

Evaluation of Cross-Reactivity between *Holoptelea integrifolia* and *Parietaria judaica*

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Key Words

Holoptelea integrifolia · *Parietaria judaica* ·
Cross-reactivity · Allergens

Abstract

Background: *Holoptelea integrifolia* and *Parietaria judaica* belong to the family Urticaceae, but are geographically distantly located. *H. integrifolia* is an important pollen allergen of India and sensitizes almost 10% of the atopic population in Delhi. *P. judaica*, on the other hand, is a very dominant pollen allergen of the Mediterranean region, sensitizing almost 80% of the allergic population. Since both these important pollen allergens belong to the family Urticaceae, the objective of the present study was to assess cross-reactivity between these two pollen allergens from different geographical regions. **Methods:** Cross-reactivity between these two pollen allergens was assessed on the basis of skin prick tests and ELISA, ELISA inhibition and immunoblot inhibition studies. **Results:** Out of 44 atopic Indian patients skin prick tested with *H. integrifolia* extract, 34% were found to be sensitized. All the patients sensitized to *H. integrifolia* also showed varying degrees of skin positivity to *P. judaica* pollen extract. ELISA and ELISA inhibition studies suggested strong cross-reactivity between *H. integrifolia*

and *P. judaica* pollen. Immunoblot inhibition studies revealed that 14-, 16-, 28-, 38-, 42- and 46-kDa proteins are the cross-reactive proteins in *H. integrifolia* and *P. judaica*. However, Par j 1, the major allergen of *P. judaica*, is absent in *H. integrifolia* pollen. **Conclusion:** *H. integrifolia* and *P. judaica* pollens share cross-reactive as well as unique epitopes. The major allergen of *P. judaica*, Par j 1, seems to be absent in *H. integrifolia* pollen allergen.

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Introduction

One of the most important causes of respiratory allergy in India and elsewhere are pollen from different plant species [1]. Cross-reactive common proteins/polypeptides in different allergenic sources with IgE-binding properties have been established. This information has been exploited for precise diagnosis and efficient therapy of allergic subjects.

Allergenic cross-reactivity among geographically distantly located pollen allergens belonging to the same family was studied, establishing that structural similarity and not co-sensitization between proteins results in cross-reactivity. Allergen cross-reactivity was observed in in vitro

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studies among three botanically related members of the Euphorbiaceae family, namely *Hevea brasiliensis*, seeds and pollen of *Ricinus communis* (castor bean) and pollen of *Mercurialis annua*, in the different populations of France and Finland [2]. Cross-reactivity between pollen of *Parthenium hysterophorus* and *Ambrosia* sp. from the Asteraceae family was established by RAST inhibition in two groups of patients from India and the USA [3]. *Parietaria judaica* is a very important pollen allergen from the Mediterranean region of Europe. It has been reported that up to 80% of the pollinosis patients from the Mediterranean regions are sensitized to *Parietaria* allergens [4]. The different species of *Parietaria* are known to cross-react among themselves [5], but cross-reactivity of *Parietaria* with other members of the Urticaceae, such as *Urtica dioica* and *Boehmeria nivea*, is observed to be poor [6–8]. This establishes that the allergenic proteins of *Parietaria* are unique. *Holoptelea integrifolia*, another member of the family Urticaceae, is an important sensitizer in patients with respiratory allergy in India, as shown by an epidemiological survey carried out on the adult population of India [9, 10]. *H. integrifolia* is native to Asia and Africa, and is not found in the Mediterranean region, whereas *P. judaica* occurs in the Mediterranean areas of Europe, America, Australia as well as in Asian and African countries bordering the Mediterranean [11], but does not grow in India. The present investigation is therefore aimed at assessing cross-reactivity between these two clinically important pollen allergens belonging to the same family, but geographically distantly located.

Material and Methods

Pollen Samples

Holoptelea is a small genus of trees distributed in tropical and subtropical parts of Asia and Africa; only one species, i.e. *H. integrifolia*, is present in India [12].

H. integrifolia is a large deciduous tree distributed widely throughout India, particularly in the northern and western parts of the country [13]. It is mainly used as a source of timber and is planted along roadsides and in gardens. It blossoms for a short period from mid February to early March. Pollen of *H. integrifolia* was collected during its blooming season from the Delhi region. The pollen of *P. judaica* was provided by Stallergenes, Marseilles, France. Pollen samples of >90% purity were further processed for antigen extraction.

Preparation of Antigenic Extracts

Pollen from both *H. integrifolia* and *P. judaica* was treated with diethyl ether to remove the irritants and non-specific lipids. *H. integrifolia* pollen extraction was carried out in 50 mM NH₄HCO₃ buffer pH 8.0 (1:20 w/v) for 14–16 h at 4°C as described by Malik et al. [14], with slight modifications.

Table 1. General clinical data

Symptom	Patients	
	Indian (n = 44) ^a	French (n = 25) ^b
Bronchial asthma with allergic rhinitis	26	–
Bronchial asthma	9	11
Allergic rhinitis	8	9
Allergic rhinitis with conjunctivitis	1	4
Urticaria ^c	1	1
	44	25

^a 27 males, 17 females.

^b 14 males, 11 females.

^c One patient in the Indian group was suffering from cough variant of asthma with rhinitis.

P. judaica pollen extraction was carried out as described by Ayuso et al. [15], with slight modifications. *P. judaica* pollen was extracted in 50 mM NH₄HCO₃ buffer pH 8.0 (1:20 w/v) with 1 mM phenyl methyl sulphonyl fluoride for 14–16 h at 4°C. After extraction, the *H. integrifolia* and *P. judaica* pollen extracts were clarified by centrifugation at 27,000 g for 30 min at 4°C. The supernatant was dialyzed with a visking dialysis tube (7-kDa cut-off point; Spectra/Por, USA) against distilled water with several changes at 4°C for 24 h. The extracts were centrifuged again. The protein extract was then passed through 0.45-µm membrane filters (Millipore, USA), dispensed in small aliquots, lyophilized (FD 3 Heto make lyophilizer) and stored at –20°C for further experimentations.

Protein Estimation

The protein content was estimated by the method of Lowry et al. [16], with slight modifications. Briefly, soluble protein of both *H. integrifolia* and *P. judaica* extracts was precipitated with 15% phosphotungstic acid in 10% hydrochloric acid for 30 min at room temperature. The pellet was separated from the supernatant by centrifugation and dissolved in 2% sodium hydroxide solution. The protein was then diluted appropriately with distilled water and estimated.

The quality of the extracts was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie Brilliant Blue staining [17].

Skin Prick Test

Patients reporting to the allergy clinic of Vallabhbhai Patel Chest Institute from Delhi and adjoining areas were selected for a skin prick test (SPT). The clinical data of the 44 Indian and 25 French patients included in the study are presented in table 1. All patients recruited to the study had a history of pollen allergy (i.e. the presence of at least one of the following symptoms during the blooming period: conjunctivitis, rhinitis, asthma or asthma with rhinitis). SPTs were carried out from February to March, coinciding with the blooming season for *H. integrifolia*.

Informed written consent was obtained from all the subjects included in the study.

Crude aqueous extracts of *H. integrifolia* and *P. judaica*, which were used for SPT, were standardized on a w/v and protein concentration basis. The test was performed with *H. integrifolia* and *P. judaica* extracts in 50% glycerinated buffer saline (1:10 w/v) on the volar surface of the forearm with 26-gauge disposable needles. Besides pollen antigens, histamine (Sigma, USA) 1 mg/ml was used as positive control, while glycerinated buffer saline was used as negative control. The wheal diameter and erythema were measured after 15–20 min and graded according to the criteria outlined by Singh et al. [18]. A wheal diameter ≥ 3 mm was considered 1+, while ≥ 5 mm (and/or a positive control equivalent) were considered strongly positive ($\geq 2+$).

SPT was also carried out with common inhalant allergens of *Morus alba*, *Prosopis juliflora*, *Amaranthus spinosus*, *Artemisia scoparia*, *Cannabis sativa*, *Chenopodium album*, *Xanthium strumarium*, *Cynodon dactylon* and *Imperata cylindrica*, besides *H. integrifolia* and *P. judaica* extracts.

Furthermore, SPT was carried out with *P. judaica* extract on 25 French patients, but these patients were not tested against *H. integrifolia* extract.

Technique

Human Sera. Blood samples from 15 Indian patients showing marked skin positivity ($\geq 2+$) to *H. integrifolia* and *P. judaica* pollen extracts, were collected and allowed to clot at room temperature for 1 h. The expressed sera were separated by centrifugation and kept in small aliquots at -70°C until further use. Sera from 10 healthy, non-atopic subjects were collected to act as controls.

At Marseilles, blood samples were also collected from 25 patients tested with *P. judaica* extract from the Mediterranean region. They were lyophilized for better transportation and kept at 4°C .

ELISA. Allergen-specific IgE in the sera of patients with positive SPT to *H. integrifolia* and *P. judaica* was estimated using the ELISA technique according to the method outlined by Sepulveda et al. [19]. Briefly, 20 $\mu\text{g}/100 \mu\text{l}$ of *H. integrifolia* and *P. judaica* pollen extracts diluted in 0.1 M carbonate-bicarbonate buffer (pH 9.6) were coated onto a polypropylene maxisorp plate (Nunc, Denmark). The plate was incubated overnight (O/N) at 4°C , followed by repeated washings and then blocked with 1% bovine serum albumin (BSA; SRL) in phosphate-buffered saline (PBS; pH 7.4) for 2 h at room temperature. After repeated washings, patients' sera were added to the plate, diluted in 0.05% BSA-PBS (1:10) and incubated O/N at 4°C . The plates were washed with PBS with 0.05% Tween 20 (PBST), and anti-human IgE alkaline phosphatase (Sigma) diluted in 1% BSA-PBST (1:1,000) was added onto the plate. The plate was then developed with *p*-nitrophenyl phosphate substrate supplied by Sigma. The reaction was terminated with 5 N NaOH, and plates were read at 410 nm. The ODs were compared with the mean ODs of healthy volunteers who served as controls, according to the criteria of Calenoff et al. [20].

ELISA Inhibition. ELISA inhibition was carried out to detect the cross-reactive antibodies in the sera of the Indian patients showing skin positivity and raised IgE against *H. integrifolia* and *P. judaica* pollen allergens. Sera of patients were pooled by equal volume for further experimentation. The pooled sera of the patients hypersensitive to *H. integrifolia* (1:5 dilution) were pre-incubated with increasing concentrations (5–1,280 ng/ml) of *H. integrifolia* and *P. judaica* antigen extracts O/N at 4°C . The *H. integrifolia* extract was coated onto the plate, and after blocking, the pre-absorbed sera were added to the plate; ELISA was then carried out as already described above.

The pooled sera from the French patients hypersensitive to *P. judaica* pollen extract were inhibited with increasing concentrations of *H. integrifolia* and also of *P. judaica* extracts (5–1,280 ng/ml). As a control, patients' sera were incubated with increasing concentrations of BSA. The inhibition curve was plotted on a semi-log graph. A 50% inhibition concentration was taken as cut-off for analysis.

Anti-Par j 1 ELISA. Par j 1 is the major allergen present in *P. judaica*. To detect Par j 1-like proteins in *H. integrifolia* extracts, anti-Par j 1 ELISA was carried out as described by Ayuso et al. [21], with slight modifications. Anti-Par j 1 MoAb (donated by Dr. Florentino Polo, Madrid, Spain) was coated onto the 96-well plates (Nunc) at a concentration of 10 $\mu\text{g}/\text{ml}$ in PBS for 1 h at 37°C . The plates were then blocked with 1% BSA in PBS for 30 min at 37°C . After washing, 50 μl of *H. integrifolia* and *P. judaica* pollen extracts diluted in 0.5% BSA-PBST were added to the plate in increasing concentrations and incubated for 1 h at room temperature. The plates were washed with PBST and incubated with patients' sera (1:10 dilution), showing very high IgE values against *H. integrifolia* and *P. judaica* extracts, respectively, and incubated O/N at 4°C . Thereafter, the wells were washed and incubated for 2 h at 37°C with anti-human IgE labelled with peroxidase (1:1,000 diluted; Sigma). The plates were washed four times with PBST and developed with *o*-phenylenediamine (Sigma) in 50 mM citrate phosphate buffer, pH 4.2, containing H_2O_2 (0.05%). The reaction was terminated after 20 min with 2 M HCl, and the plates were read at 492 nm.

Immunoblotting. Immunoblotting was carried out according to the protocol of Towbin et al. [22]. *H. integrifolia* and *P. judaica* pollen extracts were loaded (120 μg of protein/well) onto the gel, separated by SDS-PAGE and blotted onto the nitrocellulose membrane. The strips were then blocked with 3% BSA in 50 mM PBS for 1 h at 37°C . The pooled sera of the Indian patients hypersensitive to *H. integrifolia* were diluted in 0.1% BSA-PBST (1:5) and incubated with nitrocellulose-blotted allergens (*H. integrifolia* and *P. judaica* pollen extracts). After repeated washings with PBST, the strips were incubated with anti-human IgE (Sigma) conjugated with horseradish peroxidase (1:1,000 diluted in 1% BSA-PBST) for 1 h at 37°C . The strips were then washed thoroughly with large amounts of PBST and developed with 3,3-diaminobenzidine (Sigma; 10 mg/20 ml PBS containing 30 μl of H_2O_2) for 5–10 min. The strips were washed with distilled water, dried and stored in the dark.

Immunoblot Inhibition. Immunoblot inhibition was carried out to identify the cross-reactive proteins in the *H. integrifolia* and *P. judaica* extracts. The sera of the patients hypersensitive to *H. integrifolia* and *P. judaica* pollen were diluted in 0.1% BSA-PBST (1:5) and pre-incubated O/N at 4°C with increasing concentrations of *H. integrifolia* and *P. judaica* extracts (10–1,000 μg). Patients' sera were also incubated with 40 μg of BSA as controls. The pre-adsorbed sera were then incubated O/N at 4°C with nitrocellulose-blotted allergens (*H. integrifolia* and *P. judaica* pollen extracts). The nitrocellulose-blotted allergens were incubated with sera from healthy volunteers, to serve as controls. Immunoblotting was then carried out as described earlier.

Table 2. SPT results with *H. integrifolia* and *P. judaica* on 44 patients showing identical and non-identical skin reactions on the same subjects

<i>H. integrifolia</i> SPT grading	<i>P. judaica</i> SPT grading			
	negative	1+	2+	3+
Negative	15	1		
1+	6	7		
2+		4	4	2
3+			3	2

Total identical reactions: 28/44 (63.6%).
Total identical positive reactions: 13/44 (29.5%).
Total identical negative reactions: 15/44 (34.09%).
Identical significant positivity ($\geq 2+$ skin reactions): 6/44 (13.63%).

Results

Skin Reactivity

Out of a total of 44 patients tested, 34.09% (15/44) were found to be sensitized to *H. integrifolia* extract. All the patients sensitized to *H. integrifolia* also showed skin positivity against *P. judaica* extract. Four of the patients who were sensitized to *H. integrifolia* showed slightly less skin reactivity to *P. judaica* than the positive control (histamine) and were ranked 1+ according to the criteria of Singh et al. [18].

The skin reactivity patterns to *H. integrifolia* and *P. judaica* pollen allergens in a total of 44 Indian patients correlated significantly ($r^2 = 0.7102$, $p < 0.001$).

Among the other common allergens tested, the highest sensitization was observed to *Cynodon dactylon*, with 29.54% of patients showing skin positivity. It was followed by *Cannabis sativa*, *Chenopodium album* and *Artemisia scoparia* with 20.45%, while skin positivity to other allergens varied from 10.9 to 13.63%.

The skin reactivity patterns to *H. integrifolia* and *P. judaica* pollen allergens in the Indian patients are evaluated in table 2 for a comparison of identical positive/negative reactions obtained from each patient to both these extracts. Out of a total of 44 patients tested, 63.6% showed identical skin reactivity, i.e. the intensity of the skin reactions to both *H. integrifolia* and *P. judaica* was similar in the same patient, whereas 29.5% of the patients showed identical skin positivity.

Specific IgE Evaluation

The specific IgE concentrations in the sera from *H. integrifolia* SPT-positive cases against *H. integrifolia* and also *P. judaica* in the Indian patients are shown in figure 1a. Raised specific IgE to *H. integrifolia* and *P. judaica* was shown by the in vitro ELISA method in 11/15 SPT-positive patients. As is evident from figure 1a, the ODs varied from 0.116 to as high as 2.015. The ODs were compared with those of healthy volunteers (control) according to the criteria of Calenoff et al. [20]. Means ± 2 SD of the ODs of controls were calculated, and patients showing ODs greater than means ± 2 SD were considered as positive. The ODs of controls ranged from 0.01 to 0.032, with a mean ± 2 SD of 0.0184 ± 0.022 . Interestingly, in almost all of the *H. integrifolia*-positive patients, raised IgE against *P. judaica* was observed to varying degrees.

IgE concentration in the sera of the *P. judaica*-hypersensitive patients from Marseilles are shown in figure 1b. Out of a total of 19 patients who were SPT positive to *P. judaica*, 11 showed raised IgE titres against *P. judaica* pollen extract by the in vitro ELISA method. Raised specific IgE are presented in terms of ODs, which ranged from 0.240 to 1.253 against *P. judaica* pollen. The values were compared with those of the healthy volunteers, which ranged from 0.003 to 0.034, with a mean value of 0.026 ± 0.024 . When the sera from *P. judaica*-positive patients were challenged with *H. integrifolia* besides *P. judaica*, poor or negligible levels of *H. integrifolia*-specific IgE antibodies were detected in the sera of the French *P. judaica*-positive patients.

ELISA Inhibition

The pooled sera from Indian patients hypersensitive to *H. integrifolia* pollen were inhibited by *H. integrifolia* and *P. judaica* pollen proteins at different concentrations, as analysed in figure 2a. It is clear from the figure that with an increase in antigen concentration, increased percent inhibition is recorded. The 50% inhibition with *H. integrifolia* pollen protein as inhibitor was reached at 50 ng/ml, while with *P. judaica* as inhibitor, 68 ng/ml of protein was required.

When the pooled sera from French patients hypersensitive to *P. judaica* were inhibited with increasing concentration of *H. integrifolia* and *P. judaica* pollen proteins, 50% inhibition was reached at 45 ng/ml with *P. judaica* pollen protein as inhibitors. However, *H. integrifolia* pollen proteins as inhibitor provided a maximum of only 30% inhibition, even at the highest concentration, and inhibition never reached the 50% inhibition level (fig. 2b).

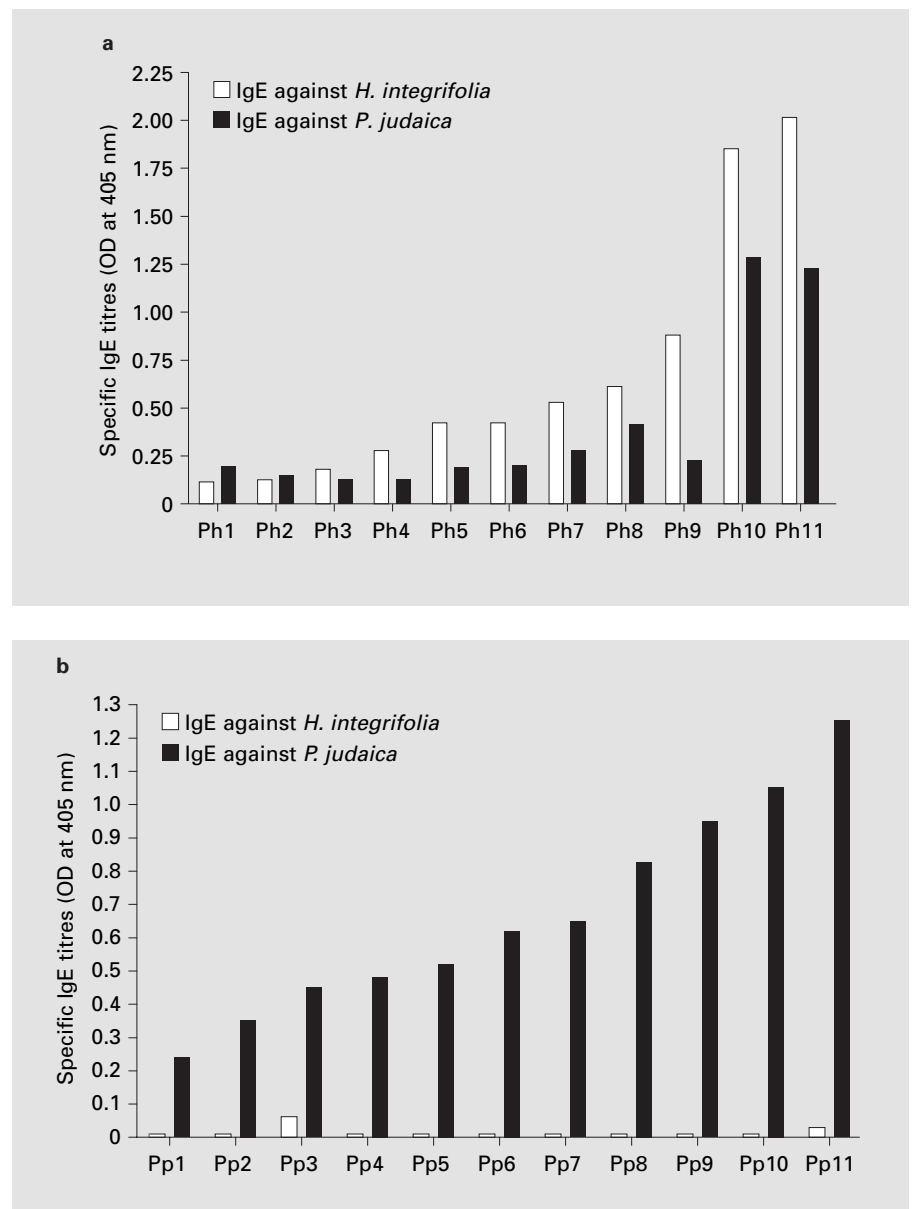


Fig. 1. Histograms showing raised specific IgE titres (OD at 450 nm) in the sera from 11 Indian patients sensitive to *H. integrifolia* and *P. judaica* pollen out of a total of 15 Indian patients with marked skin positivity to *H. integrifolia* (**a**) and from 11 French patients sensitive to *P. judaica* and *H. integrifolia* pollen out of a total of 19 French patients showing marked skin positivity to *P. judaica* (**b**).

The curve with BSA at all concentrations showed almost negligible inhibition.

Anti-Par j 1 ELISA

To analyse the Par j 1-like proteins in the *H. integrifolia* extract, an anti-Par j 1 ELISA was carried out. The anti-Par j 1 ELISA showed an increasing concentration of Par j 1 in the *P. judaica* extract with increasing protein concentration (fig. 3). Interestingly, Par j 1, which is the major allergen of *P. judaica*, could not be detected in the *H. integrifolia* extract, even at a 10-gram concentration

(data not shown), indicating the absence of Par j 1-like proteins in *H. integrifolia* pollen.

SDS-PAGE Profile of *H. integrifolia* and *P. judaica* Extracts

SDS-PAGE protein profiles of *H. integrifolia* and *P. judaica* pollen extracts are presented in figure 4a. Twenty-four bands in each of the *H. integrifolia* and *P. judaica* extracts were visible in the molecular weight range of 14–115 kDa, after staining with Coomassie Brilliant Blue. The prominent bands staining intensely with Coomassie

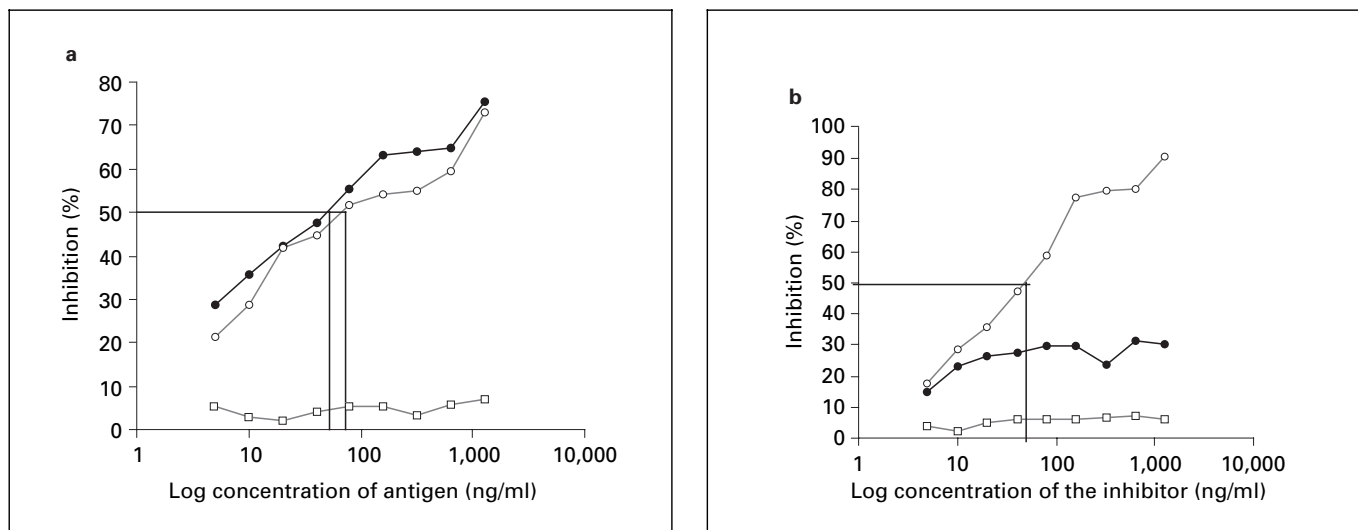


Fig. 2. IgE inhibition ELISA of pooled sera from Indian patients sensitive to *H. integrifolia* (a) and pooled sera from French patients sensitive to *P. judaica* (b) with *H. integrifolia* (●) and *P. judaica* (○) pollen extracts. *H. integrifolia* (a) or *P. judaica* (b) extract was coated (20 µg/well) and incubated separately with a pre-incubated mixture of pooled sera from patients hypersensitive to *H. integrifolia* (a) or *P. judaica* (b) (1:5 v/v) and 5–1,280 ng/ml of *H. integrifolia* and *P. judaica* antigens. □ = BSA.

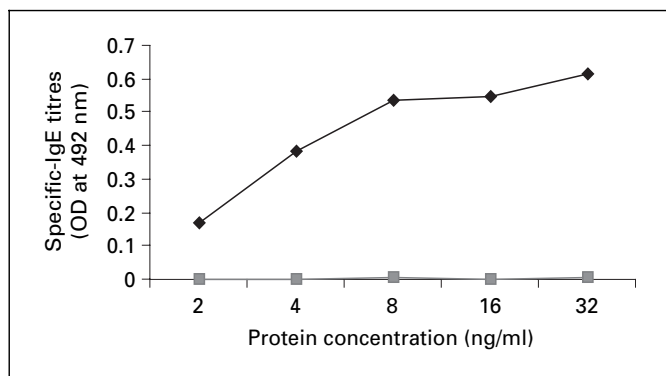


Fig. 3. ELISA dose-response curve with the anti-Par j 1 MoAb as antigen with *P. judaica* (◆) and *H. integrifolia* (■) extracts.

Blue observed in the *H. integrifolia* extract were 14, 16, 17, 18, 46, 55, 60, 66 and 95 kDa, while in the case of the *P. judaica* extract, the prominent bands were 16, 18, 46, 55, 60, 66, 70 and 115 kDa.

Immunoblot Profile of *H. Integrifolia* and *P. judaica* Extracts

The immunoblot profile of *H. integrifolia* and *P. judaica* extracts, when probed with the pooled sera of the

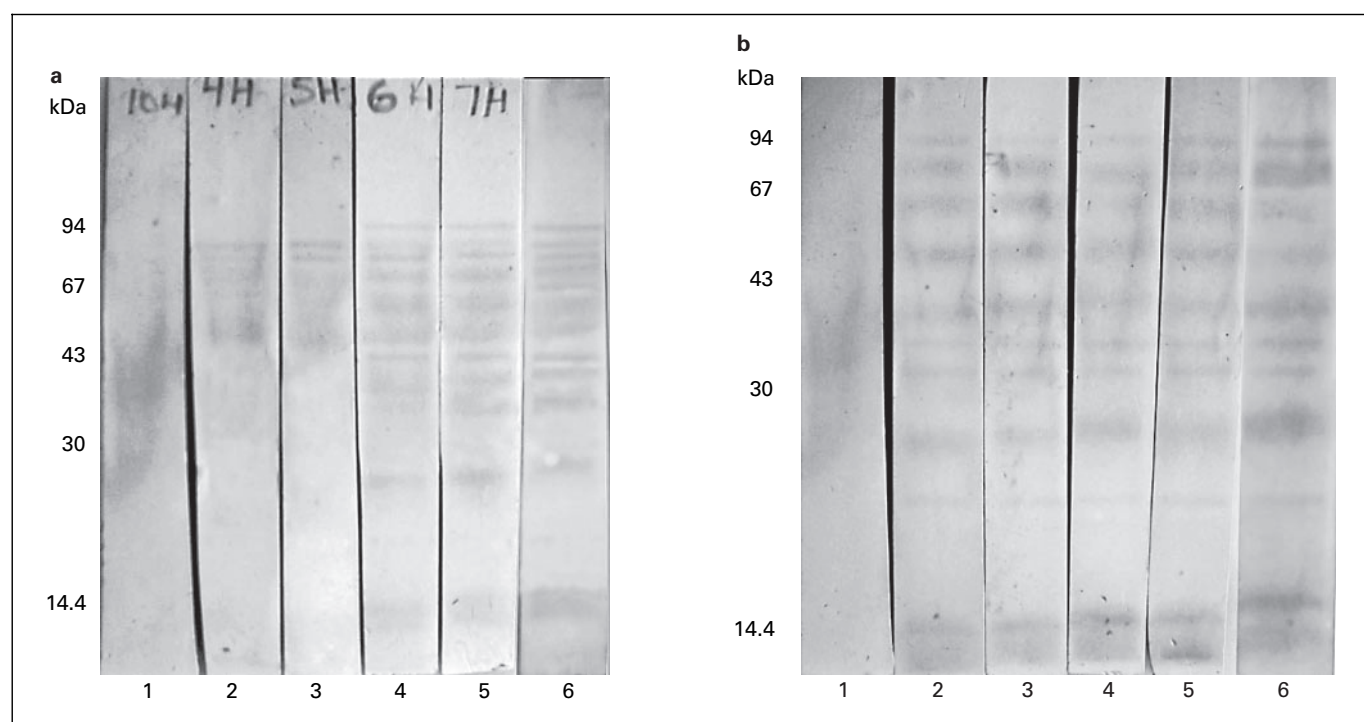
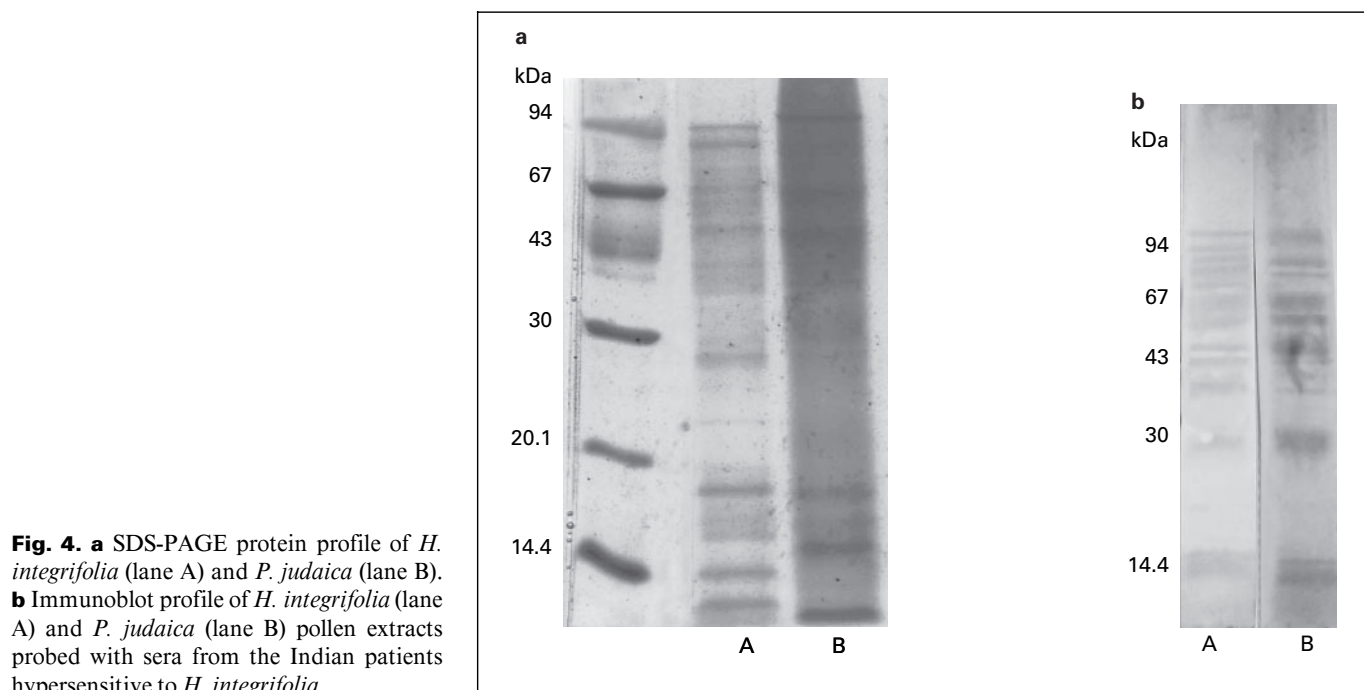
Indian patients hypersensitive to *H. integrifolia* pollen, is presented in figure 4b. A total of 19 IgE-binding components were identified in the *H. integrifolia* extract and 15 in the *P. judaica* extract. The prominent bands recognized in the *H. integrifolia* extract were 14, 28, 46, 60 and 66 kDa, while in the *P. judaica* extract, 14-, 28-, 46-, 60-, 66-, 80- and 95-kDa proteins were recognized.

Immunoblot Inhibition

When the pooled sera of the patients hypersensitive to *H. integrifolia* pollen from India were inhibited with increasing concentrations (10–1,000 µg) of *P. judaica* extract as inhibitor, cross-reactive IgE-binding fractions of *H. integrifolia* disappeared (fig. 5a). Fading and disappearance of the bands are clearly evident with increasing concentrations of *P. judaica* as inhibitor.

Protein fractions of 14, 16, 28, 38, 42 and 46 kDa completely disappeared at concentrations ≥ 100 µg of *P. judaica* as inhibitor, while 55-, 60- and 66-kDa proteins gradually faded in a dose-dependent manner. Protein bands of higher molecular weight, namely ≥ 70 kDa, were not inhibited with increasing concentrations of *P. judaica* as inhibitor except for a 95-kDa band.

Conversely, when the pooled sera from French patients hypersensitive to *P. judaica* pollen were incubated with increasing concentrations of *H. integrifolia* as inhibitor,



an inhibition pattern with increasing concentrations of *H. integrifolia* extract was not observed, even when the concentration was increased to 5,000 µg of *H. integrifolia* pollen extract (fig. 5b).

Discussion

The genera *Holoptelea* and *Parietaria* belong to the division Magnoliophyta, the class Magnolopsida, the order Urticales and the family Urticaceae. The phylogenetic relationship has been used to infer various degrees of cross-reactivity among botanically related plants. As many respiratory allergy patients from India visiting Europe complain of developing allergic symptoms during the pollination season of *Parietaria* (spring to autumn), it was thought appropriate to study cross-reactivity between pollen of *P. judaica* and *H. integrifolia*, as both belong to the Urticaceae.

The skin reactivity pattern showed that patients from India who were primarily sensitized to *H. integrifolia* pollen, as evidenced by significant skin reactivity, also showed significant skin reactivity to *P. judaica* pollen. A high correlation between skin reactivity patterns to *H. integrifolia* and *P. judaica* pollen allergens was observed in the Indian patients ($p < 0.001$). As these patients have never been exposed to *P. judaica* pollen, this indicates the presence of cross-reactive proteins in these two pollen allergens.

The in vitro ELISA results further demonstrated the presence of specific IgE to *P. judaica* pollen in the sera of hypersensitive patients sensitized to *H. integrifolia* pollen in the Indian patient group. As observed in the skin sensitivity pattern, we recorded 98% correlation between the IgE values against the *H. integrifolia* and *P. judaica* pollen allergens in the Indian patients. When the pooled sera from the Indian patient group were inhibited by *H. integrifolia* and *P. judaica* pollen extracts, strong cross-reactivity was observed among the two pollen allergens. Only 68 ng/ml of *P. judaica* pollen extract were required for 50% inhibition, as compared with 50 ng/ml for *H. integrifolia* extract. However, when the pooled sera from the French *P. judaica*-allergic patients were inhibited by *H. integrifolia* extract, the inhibition level never crossed 30%, even when we increased the inhibitor concentration to the highest concentration. This indicates that, although there is a similarity between the *H. integrifolia* and *P. judaica* pollen proteins, the specific IgE present in the sera of *P. judaica*-hypersensitive patients in the French population is directed towards some unique epitopes of *P. judaica* pollen which are absent in *H. integrifolia* pollen.

Our observations are in agreement with those of Barletta et al. [23] on cross-reactivity between *Cupressus arizonica* and *Cupressus sempervirens*, which belong to the Cupressaceae. They demonstrated that 50% inhibition was reached with 140 ng/ml of *C. arizonica*, but even a concentration of 20 µg/ml of *C. sempervirens* did not achieve 50% inhibition, and a maximum of 30% inhibition was reached when *C. arizonica* was coated onto the plate. This proved that cross-reactive epitopes are qualitatively similar but quantitatively different in the two extracts.

Halmepuro et al. [24] have also reported that in RAST inhibition experiments the binding of IgE from patient's sera to apple discs was completely inhibited by pre-incubation with birch pollen allergen, while apple allergen partially inhibited the binding of IgE to birch pollen discs. These studies clearly demonstrated the presence of shared as well as unique epitopes in these cross-reactive allergens, as also observed in the present study with *H. integrifolia* and *P. judaica* pollen allergens.

Accounting for more than 80% of the IgE-binding capacity of the whole extract, Par j 1 is the most important allergen of *P. judaica*. Ayuso et al. [21] have demonstrated that Par j 1-like proteins are genus-specific proteins present in *Parietaria officinalis* and *Parietaria mauritanica*, but absent in *Urtica dioica*, another member of the Urticaceae. This seems to be a plausible explanation for the absence of specific IgE-binding protein fractions against *H. integrifolia* pollen allergen in the sera of French patients hypersensitive to *P. judaica*. Most of the specific IgE in the sera of the French patients is directed to Par j 1, which was demonstrated to be absent in *H. integrifolia* extract by anti-Par j 1 ELISA.

Immunoblot inhibition experiments further confirmed that high-molecular-weight proteins of >70 kDa are not cross-reactive between *H. integrifolia* and *P. judaica* except for a 95-kDa protein.

The blot clearly showed complete disappearance of an approximately 14-kDa protein band with increasing concentrations of *P. judaica* as inhibitor. Independent workers have demonstrated that the major allergens of *P. judaica* are Par j 1 and Par j 2, which belong to the family of plant proteins known as non-specific lipid transfer proteins (nsLTPs) and occur in pollens of various plants and fruits [25]. In a recent study carried out on the identification of cross-reactive allergens of *P. judaica*, Stumvoll et al. [4] identified profilin and 2 EF-hand calcium-binding allergen as cross-reactive proteins in *P. judaica* and other weed species.

In the present study, we have demonstrated that Par j 1-like proteins with a molecular weight of approximately 14 kDa are absent in *H. integrifolia*; therefore, the 14-kDa cross-reactive protein in *P. judaica* and *H. integrifolia* seems to be profilin.

The other cross-reactive protein bands in *P. judaica* and *H. integrifolia* were 16, 28, 38, 42 and 46 kDa. Ford et al. [26] have reported 9 IgE-binding components in the molecular weight range of 10–80 kDa in the pollen of *P. judaica*. The IgE-binding proteins identified were 18-, 23-, 26-, 32-, 38-, 40-, 43- and 80-kDa bands. The difference in the molecular weight observed in the protein could be due to the different electrophoretic system used. These allergens have been identified in the *P. judaica* extract; however, except for a 23-kDa allergen, all the other allergens were recognized by less than 16% of the total patients tested and hence have not been much worked upon. Thus, it seems that cross-reactive proteins in *H. integrifolia* and *P. judaica* are not very important sensitizers for the Mediterranean population, while Indian allergic patients strongly reacted to these cross-reactive proteins present in *P. judaica* extract.

Two of the separate studies confirmed the importance of the geographic distribution in sensitization patterns. Using a panel of recombinant cherry allergens for component-resolved in vitro and in vivo diagnosis, Scheurer et al. [27] compared populations from Italy and Germany, while Ballmer-Weber et al. [28] compared populations from Spain and Switzerland and confirmed the apparent geographic differences in sensitization patterns. The Bet v 1 homologue in the cherry (Pru av 1) was the dominating allergen in Swiss and German patients, whereas sensitization to the LTP (Pru av 3) of cherry was almost exclusively detected in the Mediterranean patients.

We also observed a fading of the 55-, 60-, 66- and 95-kDa bands with increasing concentrations of *P. judaica* as inhibitor. High-molecular-weight allergens have not been identified in *P. judaica*, but Stumvoll et al. [4] have reported cross-reactive high-molecular-weight components (40–100 kDa) in *P. judaica* and mugwort pollen allergens, which have not yet been characterized.

In conclusion, we demonstrated cross-reactivity between *H. integrifolia* and *P. judaica*, both by in vitro and in vivo methods, as several cross-reactive proteins in these two pollen allergens in the molecular weight range of 14–95 kDa have been identified. Interestingly, the major allergen of *P. judaica*, Par j 1, is absent in *H. integrifolia*. It seems that patients from Mediterranean regions are primarily sensitized to nsLTPs, which are absent in *H. integrifolia*, while patients of Indian origin are sensitized to other shared common proteins of *H. integrifolia* and *P. judaica*. Therefore, patients sensitized to *H. integrifolia*, visiting Mediterranean regions during the pollination season of *P. judaica*, are likely to get allergic symptoms. The present study showed different sensitization patterns to the two cross-reactive pollen allergens belonging to the Urticaceae in Indian and French populations.

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