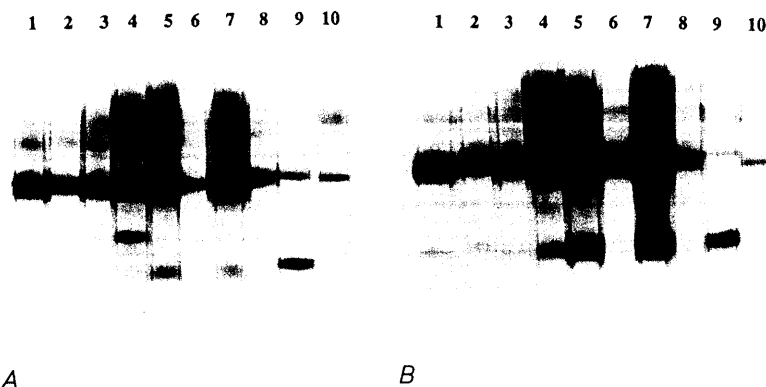


Fig. 6 Detection of IgE-binding components in pollen extracts of A) *A. vulgaris* and B) *A. princeps* after separation by SDS-PAGE and im-

munoblotting using a panel of Korean patient sera with clinical *Artemisia* allergy.

Patient number	IgE binding components in <i>A. princeps</i> extract, MW in kD						IgE-binding components in <i>A. vulgaris</i> extract, MW in kD					
	64	40	37-38	24-27	16.5	11.5-13	66-68	50	40-42	25-28	11.5-13	
1			x	xxx		x			x	xxx	xx	
2			x	xxx		x			x	xxx	xx	
3			x	xxx	x	x			x	xxx	xx	
4		x	x	xxx	xx	xx	xx	x	x	xxx	xx	
5			x	xxxx		xxx			x	xxxx	xx	
6				xx			xx			xx	xx	
7		x		xxx		xx	x		x	xxx	x	
8	x	x		xx			xx		x	xx	x	
9				xx		xxx				xx	xxx	
10	xx	x		xx		x	xx		x	xx		
Total	2	4	5	10	2	8	5	1	8	10	9	

xxxx Very strong reaction, x Weak reaction.

Table 2 Korean patient sera with IgE towards components present in extracts of *A. vulgaris* and *A. princeps* when separated by SDS-PAGE.

pl
3.5

showed that both groups of patients had the same pattern of reactivity towards both extracts. This means that an allergy acquired towards an *Artemisia* sp. in e.g. Norway, makes the person susceptible towards pollen from other *Artemisia* species growing in other parts of the world.

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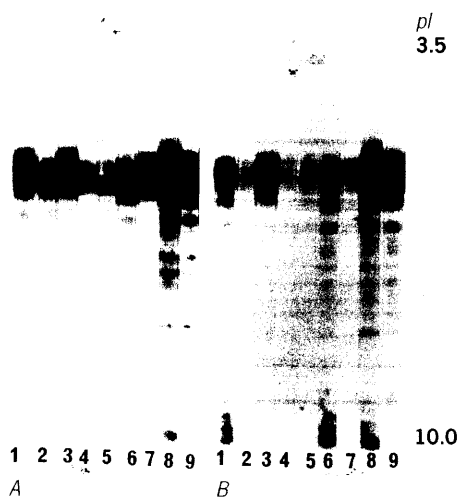


Fig. 7 Detection of IgE binding components in pollen extracts of **A)** *A. vulgaris* and **B)** *A. princeps* after separation by IEF and immunoblotting using a panel of Norwegian patient sera with clinical allergy towards *A. vulgaris*.

Conclusion

Comparison of the allergen extracts from pollen of six different *Artemisia* species by means of SDS-PAGE, IEF, immunoblotting, FRIE, chemical composition with emphasis on the carbohydrate moiety, and also morphological studies on the pollen grains, showed an extensive degree of similarity and cross-reactivity between the different species investigated. The main allergens present in *A. vulgaris* extracts were also present in the other extracts. Screening of both Korean and Norwegian patient sera against extracts from *A. vulgaris* and *A. princeps*

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