



Antigenic cross-reactivity between *Schistosoma mansoni* and pollen allergens from the birch tree (*Betula verrucosa*) and Timothy grass (*Phleum pratense*): involvement of shared glycan epitopes and implications for the hygiene hypothesis

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ABSTRACT

Previous studies have shown that schistosome infection can protect against allergic symptoms, but the underlying mechanisms are still not fully understood. Here we have shown that rabbit IgG antibodies raised against *Schistosoma mansoni* soluble egg antigens (SmSEA) are cross-reactive with a wide array of molecules in Timothy grass pollen (TGP) and birch tree pollen (BTP). Five of the cross-reactive pollen molecules (two from TGP and three from BTP) were selected randomly and identified by tandem mass spectrometric (TMS) analysis to be, respectively, the TGP allergens Phl p 1 and Phl p 5b, and BTP glutathione S-transferase (GST), and the BTP allergens Bet v 1 and Bet v 6.0102. Rabbit anti-SmSEA IgG antibodies that cross-reacted with each of the five allergens were found to be reactive with three major *S. mansoni* egg antigens, IPSE/alpha-1, omega-1 and kappa-5. Pairwise alignment of the amino acid sequences of each of the five TMS-identified pollen allergens with each of the three egg antigens revealed a low level of amino acid sequence identity. Further experiments indicated that the schistosome antigen/allergen cross-reactivity was mostly due to similar glycans present in helminths and plants, but not in mammals: so called cross-reactive carbohydrate determinants (CCDs). Previously, CCDs have been implicated in the cross-reactivity between many plants and invertebrates. Furthermore, pollen-induced anti-CCD IgGs have been found in sera of patients undergoing allergen-specific immunotherapy (SIT) and implicated in the treatment of the allergy. Thus, our finding provides not only possible explanations for the allergy-protective effect of helminth/schistosome infections as explained by the hygiene hypothesis, but also a potential starting point for improved SIT.

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1. Introduction

In recent decades populations benefitting from well-developed health systems have suffered from a steep rise in the occurrence of allergies, asthma and other disorders of the immune system of humans (Bach, 2002). The 'hygiene hypothesis' is often offered as an explanation, i.e., due to an increase in healthy living conditions and/or prevention of infection by vaccinations and drugs, populations have suffered less from parasitic and microbial

infections and the patterns of maturation of human immune systems are therefore now different from those that pertained to pre-hygienic, infection-rife eras (Rook and Stanford, 1998). A consequence of this difference is an untimely and pathological immune response to environmental and air-borne antigens that are responsible for asthma and allergies, and which 'normal' human immune responses would have perceived as innocuous. The hygiene hypothesis may also have relevance to autoimmune and inflammatory diseases such as inflammatory bowel disease and Type 1 diabetes (Zaccone et al., 2006; Koloski et al., 2008). In helminth-endemic countries in particular, an inverse correlation has been observed between infection with helminths including schistosomes, and sensitisation to allergies (Yazdanbakhsh and

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Wahyuni, 2005; Rujeni et al., 2012), for which situation the hygiene hypothesis has been evoked as an explanation (Rook, 2007).

Immune responses that cause allergic reactions and that are also induced by helminth infections are so-called Th2-type responses (Steinke et al., 2008; Allen and Maizels, 2011). These are mostly characterised by involvement of Th2 cells, the production of IgE antibodies, the activation of granulocytes such as mast cells, basophils and eosinophils, and the release of inflammatory factors such as histamine and cytokines such as IL-4, IL-5, and IL-13 (Steinke et al., 2008; Allen and Maizels, 2011). When helminth infections advance to chronicity, Th2-type responsiveness may become modulated or modified and characterised by the activities of regulatory T- (Treg) and B-cells, and the production of cytokines such as IL-10 and TGF- β (Caldas et al., 2008). The immunomodulated state may facilitate parasite survival and possibly also that of the host (van Die and Cummings, 2006).

The above-mentioned immunomodulatory mechanisms by which parasites down-regulate excessive helminth-induced Th2 responsiveness have been reported to have a suppressive effect on allergic sensitisation (Schnoeller et al., 2008). The processes by which this occurs have not been fully elucidated, although one widely accepted explanation pertinent to the hygiene hypothesis is that chronic helminth infection, for example with *Schistosoma mansoni*, results in the activation of Treg cells and production of cytokines such as IL-10 and TGF β (Araujo et al., 2004). IL-10 and TGF- β are anti-inflammatory cytokines which may have a dampening effect on atopic diseases such as allergy and asthma (Araujo et al., 2004).

So-called 'blocking antibodies' may provide an alternative explanation to account for the negative correlation between helminth infection and allergy. Helminth infections induce the production of copious amounts of IgE, much of which probably lacks the function of specific antibodies, but it could saturate Fc ϵ receptors on mast cells and basophils, and thus 'block' the cross-linking of such receptors by allergen-induced specific IgE antibody (Araujo and de Carvalho, 2006). There is, however, evidence against this possibility (Mitre et al., 2005).

On the other hand, many proteins in plants and invertebrates including helminths are glycoproteins and the glycanic residues have been reported to be major targets of IgE antibodies that are induced after an insect sting or exposure to plant pollens or food (Tretter et al., 1993; Aalberse and van Ree, 1997; van Ree et al., 2000; Kwaasi et al., 2002; Bencurova et al., 2004; Wicklein et al., 2004; Paschinger et al., 2009). These glycan epitopes are present in identical form in many different plants and invertebrates and have been termed cross-reactive carbohydrate determinants (CCDs) (van Ree, 2002; Wicklein et al., 2004). IgE anti-CCD antibodies are, however, generally considered to be of little clinical relevance (Altmann, 2016).

We have recently begun to investigate the antigenic cross-reactivity between *S. mansoni* and allergens from different organisms, and have to date demonstrated cross-reactivity between the helminth's molecules and the allergens Hev b 7 from latex (Doenhoff et al., 2016), and Ara h 1 from peanut (Igetei et al., 2017). Because pollen allergens are of considerable public health importance as agents of allergic sensitisation in humans and reported to trigger IgE sensitisation in ~40% of all allergic patients (Freidhoff et al., 1986; Stumvoll et al., 2002), we have extended the aforementioned studies to an exploration of the antigenic relationships between *S. mansoni* soluble egg antigens (SmSEA) and allergens from birch tree pollen (BTP, *Betula verrucosa*) and Timothy grass pollen (TGP, *Phleum pratense*).

To identify cross-reactive antigens/allergens we used rabbit IgG antibodies reactive against *S. mansoni* egg antigens (anti-SmSEA) to probe western immunoblots carrying aqueous-soluble

constituents of BTP and TGP. Five cross-reactive antigens from the pollen extracts, three from BTP and two from TGP, were purified separately by means of SDS-PAGE and analysed in tandem mass spectrometry (TMS). The rabbit anti-SmSEA IgG antibodies that were cross-reactive with each of the five purified pollen allergens were purified separately by acid elution from immunoblots carrying each of the antigenically cross-reactive allergen molecules. The acid-eluted antibodies were used to probe immunoblots of SmSEA to identify the *S. mansoni* egg antigens that may have induced production of the allergen cross-reactive antibodies in the rabbits.

Some patients suffering from allergic rhinitis towards pollen are unable to eat fruits or vegetables implicated in the so-called 'oral allergen syndrome' (OAS) (Kwaasi et al., 2002; Cadot et al., 2003). Therefore, the reactivity of the eluted anti-SmSEA IgG antibody preparations eluted from the five pollen molecules was tested on extracts of five plants implicated in OAS. The characteristics of epitopes involved in the cross-reactivity between the antigens of *S. mansoni*, the two pollen extracts and constituents of the five plants involved in OAS were investigated for the involvement of homologous peptides and/or CCDs using bioinformatics and the effects of treatment with a mild solution of sodium metaperiodate.

The possibility that anti-*S. mansoni* IgG antibodies that are cross-reactive with allergens could somehow block allergic reactions is discussed as a possible explanation for the hygiene hypothesis.

2. Materials and methods

Except when stated otherwise, all chemicals, reagents and buffers used in all techniques are of analytical grade and were purchased from Sigma–Aldrich, Poole, Dorset, England.

2.1. Preparation of SmSEA extracts

The life cycle of a Puerto-Rican isolate of *S. mansoni* was maintained by continuous passages through the aquatic snail *Biomphalaria glabrata* and CD1 strain random-bred mice. Experiments that used mice for production of *S. mansoni* antigens and rabbits for production of antisera were approved by the Ethical Review Committee of the universities in which these materials were produced and the work was carried out in strict accordance with the regulations set out in the UK Animals (Scientific Procedures) Act, 1986, (Project licence numbers PPL 40/3024 and 40/3595). Animals were euthanised using a lethal dose of pentobarbitone anaesthetic.

SmSEA were extracted from eggs from the livers and intestines of infected mice harbouring adult worms as previously described by Doenhoff et al. (1988). The soluble extract was split into 1 mg aliquots in 0.5 ml Eppendorf vials prior to lyophilisation, and stored at -80°C until use.

2.2. Preparation of pollen and other plant allergen extracts

Soluble extracts of BTP and TGP were prepared by incorporating 50 mg of each pollen powder in 1 ml of 0.05 M Tris–HCl buffer, pH 7.5, and extracted as described previously (Schenk et al., 2009). The soluble extracts obtained after centrifugation were split into 100 μl aliquots in 0.5 ml tubes and stored at -20°C until use.

Peanut (*Arachis hypogaea*) extract was prepared from fresh peanut seeds bought from a grocery shop and protein extraction was done as described previously (Doenhoff et al., 2016).

All other fruits used for antigen extraction, namely melon (*Cucumis melo* spp.), banana (*Musa* spp.), tomato (*Lycopersicon esculatum*) and kiwi fruit (*Actinidia deliciosa*) were bought ripe from grocery stores and were prepared in the same way as described for peanut.

2.3. Preparation of mouse tissue extracts: blood, kidney and spleen

Mouse blood was extracted as previously described by Darani and Doenhoff (2008). Extracts of mouse kidney and spleen were prepared by homogenisation, sonication and centrifugation using the same extraction methods described previously (Doenhoff et al., 2016).

2.4. Protein concentration in SmSEA and plant extracts

Estimates of protein concentrations in SmSEA and in extracts of pollens and other plants were performed using the adapted Bio-Rad DC (Watford, UK) protein assay method (Lowry et al., 1951), with BSA as standard.

2.5. Preparation of rabbit polyspecific anti-SmSEA, anti-normal mouse serum (NMS), anti-CFA and monospecific egg antisera

Polyspecific antisera used in the study were raised against SmSEA by weekly immunisation of rabbits as described previously (Dunne et al., 1986; Modha et al., 1988). A polyspecific rabbit anti-NMS antiserum was prepared as described previously (Darani and Doenhoff, 2008). The serum pool was divided into 5 ml aliquots and stored at -20°C . Rabbit anti-CFA antiserum was prepared as described for anti-NMS except that rabbits were injected with repeated weekly doses of 1 ml emulsions of CFA alone. Rabbit antisera monospecific for the three egg antigens, IPSE/alpha-1, omega-1 and kappa-5, were prepared as described previously (Dunne et al., 1986).

2.6. One dimensional (1-D) SDS-PAGE and western immunoblotting

SDS-PAGE methodology was adapted from Laemmli (1970) and performed as described by Studier (1973) using 15% polyacrylamide. Western immunoblotting was adapted from Doenhoff et al. (2016) and performed as described previously by Towbin et al. (1979).

2.7. Staining and purification of electrophoresed proteins in SDS-PAGE

SDS-PAGE gels containing electrophoresed proteins were stained for protein visualisation as previously described in Doenhoff et al. (2016) using SimplyBlue SafeStain (Invitrogen, Carlsbad, CA, USA).

Purification of all proteins was achieved by excision of the band of interest from an array of molecules that are present on a destained gel, followed by overnight incubation in buffer, centrifugation and re-electrophoresing of the eluate in a second SDS-PAGE as recently described (Doenhoff et al., 2016; Igetei et al., 2016).

2.8. Purification of cross-reactive anti-SmSEA IgG antibodies by acid elution

Anti-SmSEA antibodies that were cross-reactive with five pollen allergens were purified by acid elution using an adapted method from Rybicki (1986), modified as recently described (Doenhoff et al., 2016).

2.9. Treatment of nitrocellulose paper (NCP) with sodium meta-periodate

The technique was adapted from Doenhoff et al. (2016) and performed as described earlier (Hamilton et al., 1999; Eberl et al., 2001).

2.10. TMS analysis of protein samples and data analysis

Analysis of purified antigen extracts was achieved using the LC-electrospray ionisation (ESI)-tandem on a Q-TOF2 mass spectrometer fitted with a nanoflow ESI source (Waters UK, Elstree, UK) (Papayannopoulos, 1995; Steen and Mann, 2004), adapted as recently described (Doenhoff et al., 2016; Igetei et al., 2016).

TMS-identified proteins were further investigated and analysed on the NCBI database (National Centre for Bioinformatics Information, USA) using the Basic Local Alignment Search Tool (BLAST) <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. Alignment of two amino acid sequences was done using the pairwise sequence alignment tool (<http://www.ebi.ac.uk/Tools/psa/>).

The prediction of potential glycosylation sites on TMS-identified allergens was done using GlycoEP software (Chauhan et al., 2013) and ExPASy – Bioinformatics Resource Portal (GlycoMod) tool (<http://web.expasy.org/cgi-bin/glycomod/glycomod.pl>) as described previously (Doenhoff et al., 2016).

3. Results

3.1. Reactivity of rabbit anti-SmSEA IgG antibodies on aqueous extracts of TGP and BTP

The cross-reactivity between *S. mansoni* antigens and those in TGP and BTP was investigated by electrophoresing aqueous extracts of each of the pollens in SDS-PAGE and subsequently probing electrobots of the extracts with a rabbit anti-SmSEA serum or a rabbit anti-CFA serum for IgG reactivity. Fig. 1 illustrates that IgG antibodies raised against SmSEA were cross-reactive with molecules in TGP and BTP.

Fig. 1A, lane 1, shows reactivity between the rabbit IgG antibodies and molecules in TGP which range in molecular mass from ~ 10 kDa to 150 kDa. No reactivity against the grass pollen was seen in negative control blots incubated with the buffer that was used to dilute the rabbit serum (Fig. 1A, lane 2) or with the rabbit anti-CFA antiserum (Fig. 1A, lane 3).

Fig. 1B–D illustrate steps in the identification and purification of two randomly selected schistosome-cross-reactive TGP molecules of approximately 33 and 35 kDa, respectively. Fig. 1B shows a replica of the SDS-PAGE that was used to prepare the western blot in Fig. 1A, but which was stained in SimplyBlue SafeStain. Two protein bands appearing above the level of the 30 kDa molecular weight marker and estimated to be 33 and 35 kDa, respectively, were purified separately by excision of each band from the gel and the protein contained therein eluted by incubation overnight in buffer. Each of the resultant supernatants/eluates was re-electrophoresed and probed with the same rabbit anti-SmSEA antiserum in a western immunoblot to test for cross-reactivity (Fig. 1C). Samples of the two purified molecules illustrated in Fig. 1C, were subjected to SDS-PAGE and stained in SimplyBlue SafeStain to visualise both bands (Fig. 1D) and thus allow their excision and analysis in TMS.

Fig. 1E, lane 1, also revealed extensive cross-reactivity of BTP antigens with IgG antibodies using the same rabbit anti-SmSEA serum as for TGP. This reactivity was not observed with either the antibody-diluting buffer (TBS solution), or an anti-CFA antiserum (Fig. 1E, lanes 2 and 3, respectively). Three cross-reactive antigens in extracts of BTP with molecular masses of ~ 32 kDa, ~ 28 kDa and ~ 17 kDa, respectively, (identified by arrows in Fig. 1F) were randomly selected for further investigation, and thus were purified separately from SDS-PAGE gels. Samples of each of the three purified BTP molecules were electrophoresed in duplicate in SDS-PAGE, the first replicate being probed in a western immunoblot with IgG antibodies raised against SmSEA to confirm the

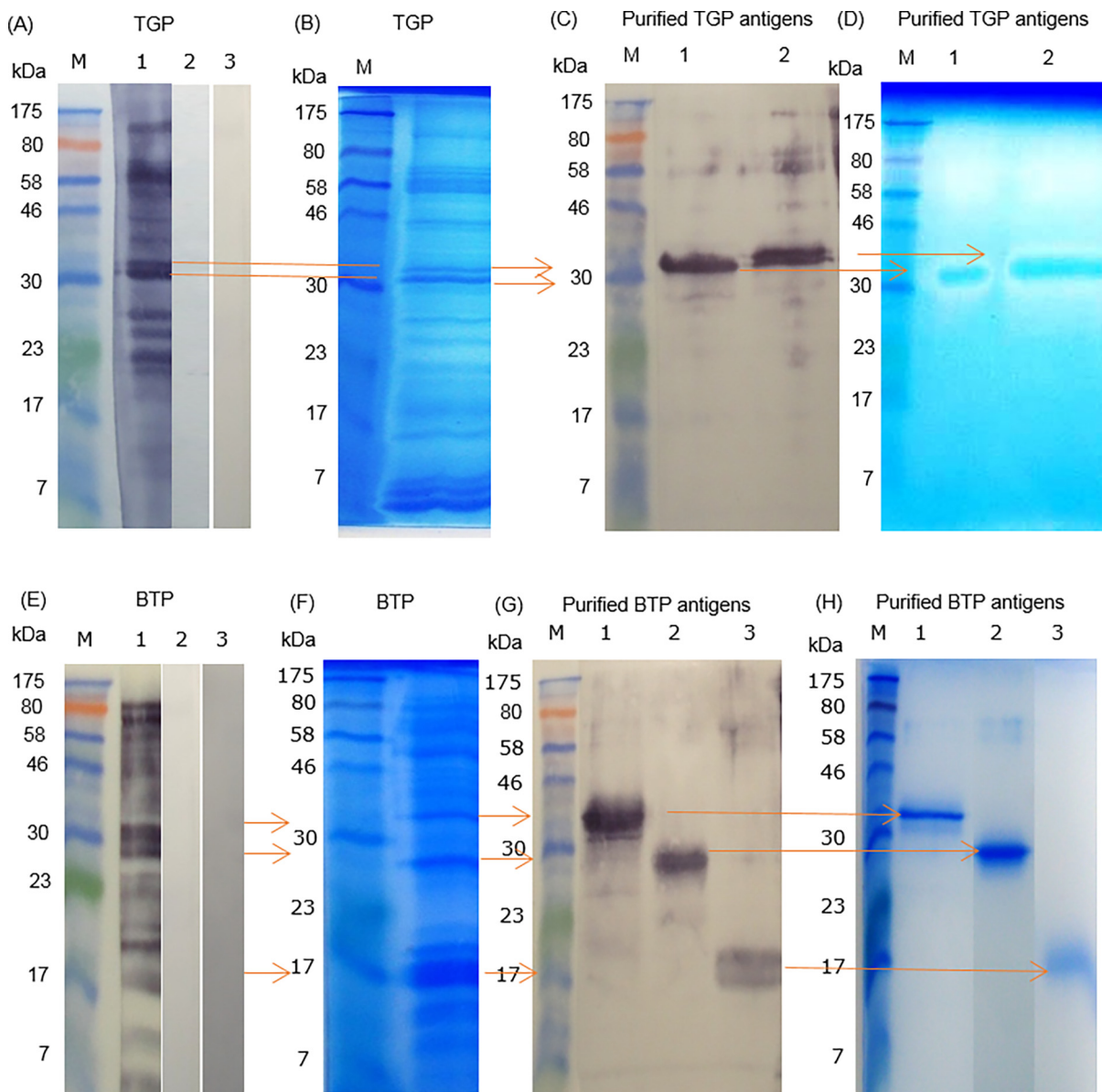


Fig. 1. Identification and purification of five pollen antigens in Timothy grass pollen (TGP) and birch tree pollen (BTP) that are cross-reactive with IgG antibodies in a rabbit anti-*Schistosoma mansoni* soluble egg antigen (SmSEA) serum. (A) Western immunoblot in which an aqueous extract of TGP was probed or incubated with: (lane 1) rabbit anti-SmSEA serum, (lane 2) diluting buffer alone (TBS), (lane 3) rabbit anti-CFA serum. (B) A replicate of 'A' stained with SimplyBlue SafeStain for identification, excision and elution of ~33 kDa and ~35 kDa protein bands. (C) Western blot of purified (lane 1) ~33 kDa, (lane 2) ~35 kDa TGP antigens probed with anti-SmSEA. (D) A replicate of 'C' stained with SimplyBlue SafeStain for tandem mass spectrometric (TMS) analysis. Protein as 0.018 mg and 0.015 mg was loaded in lanes 1 and 2, respectively. (E) Western immunoblot in which an aqueous extract of BTP was probed or incubated with: (lane 1) rabbit anti-SmSEA serum, (lane 2) diluting buffer (TBS), (lane 3) rabbit anti-CFA serum. (F) A replicate of 'E' stained with SimplyBlue SafeStain for excision and elution of ~35 kDa, ~28 kDa and ~17 kDa protein bands. (G) Western blot of purified (lane 1) ~35 kDa, (lane 2) ~28 kDa, (lane 3) ~17 kDa BTP molecules probed with rabbit anti-SmSEA. (H) A replicate of 'G' stained with SimplyBlue SafeStain for MS. Protein as 0.018 mg, 0.005 mg and 0.019 mg was loaded in each of lanes 1, 2 and 3, respectively. The ratio of all rabbit antisera to dilution buffer was 1:100.

molecules were still antigenically cross-reactive, while the second replicate was stained in Simply-Blue SafeStain for another procedure involving excision and analysis in TMS (Fig. 1H).

The two bands purified from TGP (Fig. 1D) and the three bands purified from BTP (Fig. 1H) were excised separately into sterile tubes and analysed in TMS. A Mascot search done on each set of data derived from the purified ~33 kDa and ~35 kDa bands from TGP revealed significant peptide matches for two TGP (*Phleum pratense*) allergens, namely Phl p 5b (Swiss-Prot, MPA5B_PHLPR) (Supplementary Table S1), and Phl p 1 (NCBI, gi: 3901094) (Supplementary Table S2).

Also, Mascot searches done on the TMS-derived peptides from the three bands of ~17 kDa, ~28 kDa and ~35 kDa purified from

BTP revealed significant peptide matches for three BTP (*Betula verrucosa*) antigens/allergens identified as Bet v 1 (gi:1542869), glutathione S-transferase (BTP GST; gi: 573005958) and Bet v 6.0102 (NCBI, gi: 10764491) (Supplementary Tables S3–S5).

3.2. Purification and investigation of the IgG antibodies in a rabbit anti-SmSEA antiserum that are cross-reactive with each of the five pollen allergens

Following the identification of five cross-reactive pollen allergens, anti-SmSEA IgG antibodies that were cross-reactive with each were purified by acid elution from electroblots containing each of the purified allergens. Supplementary Fig. S1 illustrates

the method of purification of each of the five IgG antibody preparations.

The five acid-eluted rabbit anti-SmSEA IgG antibody preparations (anti-SmSEA IgG antibodies) cross-reactive with each of the five purified allergens from TGP and BTP, and denoted as anti-35 kDa Phl p 1, anti-33 kDa Phl p 5b, anti-35 kDa Bet v 6.0102, anti-28 kDa BTP GST and anti-17 kDa Bet v 1, were investigated separately on their corresponding aqueous extracts in western immunoblots (Fig. 2A and B), while the eluted anti-17 kDa Bet v 1 and anti-35 kDa Bet v 6.0102 were also used to probe an extract of TGP (Fig. 2C). Fig. 2A shows that the two antibodies eluted from TGP blots, anti-35 kDa Phl p 1 and anti-33 kDa Phl p 5b, were reactive with their putative respective allergens (Phl p 5b and Phl p 1) as well as with several other antigens in the extract of TGP (lanes 1 and 2, respectively). The reactivity in lanes 1 and 2 was thus quite similar to the reactivity of the unfractionated anti-SmSEA serum (Fig. 2A, lane 3). However, a blot of the same extract incubated with an anti-normal mouse serum (NMS) showed no reactivity (Fig. 2A, lane 4), while that incubation with acid elution buffer alone also showed no reactivity with any of the molecules in TGP (Fig. 2A, lane 5).

Fig. 2B and C show results of the investigation of the three eluted anti-SmSEA IgG antibody preparations from the three purified BTP allergens on aqueous extracts of BTP (Fig. 2B) as well as the eluted anti-17 kDa Bet v 1 and anti-35 kDa Bet v 6.0102 on an extract of TGP (Fig. 2C). Thus, results in Fig. 2B, lanes 1–3, showed that the three eluted IgG antibodies were reactive with each of their respective antigens and other antigens of BTP, an outcome very similar to that with anti-SmSEA serum in lane 4. However, a blot of the same extract incubated with an anti-normal mouse serum (NMS) showed no reactivity (Fig. 2B, lane 5), while that incubated with acid elution buffer alone also gave no reactivity with any of the molecules in BTP (Fig. 2B, lane 6). Interestingly,

the anti-SmSEA IgG antibody preparations eluted from Bet v 1 and Bet v 6.0102, respectively, probing an extract of TGP were found to be cross-reactive with TGP molecules (Fig. 2C, lanes 1 and 2), seemingly very similarly to the anti-SmSEA serum in Fig. 2A lane 3, while no reactivity was observed with acid-elution buffer alone (Fig. 2C, lane 3).

Supplementary Fig. S2 shows results of control experiments whereby the five acid-eluted rabbit anti-SmSEA IgG antibody preparations cross-reactive with each of the five purified allergens from TGP and BTP were investigated separately on three extracts of mouse tissues, namely blood, kidney and spleen. Supplementary Fig. S2A–C show that the two eluted anti-SmSEA IgG antibody preparations from the two purified TGP allergens, as well as the anti-SmSEA serum, showed no reactivity with extracts of three mouse tissues, namely blood, kidney and spleen (lanes 1–3, Supplementary Fig. S2A–C). A control lane probed with a rabbit anti-NMS antiserum did show reactivity with some antigens in each of the mouse extracts (lane 4 in each of Supplementary Fig. S2A–C). No reactivity was observed by incubation of the acid elution buffer alone with any of the three tissue extracts from mouse (lane 5 in each of Supplementary Fig. S2A–C). Supplementary Fig. S2D and F show results of the investigation of the three eluted anti-SmSEA IgG antibody preparations from the three purified BTP allergens on three tissue extracts from mouse – blood, kidney and spleen – (a replication of the experiment with TGP illustrated in Supplementary Fig. S2A–C). The use of the three purified antibodies as well as the anti-SmSEA serum to probe three extracts of mouse tissues (blood, kidney and spleen) to test their cross-reactivity showed no reactivity with any the three extracts (lanes 1–4 in Supplementary Fig. S2D–F). Conversely, a control lane probed with a rabbit anti-NMS antiserum showed reactivity with some antigens in each of the three mouse tissue extracts (lane 5 in each of Supplementary Fig. S2D–F), but the acid-elution buffer

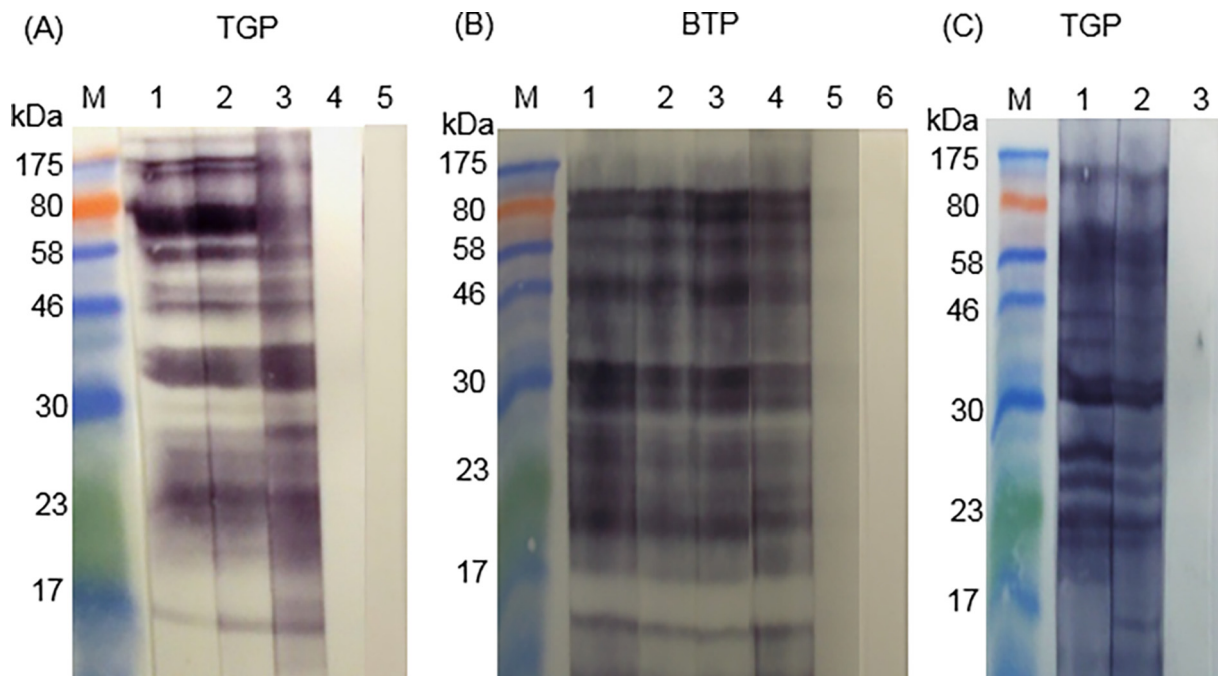


Fig. 2. Western immunoblot investigating reactivity of five acid-eluted rabbit anti-*Schistosoma mansoni* soluble egg antigen (SmSEA), allergen cross-reactive IgG antibody preparations on aqueous extracts of Timothy grass pollen (TGP) and birch tree pollen (BTP). (A) TGP probed with: (lane 1) rabbit anti-SmSEA IgG eluted from the ~33 kDa TGP molecule Phl p 5b, (lane 2) rabbit anti-SmSEA IgG eluted from the ~35 kDa TGP molecule Phl p 1, (lane 3) rabbit anti-SmSEA, (lane 4) anti-normal mouse serum (NMS), (lane 5) incubated in acid elution buffer only. (B) BTP probed with: (lane 1) rabbit anti-SmSEA IgG eluted from the ~35 kDa BTP molecule Bet v 6.0102, (lane 2) rabbit anti-SmSEA IgG eluted from the ~28 kDa BTP GST molecule, (lane 3) rabbit anti-SmSEA IgG eluted from the ~17 kDa BTP molecule Bet v 1, (lane 4) rabbit anti-SmSEA, (lane 5) anti-NMS, (lane 6) incubated in acid elution buffer only. (C) TGP probed with: (lane 1) rabbit anti-SmSEA IgG eluted from the ~17 kDa BTP molecule Bet v 1, (lane 2) rabbit anti-SmSEA IgG eluted from the ~35 kDa BTP molecule Bet v 6.0102, (lane 3) incubated in acid elution buffer only.

alone gave no reactivity on any of the three extracts from mouse (lane 6 in [Supplementary Fig. S2D–F](#)).

3.3. Identification of *S. mansoni* egg antigens that may have induced the production of anti-SmSEA IgG antibodies in the rabbit that are cross-reactive with the five purified pollen allergens

Each of the five IgG antibody solutions prepared by acid elution from TGP (two solutions) and BTP (three solutions) were tested for reactivity on extracts of SmSEA in western immunoblots to identify the SEAs that potentially induced these antibodies. [Fig. 3A](#), lanes 1–5 shows that all five acid-eluted IgG antibody preparations were reactive with a range of SmSEAs. The acid-eluted antibodies reacted against SmSEA similarly to an anti-SmSEA serum ([Fig. 3A](#), lane 6). The reactivity of the eluted antibodies against SmSEAs showed some similarity to those of rabbit antisera raised against, respectively, omega-1, IPSE/alpha-1 and kappa-5, which were used as positive controls ([Fig. 3A](#), lanes 7, 8 and 9, respectively). No reactivity against egg antigens was observed with an anti-CFA antiserum used in [Fig. 3A](#), lane 10, nor with the acid elution buffer alone (lane 11).

To further ascertain the identities of the three pollen cross-reactive *S. mansoni* egg antigens putatively identified in [Fig. 3A](#), purified samples of two of the three egg antigens (IPSE/alpha-1 and kappa-5) were probed with the five eluted IgG preparations. Results in [Fig. 3B, C](#) demonstrate that all five eluted antibodies were reactive with each of the two purified egg antigens, thus showing similar reactivity to an anti-SmSEA antiserum and two antisera specific for IPSE/alpha-1 and kappa-5 respectively ([Fig. 3B, C](#), lanes 1–7). No reactivity of the two purified egg antigens was observed with an anti-CFA antiserum used in [Fig. 3B](#) and [C](#), lane 8, nor with the acid-elution buffer alone used in lane 9.

3.4. Investigation of peptide sequence consensus between the three egg antigens in SmSEA and the five TMS-identified pollen allergens

Following the observed cross-reactivity between the five acid-eluted IgG antibody preparations and the three antigens of SmSEA, alignments of the amino acid sequences of the five allergens: Phl p

1 (GenInfo Identifier (GI): 3901094, CAA81613.1), Phl p 5b (MPA5B_PHLPR, or GI: 2851457, Q40963.2), Bet v 6.0102 (GI: 10764491, AAG22740.1, AF282850_1), GST from BTP (GI: 568853839, XP006480546.1) and Bet v 1 (GI: 1542873, CAB02161.1) were done with each of the three identified egg antigens: IPSE/alpha-1 (GI: 28894857, AAK26170.1), omega-1 (GI: 82400504, ABB73003.1) and kappa-5 (GI:62462031, AAX83114.1) to check for peptide sequence identity/homology. These were done using the pairwise sequence alignment tool from the EMBL-EBI database. Peptide identities between Phl p 1 and IPSE/alpha-1, omega-1 and kappa-5 were 6.9%, 4.0% and 6.7%, respectively ([Supplementary Figs. S3–S5](#)); Phl p 5b and the three corresponding egg antigens were 0.5%, 1.0% and 0.2%, respectively ([Supplementary Figs. S6–S8](#)); Bet v 1 and the three egg antigens were 2.9%, 4.7% and 5.9%, respectively ([Supplementary Figs. S9–S11](#)), BTP GST and the three corresponding egg antigens were 4.9%, 12.3% and 8.1%, respectively ([Supplementary Figs. S12–S14](#)) and for Bet v 6.0102 were 7.5%, 1.6% and 2.5%, respectively ([Supplementary Figs. S15–S17](#)). Cumulatively, these results show a very low level of amino acid sequence consensus between the five TMS-identified pollen allergens and the three immunodominant *S. mansoni* egg antigens.

3.5. The cross-reactivity between anti-SmSEA antibodies and pollen allergens is abrogated by treatment with mild sodium meta-periodate

In view of the above results indicating low sequence consensus between the five allergens and the three *S. mansoni* egg antigens, an alternative possibility, namely that the cross-reactivity was due to CCDs, was investigated. The effect of mild sodium meta-periodate on the cross-reactive epitopes of each of the five allergens and other molecules contained in aqueous extracts of TGP and BTP, respectively, was tested ([Fig. 4](#)). [Fig. 4A](#) shows that the reactivity of anti-SmSEA with Phl p 1, Phl p 5b and other molecules in an aqueous extract of TGP observed in lanes 1–3 were partially inhibited by meta-periodate treatment, as observed in lanes 4–6. [Fig. 4B](#) revealed that the cross-reactivity observed between anti-SmSEA and the three identified BTP allergens (lanes 1–3) and other

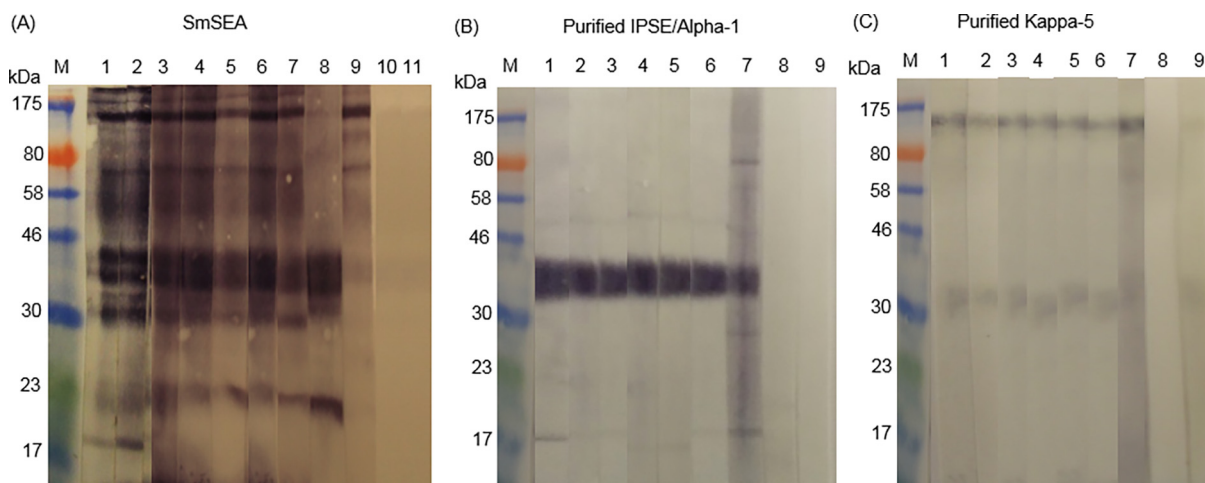


Fig. 3. Western immunoblot reactivity of acid eluted anti-*Schistosoma mansoni* soluble egg antigen (SmSEA) cross-reactive Ig antibody preparations on aqueous extracts of (A) SmSEA and (B) and (C) purified *S. mansoni* egg antigens. SmSEA probed with: (lane 1) anti-SmSEA IgG eluted from Timothy grass pollen (TGP) allergen Phl p 5b, (lane 2) anti-SmSEA IgG eluted from TGP allergen Phl p 1, (lane 3) anti-SmSEA IgG eluted from birch tree pollen (BTP) allergen Bet v 6.0102, (lane 4) anti-SmSEA IgG eluted from BTP glutathione S-transferase (GST), (lane 5) anti-SmSEA IgG eluted from Bet v 1, (lane 6) rabbit anti-SmSEA, (lane 7) rabbit anti-*S. mansoni* egg antigen omega-1, (lane 8) rabbit anti-*S. mansoni* egg antigen IPSE/alpha-1, (lane 9) rabbit anti-*S. mansoni* egg antigen kappa-5, (lane 10) incubated in antibody dilution buffer alone, (lane 11) rabbit anti-CFA serum. 0.010 mg of SmSEA was loaded per lane. (B) Purified IPSE/alpha-1 probed with: (lane 1) rabbit anti-SmSEA IgG eluted from TGP Phl p 5b, (lane 2) anti-SmSEA IgG eluted from TGP Phl p 1, (lane 3) anti-SmSEA IgG eluted from BTP Bet v 6.0102, (lane 4) anti-SmSEA IgG eluted from BTP GST, (lane 5) anti-SmSEA IgG eluted from BP Bet v 1, (lane 6) anti-IPSE/alpha-1, (lane 7) anti-SmSEA, (lane 8) incubated in antibody dilution buffer alone, (lane 9) anti-CFA antiserum. (C) Purified kappa-5 probed with: (lane 1) anti-SmSEA IgG eluted from TGP Phl p 5b, (lane 2) anti-SmSEA IgG eluted from TGP Phl p 1, (lane 3) anti-SmSEA IgG eluted from BTP Bet v 6.0102, (lane 4) anti-SmSEA IgG eluted from BP Bet v 1, (lane 5) anti-SmSEA IgG eluted from BTP Bet v 1, (lane 6) anti-kappa-5, (lane 7) anti-SmSEA, (lane 8) incubated in antibody dilution buffer alone, (lane 9) rabbit anti-CFA antiserum.

cross-reactive antigens in extracts of BTP in lane 4 was completely inhibited by mild meta-periodate treatment as observed in Fig. 4B, lanes 5–8. Furthermore, the purified extracts of each of the five allergens were probed with their corresponding eluted IgG antibodies and the effect of mild meta-periodate treatment was investigated. Fig. 4C demonstrates that the reactivity of the two purified TGP allergens with their corresponding eluted antibodies was also due to meta-periodate-sensitive epitopes (Fig. 4C, compare lanes 1 and 3 with lanes 2 and 4). Similarly, the reactivity of the three purified BTP allergens with each of their respective IgG antibody preparations was also inhibited by treatment with meta-periodate, suggesting the possible involvement of CCDs (Fig. 4D, compare lanes 1, 3 and 5 with lanes 2, 4 and 6).

Following the identification of the *S. mansoni* egg antigens that are putatively cross-reactive with the five pollen allergens, as well as other antigens in extracts of TGP and BTP, respectively, the reactivity of the five IgG antibody preparations with antigens in SmSEA with and without treatment with sodium meta-periodate was investigated. Fig. 5 shows that two of the egg antigens, omega-1 and kappa-5, contained sodium meta-periodate-sensitive epitopes as the oxidising agent inhibited their reactivity with each of the five antibodies. The reactivity of IPSE/alpha-1, however, showed resistance to this treatment (Fig. 5, compare lanes 1–8 with lanes 9–16). These observations are a further indication of the involvement of CCDs in the antigenic cross-reactivity between at least two *S. mansoni* egg antigens and pollen allergens.

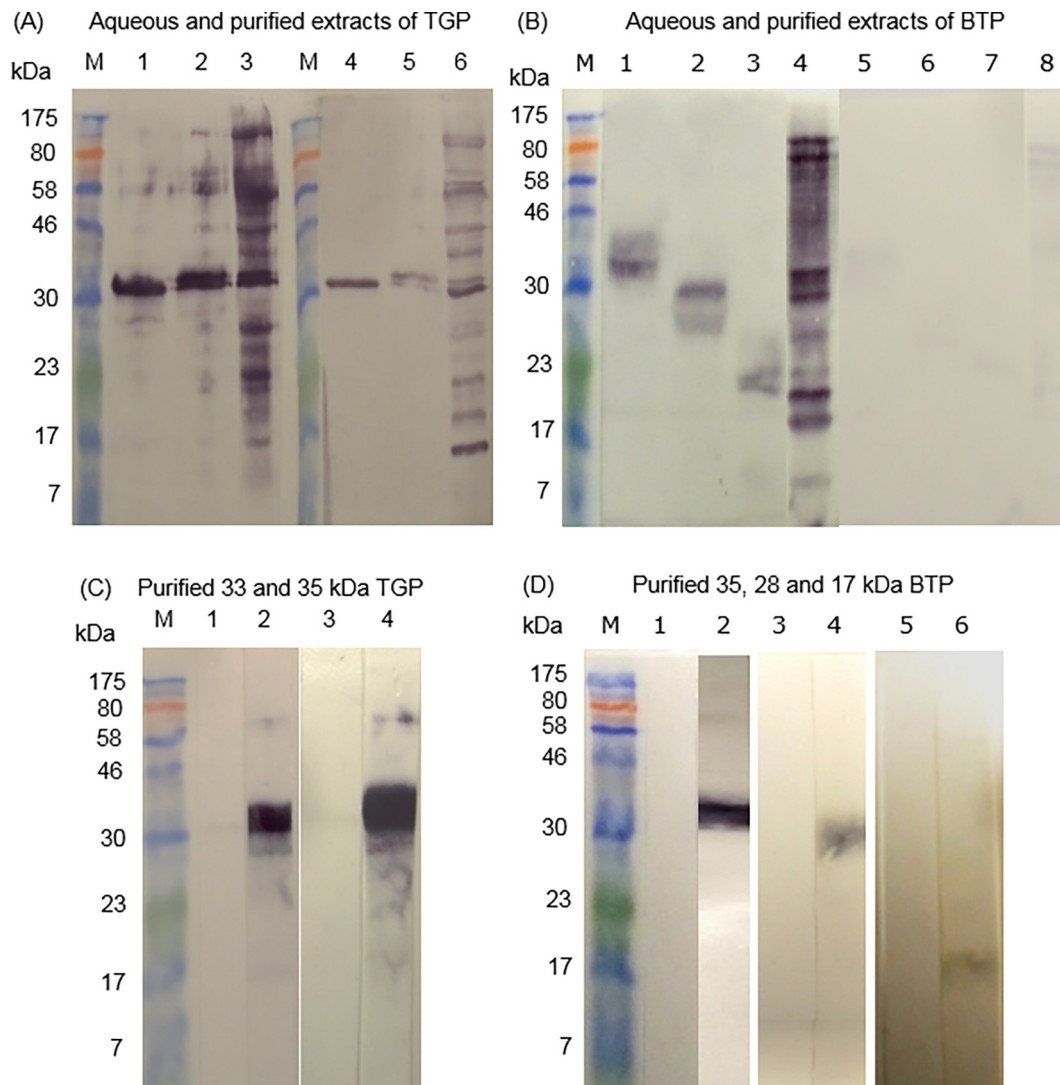


Fig. 4. Sodium meta-periodate treatment of aqueous and purified electroblotted pollen extracts prior to western immunoblotting. (A) Lanes 1 and 4 were loaded with purified 33 kDa Timothy grass pollen (TGP) allergen Phl p 5b, lanes 2 and 5 with purified 35 kDa TGP allergen Phl p 1 and lanes 3 and 6 with aqueous extract of TGP. Lanes 1–3: NCP was incubated in 50 mM sodium acetate buffer, pH 4.5. Lanes 4–6: NCP was incubated in 20 mM sodium meta-periodate dissolved in 50 mM sodium acetate buffer. Lanes 1–6 were probed with rabbit anti-*Schistosoma mansoni* soluble egg antigen (SmSEA). (B) Aqueous solutions of BTP molecules probed with anti-SmSEA antiserum: lanes 1 and 5 were loaded with purified 35 kDa BTP allergen Bet v 6.0102, lanes 2 and 6 with 28 kDa BTP GST, and lanes 3 and 7 with 17 kDa BTP allergen Bet v 1, lanes 4 and 8 with unfractionated aqueous birch pollen extract. Strips 1–4 were incubated in 50 mM sodium acetate buffer alone. Strips 5–8 were incubated in 20 mM sodium m-periodate in 50 mM sodium acetate buffer. (C) Periodate treatment of purified TGP molecules 33 kDa Phl p 5b (lanes 1 and 2), 35 kDa Phl p 1 (lanes 3 and 4). Lanes 1 and 3 were incubated in periodate solution prior to probing with, respectively, anti-SmSEA IgG eluted from TGP allergen Phl p 5b and anti-SmSEA IgG eluted from TGP allergen Phl p 1, Lanes 2 and 4 were incubated only in buffer solution prior to probing with, respectively, anti-SmSEA IgG eluted from Phl p 5b and anti-SmSEA IgG eluted from Phl p 1. (D) Periodate treatment of purified BTP molecules 35 kDa Bet v 6.0102 (lanes 1 and 2), 28 kDa BTP GST (lanes 3 and 4), and 17 kDa Bet v 1 (lanes 5 and 6). Strips 1, 3 and 5 were incubated in 20 mM sodium m-periodate in 50 mM sodium acetate buffer. Strips 2, 4 and 6 were incubated only in 50 mM sodium acetate buffer. Lanes 1 and 2 were probed with anti-SmSEA IgG eluted from Bet v 6.0102, lanes 3 and 4 with anti-SmSEA IgG eluted from 28 kDa BTP GST, lanes 5 and 6 with anti-SmSEA IgG eluted from Bet v 1.

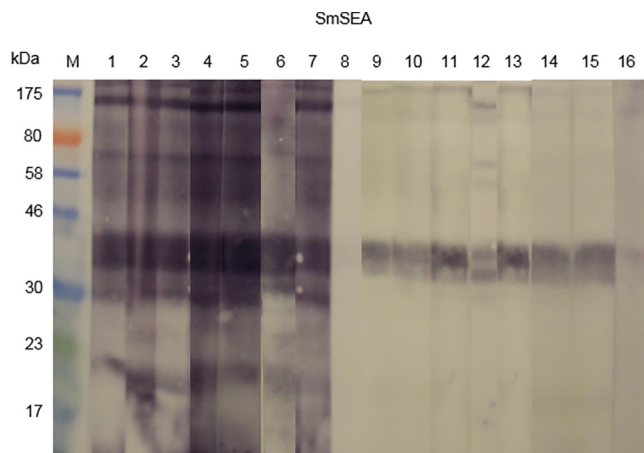


Fig. 5. Sodium meta-periodate treatment of nitrocellulose paper (NCP) containing electro-transferred *Schistosoma mansoni* soluble egg antigen (SmSEA) extract prior to western immunoblotting. Strips 1–8 were incubated in 50 mM sodium acetate buffer alone. Strips 9–16 were incubated in 20 mM sodium meta-periodate in 50 mM sodium acetate buffer solution. Strips were probed with: (lanes 1 and 9) anti-SmSEA IgG eluted from Phl p 5b, (lanes 2 and 10) anti-SmSEA IgG eluted from Phl p 1, (lanes 3 and 11) anti-SmSEA IgG eluted from Bet v 6.0102, (lanes 4 and 12) anti-SmSEA IgG eluted from 28 kDa Birch tree pollen (BTP) glutathione S-transferase (GST), (lanes 5 and 13) anti-SmSEA IgG eluted from Bet v 1, (6 and 14) rabbit anti-IPSE/alpha-1, (7 and 15) rabbit anti-SmSEA, (8 and 16) incubated only in antibody diluting buffer (TBS). A sample of 0.01 mg of SmSEA was loaded per lane.

3.6. Identification of potential glycosylation sites in the amino acid sequences of the five pollen allergens

Further analysis to investigate the possible involvement of CCDs in the cross-reactivity of *S. mansoni* antigens and the five pollen allergens was done by identifying potential glycosylation sites on each of the amino acid sequences of Phl p 5b (MPA5B_PHLPR, or GI: 2851457, Q40963.2), Phl p 1 (GI: 3901094, CAA81613.1), Bet v 6.0102 (GI: 10764491, AAG22740.1, AF282850_1), BTP GST (GI: 568853839, XP006480546.1) and Bet v 1 (GI: 1542873), respectively. Thus, the amino acid sequence of each of the allergens was examined using the GlycoEP software for the prediction of potential N and O-linked glycosylation sites (Chauhan et al., 2013). Predictions of their amino acid sequences showed that Phl p 1 has one N-linked and three O-linked potential glycosylation sites (Supplementary Tables S6 and S7, respectively); Phl p 5b has no N-linked, but 10 O-linked sites with potential for glycosylation (Supplementary Tables S8 and S9, respectively); Bet v 6.0102 has one N-linked and 10 O-linked potential glycosylation sites (Supplementary Tables S10 and S11, respectively); BTP-GST has one N-linked and seven O-linked potential glycosylation sites (Supplementary Tables S12 and S13, respectively) and Bet v 1 has one N-linked and three O-linked potential glycosylation sites (Supplementary Tables S14 and S15, respectively). Also, the amino acid sequences of all five allergens were analysed using the ExPASy software using the GlycoMod prediction tool. Results showed that Phl p 5b has no potential N-linked glycosylation site, Phl p 1 has one N-linked potential glycosylation site, while Bet v 6, BTP GST and Bet v 1 each have one N-linked potential glycosylation sites (results not shown).

3.7. Investigating the reactivity of the five eluted anti-SmSEA IgG antibody preparations on extracts of plants implicated in OAS (pollen food allergy) and sensitivity to periodate treatment

The results in Fig. 2 show that each of the anti-SmSEA IgG antibodies eluted from a pollen antigen was reactive with its corresponding antigen as well as many other antigens in the respective aqueous extracts. The five purified IgG antibody preparations

were separately used to probe western blots of aqueous extracts of five plants known to be involved in the pollen/OAS (Price et al., 2015). Thus, extracts from five different foods, tomato, peanut, kiwi fruit, melon and banana, were electroblotted and probed with each of the five eluted IgG antibodies, with and without treatment of the electroblots with meta-periodate solution prior to incubation with the primary antibodies. The results show that all five eluted anti-SmSEA IgG antibodies were reactive with a variety of antigens in each of the plant extracts and that this reactivity was inhibited by treatment with mild meta-periodate (Fig. 6A–E, compare lanes 1–5 with lanes 6–10). These observations are thus also consistent with CCDs being involved in the antigenic cross-reactivity between pollens and *S. mansoni* egg antigens.

4. Discussion

Rabbit IgG antibodies raised against SmSEA have here been shown to be cross-reactive with a wide array of molecules in TGP and BTP. Five of the cross-reactive pollen molecules (two from TGP and three from BTP) were selected randomly and identified by TMS analysis to be, respectively, the TGP allergens Phl p 1 and Phl p 5b, and BTP Bet v 1, BTP GST and Bet v 6.0102. Three of the five identified pollen allergens are important causes of human allergies. Phl p 1 and Phl p 5b are prominently allergenic in TGP as they are major targets of IgE antibodies in sera from, respectively, >95% and 80–95% grass pollen allergic patients (Johnson and Marsh, 1965; Laffer et al., 1994; Andersson and Lidholm, 2003; Radauer et al., 2008). The major allergen of BTP, Bet v 1, belongs to the pathogenesis-related-10 (PR-10) protein family (Deift et al., 2014, Radauer et al., 2008) while Bet v 6.0102 is a minor allergen belonging to the isoflavone reductase-related family of proteins and was recognised by 18% of IgE-positive sera from birch pollen-allergic patients (Karamloo et al., 1999). BTP GST has only recently been recognised as an allergen as it is recognised by 13% of IgE-positive sera from allergic patients (Deift et al., 2014).

Perhaps remarkably, the five eluted anti-SmSEA IgG antibody preparations reacted with very many other molecules in each of their respective aqueous pollen extracts and were also reactive with each of their corresponding allergens (Fig. 2). More remarkable is the cross-reactivity of the anti-SmSEA antibodies eluted from each of anti-Bet v 1 and anti-Bet v 6.0102 against TGP molecules, which is similar to that of rabbit anti-SmSEA itself against TGP (Fig. 2C). This could be due to the sharing of common epitopes between the antigens in SmSEA and many constituents of the two pollen extracts. The results of treatment with meta-periodate solution in Fig. 4 indicate that CCDs could be the reason for this considerable degree of antigenic cross-reactivity. The absence of antibody reactivity in the five purified anti-SmSEA IgG antibodies against any of the antigens/molecules in the three extracts from mouse indicate that the cross-reactivity observed herein between *S. mansoni* egg antigens and pollen allergens is not ubiquitous nor random.

Probing electroblots of SmSEA with the five acid-eluted IgG antibody preparations enabled the identification of egg antigens that potentially induced production of the cross-reactive antibodies in the SmSEA-immunised rabbit. (While results presented here have been obtained from just one rabbit anti-SmSEA antiserum, it may be noted that other rabbit anti-schistosome antisera reacted similarly – J. Igetei, unpublished observations.) Thus, the three *S. mansoni* egg antigens, IPSE/alpha-1, kappa-5 and omega-1, were identified by their reactivity with the five acid-eluted IgG antibody preparations whose bands appeared in line with the reactivity given by antisera raised against each of the three egg antigens (Fig. 3). Furthermore, samples of two egg antigens, IPSE/alpha-1 and kappa-5, which had been purified from gels of electrophoresed

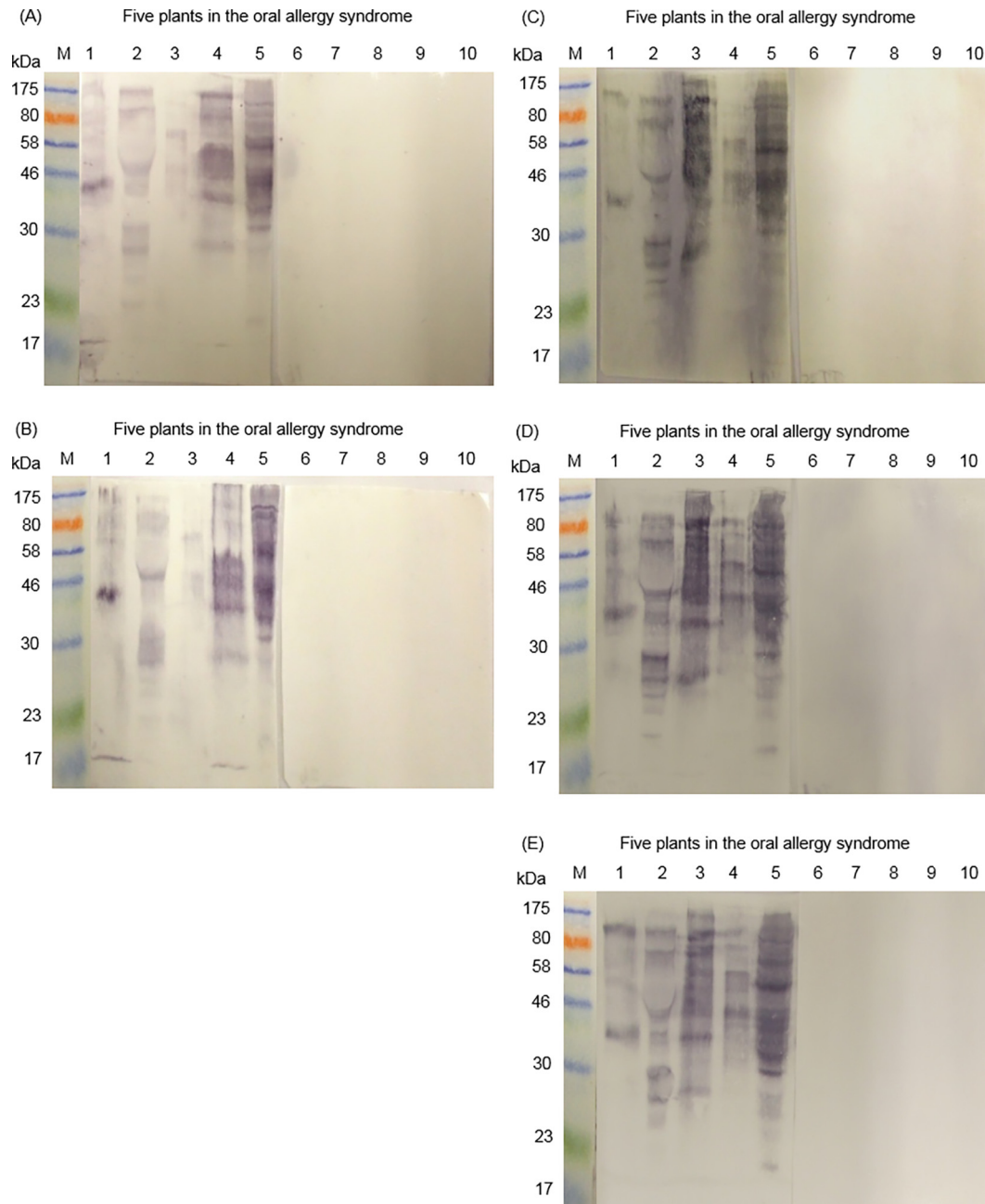


Fig. 6. Western immunoblots showing cross-reactivity of the five eluted anti-*Schistosoma mansoni* soluble egg antigen (SmSEA) IgG antibody preparations with five aqueous extracts of allergenic plants implicated in the oral allergy syndrome (OAS) and the effect of treatment with sodium meta-periodate. Blots were probed with: (A) anti-SmSEA IgG eluted from Phl p 5b, (B) anti-SmSEA IgG eluted from Phl p 1, (C) anti-SmSEA IgG eluted from Bet v 1, (D) anti-SmSEA IgG eluted from birch tree pollen (BTP) glutathione S-transferase (GST), (E) anti-SmSEA IgG eluted from Bet v 6.0102. M, molecular weight markers. Lanes 1 and 6, tomato (0.27 mg/lane); lanes 2 and 7, peanut (0.10 mg); lanes 3 and 8, kiwi (0.19 mg); lanes 4 and 9, melon, (0.24 mg); lanes 5 and 10, banana, (0.16 mg). Lanes 1–5 show controls incubated in sodium acetate buffer for 1 h prior to incubation with primary antibodies. Lanes 6–10 were treated with 20 mM sodium meta-periodate for 1 h.

SmSEA, reacted with all five acid-eluted IgG antibody preparations, as well as with respective monospecific antisera (anti-IPSE/alpha-1 and anti-kappa-5) that had been raised against them. The presence of IPSE/alpha-1 in a solution of the antigen purified by the methods used here has previously been confirmed by TMS analysis (Igetei et al., 2017), while kappa-5 was identified in an immunoblot using a rabbit anti-kappa-5 serum.

Previously, IPSE/alpha-1 has been described as belonging to the $\beta\gamma$ crystallin superfamily of proteins (Meyer et al., 2015), omega-1 has been reported as a member of the T2 family of cytotoxic ribonucleases (Fitzsimmons et al., 2005), while proteins similar to kappa-5

have not yet been found in other organisms. Phl p 5b (Bufe et al., 1995) and omega-1 (Fitzsimmons et al., 2005) have been reported to show ribonuclease activity, but an alignment of the two amino acid sequences revealed a very low level of peptide identity (1.0%, see Supplementary Fig. S7). Furthermore, the level of amino acid sequence identity between the three cross-reactive *S. mansoni* egg antigens and the four other pollen allergens was also found to be very low (Supplementary Figs. S3–S17). Previous analyses have shown that the three *S. mansoni* egg antigens share no significant amino acid sequence similarities with known proteins from any non-schistosome organisms (Schramm et al., 2009; Young et al.,

2012). Thus, it is unlikely that the cross-reactivity observed herein between the three *S. mansoni* antigens and five pollen allergens is due to peptide epitopes.

A sharing of glycan epitopes/CCDs may provide an alternative explanation for the cross-reactivity between the five-identified pollen-derived allergens, the other molecules in extracts of TGP and BTP, and the three identified *S. mansoni* egg antigens. Furthermore, the very extensive cross-reactivity of the anti-SmSEA antibodies eluted from Bet v 1 and Bet v 6.0102 respectively with TGP molecules was consistent with the involvement of CCDs. Previously, the cross-reactivity between plant allergens and some molecules in invertebrates including helminths, have been reported and suspected to be due to shared CCDs (Wilson et al., 1998; van Ree et al., 2000; Paschinger et al., 2009). IPSE/alpha-1, omega-1 and kappa-5 carry immunogenic glycan structures containing fucose and mannose residues, and xylose has been found on kappa-5 (Wuhrer et al., 2006; Meevisen et al., 2010, 2011). Phl p 1 has been described as a glycoprotein with one N-linked glycosylation site containing two mannoses, one xylose and one fucose residue (Petersen et al., 1995) and 20% of sera from patients allergic to grass pollen have been reported to possess IgE antibodies directed against these glycan epitopes (Wicklein et al., 2004). Information on the potential glycosylation of Phl p 5 is sparse, but analysis of its amino acid sequence predicts it to have no N-linked and 17 O-linked glycosylation sites. Two tools for the prediction of glycosylation patterns of proteins revealed that all three BTP allergens possessed at least one site for potential N- and/or O-linked glycosylation. Bet v 1 has been found to possess a single N-linked consensus signal peptide at codon 83 for potential glycosylation (Breiteneder et al., 1989), as also predicted here using the GlycoEP software (Chauhan et al., 2013) (Supplementary Table S14). However, previous reports on the glycosylation of Bet v 1 have been mixed; some authors have reported it to possess a single N-linked glycosylation site (Larsen et al., 1992), but others did not find any glycosylation site on that molecule (Swoboda et al., 1995). In addition to the single N-linked site for potential glycosylation on Bet v 1, our analysis revealed it to possess three sites for potential O-linked glycosylation (Supplementary Table S15), which may not have been given sufficient consideration. In this regard, it may be noted that the intensity of cross-reactivity of the anti-SmSEA antibodies was lower against Bet v 1 than against the other two birch tree pollen allergens. It is worth noting that the O-linked glycans in plants and invertebrates have been only poorly investigated to date (Wicklein et al., 2004), although O-glycan-borne epitopes on Art v 1, an allergen in mugwort pollen, are antigenic in immunoblots with IgE antibodies in sera from mugwort-allergic patients and with rabbit IgG antibodies (Himly et al., 2002). Thus, O-linked glycans may play a role in the antigenic cross-reactivity between *S. mansoni* antigens and pollen allergens. However, more investigations are needed to ascertain the respective involvements of N- and O-linked glycan epitopes in the schistosome-pollen antigenic cross-reactivity.

The putative involvement of CCDs in the antigenic cross-reactivity was corroborated by observations that the reactivity of the five eluted anti-SmSEA IgG antibody preparations against omega-1 and kappa-5 in SmSEA was inhibited by incubation in mild sodium meta-periodate solution, although reactivity against IPSE/alpha-1 was not affected (Fig. 5). Reasons for the exception of the latter egg antigen are unclear, although similar findings had been reported previously (Hamilton et al., 1999; Doenhoff et al., 2016). A possible explanation for this paradox could be the general immunoglobulin-binding activity of IPSE/alpha-1 mediated by a unique loop in the crystallin structure including aromatic and positively-charged amino acids (Meyer et al., 2015). While this IPSE/alpha-1-immunoglobulin interaction is particularly strong with regard to IgE, the egg molecule does also react with IgG

(Meyer et al., 2015). Another putative reason could be that the purified antibodies were reacting with the glycan backbone of IPSE/alpha-1, rather than the fucose-containing epitopes which are usually oxidised by sodium meta-periodate. A similar observation of periodate resistance by another glycoprotein has been reported, namely the reactivity of phytohaemagglutinin with horse radish peroxidase (HRP)-induced anti-CCD antibodies, and it was suggested that the anti-CCD antibodies were reacting with the trisaccharide backbone of the molecule rather than the periodate-degradable glycan residues containing xylose, fucose or mannose (Hino et al., 2009). Thus, while the possibility that the cross-reactivity found here is in part due to homologous peptide epitopes cannot be ruled out entirely, the presence of shared glycan epitopes between the three *S. mansoni* egg antigens and the five pollen allergens is likely the main reason for the IgG cross-reactivity observed between them. However, more direct evidence is needed to ascertain this, e.g., assessment of the antigenic reactivity of molecules that have been deglycosylated by enzymatic treatment.

Patients with the OAS (Cadot et al., 2003) suffer from pollen allergies and are also likely to be allergic to molecules in some other plants such as tomato, peanut, kiwi fruit, melon and banana. The reactivity of each of the five eluted anti-SmSEA IgG antibody preparations against the constituents of plant extracts known to be involved in the OAS is another indication of the involvement of shared epitopes. Moreover, the inhibition of all the observed cross-reactivity by incubation in a mild solution of sodium meta-periodate indicates that these shared epitopes could involve CCDs. CCDs are important immunogenic carbohydrate structures present in many plants and invertebrates (Collot et al., 2011; Hoffmann-Sommergruber et al., 2011). In particular, CCDs containing core fucose and xylose form important components of epitopes in plants and invertebrates and have been implicated in the IgE cross-reactivity observed between these groups of organisms (van Ree et al., 2000; van Ree, 2002).

In *S. mansoni*, glycans which contain core fucosylated and xylo-sylated epitopes have been reported in extracts from all life stages of the parasite (Faveeuw et al., 2003), and especially in eggs (Wuhrer et al., 2006; Meevisen et al., 2010, 2011; Homann et al., 2017). Epitopes containing core fucose and xylose are expressed more in *S. mansoni* eggs compared with other life stages and are developmentally regulated. Thus, shared glyco-epitopes present on both *S. mansoni* egg antigens and pollen allergens could be responsible for their observed antigenic cross-reactivity. Furthermore, core fucose- and/or xylose-containing epitopes present on *S. mansoni* egg glycoproteins are strong inducers of Th2 immune responses during infection with the parasite (Nyame et al., 2000; Faveeuw et al., 2003), similar to many allergens.

The involvement of glyco-conjugates in the cross-reactivity between *S. mansoni* antigens, particularly those of the eggs, and allergens could have useful implications. These findings may provide an alternative explanation for the hygiene hypothesis; that is, anti-*S. mansoni* IgG antibodies that are cross-reactive with allergens could act as blocking antibodies for anti-allergen IgE reactivity, thereby inhibiting allergic sensitisation in susceptible individuals. The possibility that IgG antibodies may somehow block the reactivity of allergenic IgE antibodies has been suggested previously (Jin et al., 2008; Siman et al., 2013; Doenhoff et al., 2016). If true, the idea could be relevant for specific immunotherapy (SIT) of allergic disorders. Currently allergic desensitisation often involves vaccine-type administration of increasing doses of extracts of individual or several allergens (Larche, 2006; Larche et al., 2006; Fujita et al., 2012). SIT is the only treatment approved by the World Health Organization (WHO) for the treatment of allergic diseases apart from allergen avoidance (Bullimore et al., 2012). Although progress has been made, SIT has disadvantages:

it can be cumbersome, there is often an absence of standardised extracts, it requires a composition related to the allergy being treated and multiple doses of injections, and could result in hypersensitive reactions to the immunotherapeutic administrations (Winther et al., 2006; Casset et al., 2012). One means by which SIT is believed to be effective is through the activation of regulatory T-cells, which in turn produce anti-inflammatory cytokines such as IL-10 and TGF- β . IL-10 production is inimical to the synthesis of Th2-type cytokines such as IL-4 and IL-13 that promote allergic inflammation and it stimulates B cells to produce allergen-specific IgG4 antibodies instead of allergenic IgE (Durham et al., 1996; Bellinghausen et al., 1997; Hamid et al., 1997; Larche et al., 2006; Fujita et al., 2012; Mobs et al., 2012; Marth et al., 2013).

IgG antibodies, particularly of the IgG4 subclass, found in sera from individuals undergoing SIT for birch pollen allergen and plant foods, have been reported to compete with IgE antibodies for specific antigenic epitopes on pollen allergens (Subbarayal et al., 2013). Furthermore, N-glycan-specific IgG4 antibodies in sera of pollen-allergic patients undergoing SIT are produced during treatment for grass pollen allergies (van Ree and Aalberse, 1995). A more recent study reported that IgG4 antibodies in sera of allergic patients induced by CCDs on plants and invertebrate glycoproteins amounted to 20% of CCD-specific IgG as well as total IgG (Jin et al., 2008). These authors further found that the total IgG, which contained IgG4, had a higher affinity for CCDs than for peptide epitopes on allergens. The mechanism of SIT involved the “blocking” of allergen specific-IgE by IgG, especially IgG4 (Subbarayal et al., 2013; Nouri-Aria et al., 2004; Fujita et al., 2012).

Schistosoma mansoni egg antigens are known to induce IgG antibodies including those of the IgG4 subclass (Silveira et al., 2002; Figueiredo et al., 2012). The modulated, Th-2-modified immune responses induced by chronic schistosome infections are also known to activate similar immune mechanisms as SIT. Thus, a possible explanation for the protective effect of schistosome infections against allergies might be that *S. mansoni* egg antigens that are antigenically cross-reactive with allergens induce IgG antibodies that somehow block the activity of allergenic IgE antibodies.

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Appendix A. Supplementary data

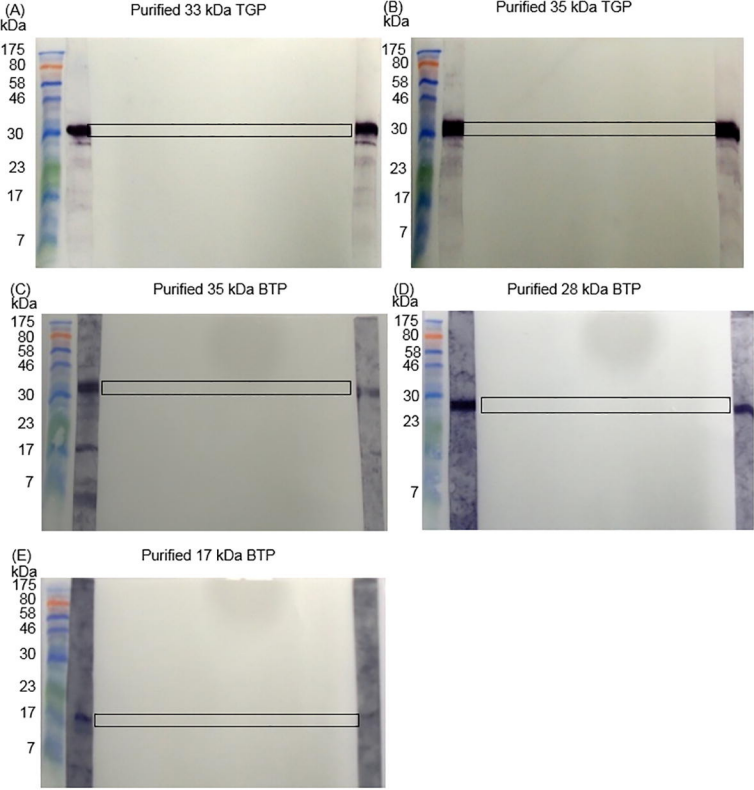
Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ijpara.2017.12.005>.

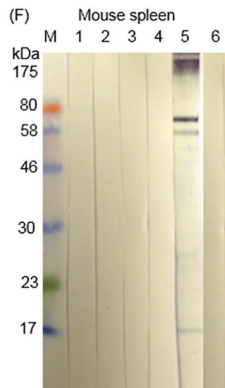
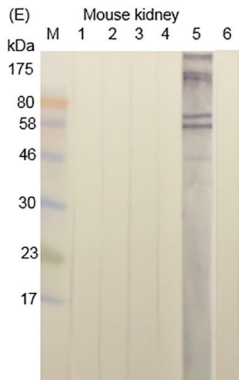
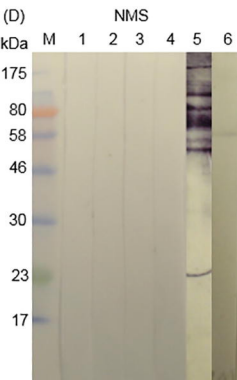
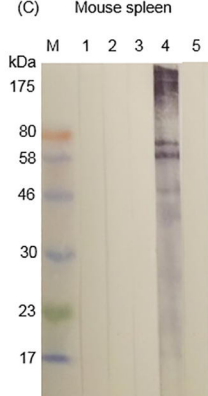
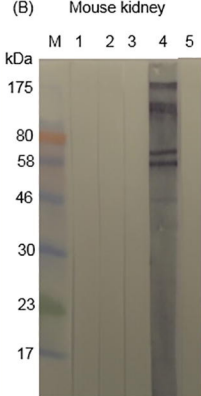
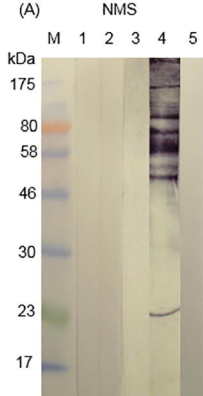
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Supplementary Fig. S3.

```

Aligned_sequences: 2
# 1: Phl p 1
# 2: IPSE/alpha-1
# Matrix: EBL0SUM62
# Gap penalty: 10.0
# Extend penalty: 0.5
#
# Lengtha: 317
# Identityb:      22/317 (6.9%)
# Similarityc:   35/317 (11.0%)
# Gapsd:        237/317 (74.8%)
# Scoree: 15.0

Phl p 1      1 MASSSSVLLVVALFAVFLGSAHGIPKVP GP NITATYGDKWLD AKSTWYG      50
IPSE/alpha-1 1 -----                                0

Phl p 1      51 KPTAAGPKDNGGACGYKDVDKPPFSGMTGCGNTPIF-----      86
                                     :|
IPSE/alpha-1 1 -----MFLIAVLSYTLISQLG      16

Phl p 1      87 -KSGRGC GSCFE-----IKCTKPEACSGEPVVVHITDD      118
      :...|...|.:      :...|...|...|...|...|..
IPSE/alpha-1 17 ITTSDSCKYCLQLYDETYERGSYIEVYKSVGSLSPWP TPGSV CVPFVNDT      66

Phl p 1      119 NEEPIAAYHFD-----LSGIAFGSM AK-----KGDEQKLR----SA      150
      ..|...|..|      ::|...|:...      ||.....|      ..
IPSE/alpha-1 67 KRERP YWYLF DN VNYTGRITGLGHGTCIDDFTKSGFKGISSIKRCIQTKD      116

Phl p 1      151 GEVEI----QFRRVKCKYPEGTKVTFHVEKGSNP NYLALLVKFVAGDGDV      196
      |:|..      :..|...|:..
IPSE/alpha-1 117 GKVE CINQPKRRRTYCRF-----      134

Phl p 1      197 VAVDIKEKGKDKWIALKESWGAIWRIDTPEVLKGPFTVRYTTEGGTKGEA      246
IPSE/alpha-1 135 -----                                134

Phl p 1      247 KDVIPEGWKADTAYESK      263
IPSE/alpha-1 135 -----                                134

```

Supplementary Fig. S4.

```
# Aligned_sequences: 2
# 1: Phl p 1
# 2: Omega-1
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Lengtha: 425
# Identityb:      17/425 (4.0%)
# Similarityc:    27/425 (6.4%)
# Gapsd:          362/425 (85.2%)
# Scoree: 6.0

Phl p 1      1  ----- 0
Omega-1      1  MMRAQSLCFLVSILVTILHVGYSQNSWDYVFSVTWPPTYCESIQCLPR 50
Phl p 1      1  ----- 0
Omega-1     51  GLRDFTIHGLWPTIFPNRQPNECTGSLRFDIRRLQGIRNELDLMWPHLKNY 100
Phl p 1      1  ----- 0
Omega-1    101  RESPSFWKHFEFEKHGLCAVEDPQVFNQYGYFKGIQLMQKLNLLKTLMKY 150
Phl p 1      1  -----MASSSSVLLVVALFAVFLGSAHGIPKVP GP NITATYG----- 38
Omega-1    151  RISPHDSRQYDTINLMNVLEREFGYNGSANCIRK---PGRRGMYHLEEV 196
Phl p 1     39  DKWLDAKSTWYGKPTAAGPKDNGGACGYKDVKPPFSGMTGCGNTPIFKS 88
Omega-1    197  HVCLNRKHEFMNCPFL-----GNCPKKFI-FPPFQ----- 225
Phl p 1     89  GRGCGSCFEIKCTKPEACSGEPVVVHITDDNEEPIAAYHFDLSGIAFGSM 138
Omega-1    226  ----- 225
Phl p 1    139  AKKGDEQKLRSAGEVEIQFRRVKCKYPEGTKVTFHVEKGSNPNYLALLVK 188
Omega-1    226  ----- 225
Phl p 1    189  FVAGDGDVVAVDIKEKGKDKWIALKESWGAIWRIDTPEVLKGPFTVRYTT 238
Omega-1    226  ----- 225
Phl p 1    239  EGGTKGEAKDVIPEGWKADTAYESK 263
Omega-1    226  ----- 225
```

Supplementary Fig. S5.

```

Aligned_sequences: 2
# 1: Ph1 p 1
# 2: Kappa-5
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Lengtha: 448
# Identityb:      30/448 (6.7%)
# Similarityc:    55/448 (12.3%)
# Gapsd:         333/448 (74.3%)
# Scoree: 38.5

Phl p 1      1 MASSSSVLLVVALFAVFLGSAHGIPKVP PGP NITATYGDKWLD AKSTWYG      50
Kappa-5      1 -----                                0

Phl p 1     51 KPTAAGPKDNGGACGYKDVDKPPFSGMTGCGNTPIFKSGRGC GSCFEIKC    100
Kappa-5      1 -----                                0

Phl p 1    101 TKPEACSGEPVVVHITDDNEEPIAAYH-----FDLSGIAFGSMAKK    141
Kappa-5      1 -----MLQ EYNLLINGVTLLISLSGLICEAQSPT      29
               . . . . | : . . | | | : . . . . .

Phl p 1    142 GDEQ--KLRSAGEVEIQFRRVKCKYPEGTKVTFHVE--KGSNP NYLALL    186
Kappa-5     30 NDEM HATISEYGRLYI-----TNIHIHYRLLIMALSPN-----    62
               . | | . . . . . | : . | . . . . . | : . . . . .

Phl p 1    187 VKFVAGDGDVVAVDIKEKGKDKWIALK--ESWGAIWRIDTPEVLKGPF TV    234
Kappa-5     63 MKFTPGEADN ILHKSEEEHQVKW-ALNYLNAARSTWKLE NEDMFK-----    106
               : | | . . | : . | . . . . . | : . . . . | | . . . . . | : . . . . .

Phl p 1    235 RYTTEGGTKGEAKDVIP-----EGWKADTAYESK-----    263
Kappa-5    107 --KTMSSYVGVNKTIVPNFCMTMLQSSARNW--DTNIQKDITYGCEVLK    152
               . | . . . . | : . | : : | . . | | | . . . .

Phl p 1    264 -----                                263
Kappa-5    153 KYSELKWGARKKLDLMTIRWLNGSDENGQSQHISDKGFNYHSKKEYLECA    202

Phl p 1    264 -----                                263
Kappa-5    203 QSVMKIHRTKAEVDCRSTVGEFLKLQQVKDTPSSKTQAIQFDKINENFNK    252

Phl p 1    264 -----                                263
Kappa-5    253 SLVELKRRVENLEINRINYLKFMNPMERVISVIDAMEEEVDHKYGVYM    300

```

Supplementary Fig. S6.

Aligned_sequences: 2

1: Phl p 5b

2: IPSE/alpha-1

Matrix: EBLOSUM62

Gap_penalty: 10.0

Extend_penalty: 0.5

Length^a: 407

Identity^b: 2/407 (0.5%)

Similarity^c: 4/407 (1.0%)

Gaps^d: 396/407 (97.3%)

Score^e: 6.0

Phl p 5b	1	AAAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQKLIEDI	50
IPSE/alpha-1	1	-----	0
Phl p 5b	51	NVGFKAAVAAAASVPAADKFKTFEAAFTSSSKAAAAKAPGLVPKLDAAYS	100
IPSE/alpha-1	1	-----	0
Phl p 5b	101	VAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVKPVTEEPGMAKI	150
IPSE/alpha-1	1	-----	0
Phl p 5b	151	PAGELQIIDKIDAAFKVAATAAATAPADDKFTVFEEAFNKAIKESTGGAY	200
IPSE/alpha-1	1	-----	0
Phl p 5b	201	DTYKCIPSLEAAVKQAYAATVAAAPQVKYAVFEAALTKAITAMSEVQKVS	250
IPSE/alpha-1	1	-----	0
Phl p 5b	251	QPATGAATVAAGAATTAAGAASGAATVAAGGYKV-----	284
		...::	
IPSE/alpha-1	1	-----MFLIAVLSYTLISQLGITTSDSCKYCL	27
Phl p 5b	285	-----	284
IPSE/alpha-1	128	QLYDETYERGSYIEVYKSVGSLPPWTPGSVCVPFVNDTKRERPYWYLF	77
Phl p 5b	285	-----	284
IPSE/alpha-1	178	NVNYTGRITGLGHGTCIDDFTKSGFKGISSIKRCIQTKDGKVECI	127
Phl p 5b	285	-----	284
IPSE/alpha-1	128	RRTYCRF	134

Supplementary Fig. S7.

Aligned_sequences: 2

1: Ph1p5b

2: Omega-1

Matrix: EBL0SUM62

Gap_penalty: 10.0

Extend_penalty: 0.5

Length^a: 485

Identity^b: 5/485 (1.0%)

Similarity^c: 7/485 (1.4%)

Gaps^d: 461/485 (95.1%)

Score^e: 6.0

Ph1 p 5b 1 AAAAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQKLIEDI 50

Omega-1 1 ----- 0

Ph1 p 5b 51 NVGFKA AVAAAA SVPAAD KFKTF EAAFT SSSKAAA AKAPGL VPKLDA AYS 100

Omega1 1 ----- 0

Ph1 p 5b 101 VAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVKPVTEEPGMAKI 150

Omega-1 1 ----- 0

Ph1 p 5b 151 PAGELQIIDKIDAAFKVAATAAATAPADDKFTVFEEAFNKAIKESTGGAY 200

Omega-1 1 ----- 0

Ph1 p 5b 201 DTYKCIPSLEAAVKQAYAATVAAAPQVKYAVFEAALTKAITAMSEVQKVS 250

Omega-1 1 ----- 0

Ph1 p 5b 251 QPATGAATVAAGAATTAAGAASGAATVAAGGYKV----- 284

$$\dots | \cdot : \dots | \dots | : \dots | | \dots$$

Omega-1 1 -----MMRAQSLCFLVSIILVTHVGYNSWDYYVFSVTPPTY 40

Ph1 p 5b 285 ----- 284

Omega-1 41 CESIQCRLPRGLRDFTHGLWPTIFPNRQPNGTSLRFDIRRLQGIRNEL 90

Ph1 p 5b 285 ----- 284

Omega-1 91 DLMWPHLKNYRESPSFWKHEFEKHGLCAVEDPQVFNQYGYFKFGIQLMQK 140

Ph1 p 5b 285 ----- 284

Omega-1 141 L NLLKTLMKYRISPHDSRQYDTINLMNVLEREFGYNGSANCIRKPGRGRM 190

Ph1 p 5b 285 ----- 284

Omega-1 191 YHLEEVHVCLNRKHEFMNCPFLGNCPKKFIFPPFQ 225

Supplementary Fig. S8.

```

Aligned_sequences: 2
1: Ph1 p 5b
2: Kappa-5
Matrix: EBLOSUM62
Gap_penalty: 10.0
Lengtha: 577
Identityb:      1/577 (0.2%)
Similarityc:    2/577 (0.3%)
Gapsd:         570/577 (98.8%)
Scoree: 2.0

Ph1 p 5b      1 AAAAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQKLIEDI      50
Kappa-5       1 -----                                0

Ph1 p 5b     51 NVGFKA AVAAA SVPAADKFKTFEAAFTSSSKAAAAPGLVPKLDAAYS     100
Kappa-5       1 -----                                0

Ph1 p 5b    101 VAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVKPVTEEPGMAKI    150
Kappa-5       1 -----                                0

Ph1 p 5b    151 PAGELQIIDKIDAAFKVAATAAATAPADDKFTVFEEAFNKAIKESTGGAY    200
Kappa-5       1 -----                                0

Ph1 p 5b    201 DTYKCIPSLEAAVKQAYAATVAAAPQVKYAVFEAALT KAITAMSEVQKVS    250
Kappa-5       1 -----                                0

Ph1 p 5b    251 QPATGAATVAAGAATTAAGAASGAATVAAGGYKV-----            284
Kappa-5       1 -----MLQEYNLLINGVTLLISLSGLIC      23
                      ....|.:.

Ph1 p 5b    285 -----                                284
Kappa-5      24 EAQSPTNDEM HATISEYGRLYITNIHIHYRLLIMALSPNMKFTPGEADNI    73

Ph1 p 5b    285 -----                                284
Kappa-5      74 LHKSEEEHQVKWALNYLNAARSTWKL ENEDMFKKTMSSYGVNKTIVPNF    123

Ph1 p 5b    285 -----                                284
Kappa-5     124 CMTMLQSSARNWDTNIQKDITYGCEVLKKYSELKWGARKKLDLMTIRWL    173

Ph1 p 5b    285 -----                                284
Kappa-5     174 NGSDENGQSQHISDKGFNYHSKKEYLECAQSVMKIHRTKAEVDCRSTVGE    223

Ph1 p 5b    285 -----                                284
Kappa-5     224 FLKLQQVKDTPSSKTQAIQFDKINENFNKSLVELKRRVENLEINRINYLK    273

Ph1 p 5b    285 -----                                284
Kappa-5     274 FMNPMERVISVIDAMEEEVDHKYGVYM      300

```

Supplementary Fig. S9.

```

Lengtha: 276
Identityb:      8/276 (2.9%)
Similarityc:   9/276 (3.3%)
Gapsd:        258/276 (93.5%)
Scoree: 13.5

Bet v 1      1  MGVFNJETETTSVIPAARLFKAFILDGDNLFPKVAPQAISSVENIEGNGG      50
IPSE/alpha-1 1  -----                                0

Bet v 1      51  PGTIKKISFPEGFPFKYVKDRVDEVDHTNFKYNYSVIEGGPIGDTLEKIS    100
IPSE/alpha-1 1  -----                                0

Bet v 1     101  NEIKIVATPDGGSILKISNKYHTKGDHEVKAEQVKASKEMRETLLRAVES    150
                  ..|.||.|
IPSE/alpha-1 1  -----MFLIAVLS                                8

Bet v 1     151  YLL-----AHSDAYN-----                                160
                  |.|      ..||:..
IPSE/alpha-1 9  YTLISQLGITTSDSCKYCLQLYDETYERGSYIEVYKSVGSLSPWTPGSV    58

Bet v 1     161  -----                                160
IPSE/alpha-1 59  CVPFVNDTKRERPYWYLFDNVNYTGRITGLGHGTCIDDFTKSGFKGISSI    108

Bet v 1     161  -----                                160
IPSE/alpha-1109 KRCIQTKDGKVEGINQPKRRRTYCRF      134

http://www.ebi.ac.uk/Tools/services/web/toolresult.ebi

```

Supplementary Fig. S10.

Length ^a : 341			
Identity ^b : 16/341 (4.7%)			
Similarity ^c : 22/341 (6.5%)			
Gaps ^d : 297/341 (87.1%)			
Score ^e : 8.5			
Omega-1	1	MMRAQSLCFLVSILVTILHVGYSQNSWDYYVFSVTPPTYCESIQRLPR	50
Bet v 1	1	-----	0
Omega-1	51	GLRDFTIHGLWPTIFPNRQPNC TGS LRFDIRRLQGIRNELDMWPHLKNY	100
Bet v 1	1	-----	0
Omega-1	101	RESPSFWKHEFEKHGLCAVEDPQVFNQYGYFKFGIQLMQKLNLLKTLMKY	150
Bet v 1	1	-----	0
Omega-1	151	RISPHDSRQYDTINLMNVLERE-----FGYNGSANCIRKP	185
			
Bet v 1	1	-----MGVFNYETETTSVIP AARLFKAFILDGD-NLFPKV	34
Omega-1	186	GRRGMYHLEEVHVCLNRKHEFMNCPFLGN----CPKKFIFP---PFQ---	225
		...:..: :	
Bet v 1	35	APQAISSVENIE-----GNGGPGTIKKISFPEGFPFKYVK	69
Omega-1	226	-----	225
Bet v 1	70	DRVDEV DHTNFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISN	119
Omega-1	226	-----	225
Bet v 1	120	KYHTKGDHEVKAEQVKASKEMRETLLRAVESYLLAHS DAYN	160

Supplementary Fig. S11.

Aligned_sequences: 2

1: K5

2: Bet v 1

Matrix: EBLOSUM62

Gap_penalty: 10.0

Extend_penalty: 0.5

Length^a: 389

Identity^b: 23/389 (5.9%)

Similarity^c: 38/389 (9.8%)

Gaps^d: 318/389 (81.7%)

Score^e: 25.5

K5	1	MLQEYNLLINGVTLLISLSGLICEAQSPNTNDEMHAISEYGRLYITNIHI	50
Bet v 1	1	-----	0
K5	51	HYRLLIMALSPNMKFTPGEADNILHKSEEHQVKWALNYLNAARSTWKLE	100
Bet v 1	1	-----	0
K5	101	NEDMFKKTSSYVGVNKTIVPNFCMTMLQQSSARNWDNIQKDITYGCEV	150
Bet v 1	1	-----	0
K5	151	LKKYSELKWGARKKLDLMTIRWLNGSDENGQSQHISDKGFNYHSKKEYLE	200
Bet v 1	1	-----	0
K5	201	CAQSVMKIHRTKAEVDCRSTVGEF-LKLQQVKDTPSSKT-QAIQFDKINE	248
		: . .:..... ::: .:	
Bet v 1	1	-----MGVFNYETETTSVIPAAARLFKAFILDGDN-	29
K5	249	NFNKSLVELKRRVENLE-----INRINY---LKFMPMERVISVIDAM	288
		:.... : .: :::	
Bet v 1	30	LFPKVAPQAISSVENIEGNGGPGTIKKISFPEGFPFKYVKDRV-----	72
K5	289	EEEVDH---KYGVYM-----	300
		: ...:	
Bet v 1	73	-DEV DHTNFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISNKY	121
K5	301	-----	300
Bet v 1	122	HTKGDHEVKAEQVKASKEMRETLRAVESYLLAHSDAYN	160

Supplementary Fig. S12.

```

Aligned_sequences: 2
1: BTP-GST
2: IPSE/alpha-1
Matrix: EBLOSUM62
Gap_penalty: 10.0
Extend_penalty: 0.5

Lengtha: 305
Identityb:      15/305 (4.9%)
Similarityc:   25/305 (8.2%)
Gapsd:        239/305 (78.4%)
Scoree: 16.0

BTP-GST      1 MADASVKEHLPTPLDATSNPPPIFDGTTRLYTCYTCPFAQRVWITRNYKG      50
IPSE/alpha-1 1 -----                                0
BTP-GST      51 LQEKIKLVPINLQNRPAWYKEKVYPENKVPALHNGKVIGESLDLIKYYVD      100
IPSE/alpha-1 1 -----                                0
BTP-GST     101 INFEGPSLLPNDDPAKKAFAEELVAYSDTFNKTVFTSFKGDVPKEAGPAFD      150
IPSE/alpha-1 1 -----                                0
BTP-GST     151 HLEKALHKFDDGPFFLGQFSAVDIVYIPFVERFQIFLLDALK-----YDI      195
                  .||      :::::.....:..|...|:..|      ||.
IPSE/alpha-1 1 -----MFL-----IAVLSYTLISQLGITTSDSCKYCLQLYDE      32
BTP-GST     196 TAGRPKLAKWIEELNKIDAYKPTKTDPKELVEFYKARFAAQQ-----      237
                  |..|      ..:|.....:..|..|.....|..|.....:..
IPSE/alpha-1 33 TYER---GSYIEVYKSVGSLSPWPVPGSVCPVFNVDTKRERPYWYLFDNV      79
BTP-GST     238 -----                                237
IPSE/alpha-1 80 NYTGRITGLGHGTCIDDFTKSGFKGISSIKRCIQTKDGKVECINQPKRRR      129
BTP-GST     238 -----      237
IPSE/alpha-1 130 TYCRF      134

```

Supplementary Fig. S13.

Aligned sequences: 2

1: BTP-GST

2: Omega1

Matrix: EBLOSUM62

Gap penalty: 10.0

Extend_penalty: 0.5

Length^a: 316

Identity^b: 39/316 (12.3%)

Similarity^c: 81/316 (25.6%)

Gaps^d: 170/316 (53.8%)

Score^e: 21.5

BTP-GST	1	-----	0
Omega-1	1	MMRAQSLCFLVLSILVTILHVGYSQNSWDYYVFSVTWPPTYCESIQRLPR	50
BTP-GST	1	-MADASVKEHLPTPLDATSNPPPIFDGTTTRYTCYTCPFAQRVWITRNYK	49
		:. .:... : ... :..	
Omega-1	51	GLRDFTIHGLWPT---IFPNRQPNCTGSLR-----FDIRRLQ	84
BTP-GST	50	GLQEKIKLVPINLQN---RPAWYKEKVYPENKVPALAHN-----	85
		:..:.. :..: : .. :..:..:..: : ..	
Omega-1	85	GIRNELDLMWPHLKNYRESPSFWKHE-FEKHGLCAVEDPQVFNQYGYFKF	133
BTP-GST	86	GKVIGESLDLIKVDINFEGPSLLPNPAKKAFAEELVAYSDTFN-----	130
		..:..: : : :..:..: ..: : :..: ..	
Omega-1	134	GIQLMQKLNLLKTL-MKYR---ISPHDSRQ-----YDTINLMNVL	169
BTP-GST	131	KTVFTSFKGDP--VKEAG-PAFDHLEKA-----LHKFDDGPFFLGQFSA	171
		 :..:..: :..: .. : :..: . ..	
Omega-1	170	EREF-GYNGSANCIRKPGRRGMYHLEEVHVCLNRKHEFMNCP-FLGN-CP	216
BTP-GST	172	VDIVYIPFVERFQIFLLDALKYDITAGRPKLAKWIEELNKIDAYKPTKTD	221
		...:..: : .	
Omega-1	217	KKFIFPPFQ-----	225
BTP-GST	222	PKELVEFYKARFAAQQ 237	
Omega-1	226	----- 225	

Supplementary Fig. S14.

Aligned_sequences: 2

1: BTP-GST

2: Kappa5

Matrix: EBLSUM62

Gap penalty: 10.0

Extend_penalty: 0.5

Length^a: 407

Identity^b: 33/407 (8.1%)

Similarity^c: 67/407 (16.5%)

Gaps^d: 277/407 (68.1%)

Score^e: 26.5

BTP-GST 1 ----- 0

Kappa-5 1 MLQEYNLLINGVTLLISLSGLICEAQSPTNDEMHTISEYGRLYITNIHI 50

BTP-GST 1 ----- 0

Kappa-5 51 HYRLLIMALSPNMKFTPGADNILHKSEEEHQVKWALNYLNAARSTWKLE 100

BTP-GST 1 -----MADASVKEHLPTPLDATSNPPPI 23
 :::|:::

Kappa-5 101 NEDMFKKTMS¹⁰¹SYVGVNKTIVPNFCMTMLQ¹¹⁰SSARN----- 135

BTP-GST 24 FDGTTRLTYCYTC----PFAQRVWITRNYKGLQEIKLVPINLQNRP--- 66

Kappa-5 136 WDTNIQKDITYGCEVLKKYSELKW-----GARKKLDLMTIRWLNGSDEN 179

BTP-GST 67 -----AWYKEKVYPENKVPAL E-HNGK-----VIGESLDLIK 97
 :.:|.|.|.....:| |..| :|||.|.|.:

Kappa-5 180 GQSQHISDKGFNYHSKKEYLECAQSVMKIHRTKAEVDCRSTVGEFLKLQQ 229

BTP-GST 98 YVDINFE⁺GPSLLPN⁺DPAKKAF⁺AEELVAYS⁺DTFN⁺KTV----- 133
 ..||:|..|:.....|:|:|:|

Kappa-5 230 VKD-----TPSSKTQAIQFDKINENFNKSLVELKRRVENLEINR 268

BTP-GST 134 --FTSFKGDPVKEAGPAFDHLEKAL-HKFDDGPFFLQGQSAVDIVYIPFV 180

Kappa-5 269 INYLKFM-NPMERVISVIDAMEEEVDHKY--GVYM----- 300

BTP-GST 181 ERFQIFLLDALKYDITAGRPKLAKWIEELNKIDAYKPTKTDPKELVEFYK 230

Kappa-5 301 ----- 300

BTP-GST 231 ARFAAQQ 237

Kappa-5 301 - - - - - 300

Supplementary Fig. S15.

```
# Lengtha: 345
# Identityb:      26/345 (7.5%)
# Similarityc:   48/345 (13.9%)
# Gapsd:        248/345 (71.9%)
# Scoree: 17.0

Bet v 6      1 MAHKSILIIIGGTGYIGKFIVEASAKSGHPTFALVRE--STVSDPVKGKL      48
                .|:....      ::|:::  .|...|..|
IPSE/alpha-1 1 -----MFLIAVL-----SYTLISQLGITTSDSCKYCL      27

Bet v 6      49 VEKFKGLGVTLLHGDLYDHESLVKAFKQVDVV-----      80
                .|:..|:..|:..|:..|:..|
IPSE/alpha-1 28 -----QLYDETYERGSYIEVYKSVGSLSPWPVPGSVCPFVNDTK      67

Bet v 6      81 -----ISTVGHLQLADQVKIIAAIKEAGNIKRFFPSEF      113
                |::||...|.. ..::|...:|...:..
IPSE/alpha-1 68 RERPYWYLFDNVNYTGRITGLGHGTCIDDF-TKSGFKGISSIKRCIQTKD      116

Bet v 6      114 GNDVDRVHAVEPAKTAFATKAEIRRKTEAEGIPYTYVSSNFFAGYFLPTL      163
                | :|...:..|      ||:|....
IPSE/alpha-1 117 G----KVECINQPK-----RRRTYCRF-----      134

Bet v 6      164 AQPGLTSPPREKVVFVFGDGNARAVFNKEDDIGTYTIRAVDDPRTLNKIVY      213
IPSE/alpha-1 135 -----      134

Bet v 6      214 IKPAKNIYSFNEIVALWEKKIGKTKLEKIYVPEEKLLKDIQESPIPINVIL      263
IPSE/alpha-1 135 -----      134

Bet v 6      264 AINHSVFVKGDHTNFEIEASFGVEASELYPDVKYTTVEEYLQQFV      308
IPSE/alpha-1 135 -----      134
```

<http://www.ebi.ac.uk/Tools/services/web/toolresult.ebi>

Supplementary Fig. S16.

```

Lengtha: 508
Identityb:      8/508 (1.6%)
Similarityc:   13/508 (2.6%)
Gapsd:        483/508 (95.1%)
Scoree: 5.0

Omega-1   1  MMRAQSLCFLVSILVTILHVGYSQNSWDYYVFSVTPPTYCESIQRLPR      50
Bet v 6   1  -----                                0

Omega-1  51  GLRDFTIHGLWPTIFPNRQPNCGTSLRFDIRRLQGIRNELDMWPHLKNY    100
Bet v 6   1  -----                                0

Omega-1 101  RESPSFWKHEFEKHGLCAVEDPQVFNQYGYFKFGIQLMQKLNLLKTLMKY    150
Bet v 6   1  -----                                0

Omega-1 151  RISPHDSRQYDTINLMNVLEREFGYNGSANCIRKPGRRGMYHLEEVHVCL    200
                |.|...:
Bet v 6   1  -----MAHKSILII--          9

Omega-1 201  NRKHEFMNCPFLGNCPKKFI-----FPPFQ-----          225
                .....:|   ||   .|.|.
Bet v 6  10  -----IGGTGYIG----KFIVEASAKSGHPTFALVRESTVSDPVKGKLVE    50

Omega-1 226  -----                                225

Bet v 6   51  KFKGLGVTLHGDLYDHESLVKAFKQVDVVISTVGHLQLADQVKIIAAIK    100

Omega-1 226  -----                                225

Bet v 6 101  EAGNIKRFFPSEFGNDVDRVHAVEPAKTAFATKAEIRRKTEAEGIPYTYV    150

Omega-1 226  -----                                225

Bet v 6 151  SSNFFAGYFLPTLAQPGLTSPPREKVVFIFGDGNARAVFNKEDDIGTYTIR    200

Omega-1 226  -----                                225

Bet v 6 201  AVDDPRTLNKIVYIKPAKNIYSFNEIVALWEKKIGKTLEKIYVPPEKLLK    250

Omega-1 226  -----                                225

Bet v 6 251  DIQESPIPINVILAINHSVFKGDHTNFEIEASFGVEASELYPDVKYTTV    300

Omega-1 226  -----          225

Bet v 6 301  EEYLQQFV          308

```

Supplementary Fig. S17.

Length^a: 564

Identity^b: 14/564 (2.5%)

Similarity^c: 23/564 (4.1%)

Gaps^d: 520/564 (92.2%)

Score^e: 26.5

K5 1 ----- 0

Bet v 6 1 MAHKSILIIIGGTGYIGKFIVEASAKSGHPTFALVRESTVSDPVKGKGLVE 50

K5 1 ----- 0

Bet v 6 51 KFKGLGVTL LHGDLYDHESLVKAFKQVDVVISTVGHLOLADOVKIIAAIK 100

K5 1 ----- 0

Bet v 6 101 EAGNIKRFFPSEFGNDVDRVHAVEPAKTAFAATKAEIRRKTEAEGIPYTYV 150

K5 1 ----- 0

Bet v 6 151 SSNFFAGYFLPTLAQPGLTSPPREKVVIFGDGNARAVFNKEDDIGTYTIR 200

K5 1 ----- 0

Bet v 6 201 AVDDPRTLNKIVYIKPAKNIYSFNEIVALWEKKIGKTLEKIYVPEEKLLK 250

K5 1 MLQEYNLLINGVTLLISLSGLI-----CEAQSPTNDEMCHAT 36

[illegible]

Bet v 6 251 DIQESPIPIN-VILAINHSVFVKGDHTNFEIEASFGVEASELYPDVKYTT 299

K5 37 ISEYGRLYITNIHIHYRLLIMALSPNMKFTPGEADNILHKSEEEHQVKWA 86

[illegible]

Bet v 6 300 VEEYLQQFV----- 308

K5 87 LNYLNAARSTWKL ENEDMFKKTMS SYVGVNKTIVPNFCMTMLQQSSARNW 136

Bet v 6 309 ----- 308

K5 137 DTNIOKDITYGCEVLKKYSELKWGARKKLDLMTIRWLNGSDENGOSOHIS 186

Bet v 6 309 ----- 308

K5 187 DKGFNYSKKEYLECAQSVMKIHRTKAEVDCRSTVGEFLKLQQVKDTPSS 236

Bet v 6 309 ----- 308

K5 237 KTQAIQFDKINENFNKSLVELKRRVENLEINRINYLKFMNPMERVISVID 286

Bet v 6 309 ----- 308

K5 287 AMEEEV~~DD~~HKYGVYM 300

Bet v 6 309 ----- 308

Supplementary Table S1. Results of the MASCOT search of tandem mass spectrometric (TMS)-derived data from the purified 33 kDa Timothy grass (*Phleum pratense*) pollen (TGP) gel band.

SwissProt: MPA5B_PHLPR

Pollen allergen Phl p 5b (Fragment) OS=*Phleum pratense* PE=1 SV=2

Mass^a: 27985 Score^b: 258 Matches: 29 (8) Sequences: 6 (2), Protein sequence coverage^c27%

Peptide match ^d	Score ^e	Expect ^f
<i>FDSFVASLTEALR</i>	82	2.2e-05
<i>IPAGELQIIDK</i>	70	0.00043
AAVAAAASVPAADK	42	0.23
TFEAAFTSSSK	29	5.2
VAATAAATAPADDKFTVFEEAFNK	26	6.8
IPAGELQIIDKIDAAFK	11	2.5e+02

Matched peptides^g

1	AAAVPRRG	RGPGRSYTA	DAGYAPATPA	AAGAAAGKAT	TEEQKLIEDI
51	NVGFKAAVAA	AASVPAADKF	KTFEAAFTSS	SKAAAAKAPG	LVPKLDAAYS
101	VAYKAAVGAT	PEAKFDSFVA	SLTEALRVIA	GALEVHAVKP	VTEEPGMAKI
151	PAGELQIIDK	IDAAFKVAAT	AAATAPADDK	FTVFEEAFNK	AIKESTGGAY
201	DTYKCIPSLE	AAVKQAYAAT	VAAAPQVKYA	VFEAALTKAI	TAMSEVQKVS
251	QPATGAATVA	AGAATTAAGA	ASGAATVAAG	GYKV	

^aThe predicted mass of a protein in Daltons

^bMascot protein score; i.e., the total of all the peptide ion scores matching a protein;

^cThe percentage of a protein sequence covered by MS-matched peptides;

^dPeptides with scores above the threshold are revealed in italics in the first column.

^eA score assigned to individual matching peptide by MASCOT based on the probability of best match;

^fChance of obtaining of obtaining an equal or higher score for a peptide.

⁹Peptides that matched the protein in MASCOT are underlined in the amino acid sequence.

OS, Organism species/name; PE, Protein existence; SV, Sequence version.

Supplementary Table S2. Results of the MASCOT search of tandem mass spectrometric (TMS)-derived data from the purified 35 kDa Timothy grass (*Phleum pratense*) pollen (TGP) gel band

NCBI nr: gi|3901094

Pollen allergen Phl p 1 [*Phleum pratense*]

Mass: 28185^a, **Score**^b: 453, **Matches:** 60(16), **Sequences:** 7(3),

Protein sequence coverage^c: 27%

Peptide match ^d	Score ^e	Expect ^f
<i>FVAGDGDVVAVDIK</i>	93	0.00017
<i>STWYGKPTAAGPK</i>	88	0.00053
<i>SAGEVEIQFR</i>	76	0.01
GSNPNYLALLVK	66	0.096
IDTPEVLK	48	5.6
ESWGAIWR	46	11
DVIPEGWK	42	28

Matched peptides

1	<u>MAS</u> SSSVLLV	VALFAVFLGS	AHGIPKVPPG	PNITATYGDK	WLDKSTWYG
51	<u>KPTAAGPK</u> DN	GGACGYKDVD	KPPFSGMTGC	GNTPIFKSGR	GCGSCFEIKC
101	TKPEACSGEP	VVVHITDDNE	EPAAAYHFDL	SGIAFGSMK	KGDEQKLRSK
151	<u>GEVEIQFRRV</u>	KCKYPEGTVK	TFHVEKGSNP	<u>NYLALLVKFV</u>	<u>AGDGDVVAVD</u>
201	<u>IKEK</u> GKDKWI	ALK <u>ESWGAIW</u>	<u>RIDTPEVLKG</u>	PFTVRYTTEG	GTKGEAKDVI
251	<u>PEGWKADTAY</u>	ESK			

^aThe predicted mass of a protein in Daltons

^bMascot protein score; i.e., the total of all the peptide ion scores matching a protein;

^cThe percentage of a protein sequence covered by MS-matched peptides;

^dPeptides with scores above the threshold are revealed in italics in the first column.

^eA score assigned to individual matching peptide by MASCOT based on the probability of best match;

^fChance of obtaining of obtaining an equal or higher score for a peptide.

^gPeptides that matched the protein in MASCOT are underlined in the amino acid sequence.

OS, Organism species/name; PE, Protein existence; SV, Sequence version.

Supplementary Table S3. Results of the MASCOT search of tandem mass spectrometric (TMS)-derived data from the purified ~17 kDa Birch tree (*Betula verrucosa*) pollen (BTP) gel band

Database: NCBI nr, gi: 1542869

Pollen allergen Bet v 1 [*Betula pendula*]

Mass^a: 17526 Score^b: 202 Matches: 6 (1) Sequences: 5 (1),

Protein sequence coverage^c: 42%

Peptide match ^d	Score ^e	Expect ^f
DRVDEVDHTNFK	69	0.029
EMGETLLR	39	37
DRVDEVDHTNFK	35	65
IVATPDGGSILK	32	1.6e+02
AVESYLLAHSDAYN	27	4.8e+02

Matched peptides

1	MGVFNYETET	TSVIPAARLF	KAFILDGDNL	FPKVAPQAIS	SVENIEGNNGG
51	PGTIKKISFP	EGLPFKYVKD	RVDEVDHTNF	KYNYSVIEGG	PIGDTLEKIS
101	NEIKIVATPD	GGSILKISNK	YHTKGDHEVK	AEQVKASKEM	GETLLRAVES
151	YLLAHSDAYN				

^aThe predicted mass of a protein in Daltons

^bMascot protein score; i.e., the total of all the peptide ion scores matching a protein;

^cThe percentage of a protein sequence covered by MS-matched peptides;

^dPeptides with scores above the threshold are revealed in italics in the first column.

^eA score assigned to individual matching peptide by MASCOT based on the probability of best match;

^fChance of obtaining of obtaining an equal or higher score for a peptide.

^gPeptides that matched the protein in MASCOT are underlined in the amino acid sequence.

OS, Organism species/name; PE, Protein existence; SV, Sequence version.

Supplementary Table S4. Results of the MASCOT search of tandem mass spectrometric (TMS)-derived data from the purified ~28 kDa Birch tree (*Betula verrucosa*) pollen (BTP) gel band.

NCBIInr, gi: 573005958, Glutathione S-transferase [<i>Betula pendula</i>]					
Mass ^a : 27022, Score ^b : 378, Matches: 24 (1), Sequences: 8 (1),					
Protein sequence coverage ^c : 39%					
Peptide match ^d		Score ^e		Expect ^f	
<i>EHLPTPLDATSNPPPIFDGTTTR</i>		70		0.026	
EAGPAFDHLEK		64		0.13	
VIGESLDLIK		49		5.2	
IDAYKPTK		48		7.3	
AFAEELVAYSDTFNK		44		11	
YDITAGRPK		41		35	
FQIFLLDALK		37		81	
VPALEHNGK		26		9.2e+02	
Matched peptides					
1	MADASVKEHL	PTPLDATSNP	PPIFDGTTRL	YTCYTCPFAQ	RVWITRNYKG
51	LQEKIKLVPI	NLQNRPAWYK	EKVYPENKVP	ALEHNGKVI	ESLDLIKYVD
101	INFEGPSLLP	NDPAKKAF	ELVAYSDTFN	KTVFTSFKGD	PVKEAGPAFD
151	HLEKALHKFD	DGPFFLGQFS	AVDIVYIPFV	ERFQIFLLDA	LKYDITAGRP
201	KLAKWIEELN	KIDAYKPTKT	DPKELVEFYK	ARFAAQ	

^aThe predicted mass of a protein in Daltons

^bMascot protein score; i.e., the total of all the peptide ion scores matching a protein;

^cThe percentage of a protein sequence covered by MS-matched peptides;

^dPeptides with scores above the threshold are revealed in italics in the first column.

^eA score assigned to individual matching peptide by MASCOT based on the probability of best match;

^fChance of obtaining of obtaining an equal or higher score for a peptide.

^gPeptides that matched the protein in MASCOT are underlined in the amino acid sequence.

OS, Organism species/name; PE, Protein existence; SV, Sequence version.

Supplementary Table S5. Results of the MASCOT search of tandem mass spectrometric (TMS)-derived data from the purified ~35 kDa Birch tree (*Betula verrucosa*) pollen (BTP) gel band.

NCBIInr, gi: 10764491

Allergenic isoflavone reductase-like protein Bet v 6.0102 [*Betula pendula*]

Mass^a: 34169, Score^b: 732 Matches: 172 (32), Sequences: 11 (6), emPAI: 0.83

Protein sequence coverage^c: 47%

Peptide match ^d	Score ^e	Expect ^f
AVFNKEDDIGTYTIR	101	2.1e-05
NIYSFNEIVALWEK	93	0.00013
QVDVVISTVGHLQLADQVK	81	0.0021
FFPSEFGNDVDR	86	0.00084
VVIFGDGNAR	77	0.0077
ILIIGGTGYIGK	76	0.0092
EDDIGTYTIR	62	0.21
DIQESPIPINVILAINHSVFK	62	0.14
GLGVTLHGDLYDHESLVK	38	41
SGHPTFALVR	30	3.7e+02
YTTVEEYLQQFV	29	4.1e+02

Matched peptides

1MAHKSILII

GGTGYIGKFI

VEASAKSGHP

TFALVRESTV

SDPVKGKLVE

51KFKGLGVTL

HGDLYDHESL

VKAFKQVDVV

ISTVGHLQLA

DQVKIIAAIK

101EAGNIKRFFP

SEFGNDVDRV

HAVEPAKTAF

ATKAEIRRKT

EAEGIPYTYV

151SSNFFAGYFL

PTLAQPGLTS

PPREKVVIFG

DGNARAVFNK

EDDIGTYTIR

201AVDDPRTLNK

IVYIKPAKNI

YSFNEIVALW

EKKIGKTLEK

IYVPEEKLLK

251DIQESPIPIN

VILAINHSVF

VKGDHTNFEI

EASFGVEASE

LYPDVKYTTV

301EEYLQQFV

^aThe predicted mass of a protein in Daltons

^bMascot protein score; i.e., the total of all the peptide ion scores matching a protein;

^cThe percentage of a protein sequence covered by MS-matched peptides;

^dPeptides with scores above the threshold are revealed in italics in the first column.

^eA score assigned to individual matching peptide by MASCOT based on the probability of best match;

^fChance of obtaining of obtaining an equal or higher score for a peptide.

⁹Peptides that matched the protein in MASCOT are underlined in the amino acid sequence.

OS, Organism species/name; PE, Protein existence; SV, Sequence version.

Supplementary Table S6. Potential N-linked glycosylated sites for Timothy grass (*Phleum pratense*) pollen allergen Phl p 1

>Phl p 1 Length = 263

MASSSSVLLVVVLFVAVFLGSAYGIPKVPPGPNITATYGDKWLDKSTWYGKPTGAGPKDNGGACGYKDVDKPPFS
GMTGCGNTPIFKSGRGCSCFEIKCTKPEACSGEPVVVHITDDNEEPIAPYHFDLSGHAFGAMAKKGDEQKLRSA
GELELQFRRVKCKYPEGTKVTFHVEKGSNPNYLALLVKYVNGDGDVVAVDIKEKGKDKWIELKESWGAIWRIDTP
DKLTGPFTVRYTTEGGTKTEAEDVIPEGWKADTSYESK

Position	Residue	Score	Prediction
32	<u>NIT</u>	0.7670472	Potential Glycosylated
60	<u>NGG</u>	-0.97708469	Non-glycosylated
82	<u>NTP</u>	-1.3149035	Non-glycosylated
119	<u>NEE</u>	-1.4222115	Non-glycosylated
179	<u>NPN</u>	-1.3878037	Non-glycosylated
181	<u>NYL</u>	-0.98800826	Non-glycosylated
191	<u>NGD</u>	-1.2761456	Non-glycosylated

Supplementary Table S7. Potential O-linked glycosylated sites for Timothy grass (*Phleum pratense*) pollen allergen Phl p 1

>Phlp1 Length = 263

MASSSSVLLVVVLFVAVFLGSAYGIPKVPPGPNITATYGDKWLDKASTWYGKPTGAGPKDNGGACGYKDVKPPFS
GMTGCGNTPIFKSGRGCGSCFEIKCTKPEACSGEPVVVHITDDNEEPIAPYHFDLSGHAFGAMAKKGDEQKLRS
GELELQFRRVKCKYPEGTKVTFHVEKGSNPNYLALLVKYVNGGDVVAVDIKEKGDKWIELKESWGAIWRIDTP
DKLTGPFITVRYTTEGGTKTEAEDVIPEGWKADTSYESK

Position	Residue	Score	Prediction
3	S	-0.00095546551	Non-glycosylated
4	S	-0.067242144	Non-glycosylated
5	S	0.11158392	Potential Glycosylated
6	S	0.09579778	Potential Glycosylated
20	S	-0.32331112	Non-glycosylated
34	T	0.064133352	Potential Glycosylated
36	T	-0.10302248	Non-glycosylated
46	S	-0.093445547	Non-glycosylated
47	T	0.011143839	Potential Glycosylated
53	T	0.22606906	Potential Glycosylated
75	S	-0.094465604	Non-glycosylated
78	T	0.046254624	Potential Glycosylated
83	T	0.11919246	Potential Glycosylated
88	S	-0.31269445	Non-glycosylated
94	S	-0.32203716	Non-glycosylated
101	T	-0.0030757463	Non-glycosylated
107	S	-0.13253565	Non-glycosylated
116	T	0.10466831	Potential Glycosylated
131	S	-0.27473512	Non-glycosylated
149	S	-0.68676712	Non-glycosylated
168	T	-0.063710994	Non-glycosylated
171	T	-0.038581421	Non-glycosylated
178	S	-0.40961583	Non-glycosylated
215	S	-0.47592039	Non-glycosylated
224	T	0.077373158	Potential Glycosylated
229	T	0.14841502	Potential Glycosylated
233	T	0.1507103	Potential Glycosylated
237	T	0.20143532	Potential Glycosylated
238	T	0.2743446	Potential Glycosylated
242	T	-0.065456417	Non-glycosylated
244	T	-0.011155067	Non-glycosylated
258	T	-0.016934199	Non-glycosylated
259	S	0.093261094	Potential Glycosylated
262	S	-0.13262934	Non-glycosylated

Supplementary Table S8. Potential N-linked glycosylated sites for Timothy grass (*Phleum pratense*) pollen allergen Phl p 5b

>Phlp5b Length = 284

AAAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQKLIEDINVGFKAAVAAAASVPAAD
KFKTFEAAFTSSSKAAAAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVHAVKPVTE
EPGMAKIPAGELQIIDKIDAAFKVAATAAATAPADDKFTVFEEAFNKAIKESTGGAYDTYKCIPSLEAAVKQAYA
ATVAAAPQVKYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAGAASGAATVAAGGYKV

Position	Residue	Score	Prediction
51	<u>N</u> VG	-1.2671764	Non-glycosylated
189	<u>N</u> KA	-0.76057737	Non-glycosylated

Supplementary Table S9. Potential O-linked glycosylated sites for Timothy grass (*Phleum pratense*) pollen allergen Phl p 5b

>Phlp5b Length = 284

AAAVPRRGPRGGPGR**S**YTADAGYAPATPAAAGAAAGKAT**T**TEEQKLIEDINVGFKAAVAAAA**S**VPAADKFK**T**FEA
A**T****S****S****S**KAAAAKAPGL**V**PKLDAAY**S**VAYKAAVGATPEAK**F****D****S**FVAS**L**TEALRVIAGALEVHAVKPV**T**EEPG**M**AKI
PAGELQIIDKIDAAFKVAATAAA**T**APADDK**F**TVF**E**AAFNKA**I**KE**S**TGGAYD**T**YKCIP**S**LEAAVKQAYAA**T**VAAAP
QVKYAVFEAAL**T**KAIT**A**M**S**EVQKV**S**QPAT**G**AAT**V**AAGAA**T**TAAAGAA**S**GAAT**V**AAGGYKV

Position	Residue	Score	Prediction
17	S	-0.037219921	Non-glycosylated
19	T	0.24243257	Potential Glycosylated
28	T	0.30599874	Potential Glycosylated
40	T	0.018684405	Potential Glycosylated
41	T	0.036401006	Potential Glycosylated
63	S	-0.003169364	Non-glycosylated
72	T	-0.080874397	Non-glycosylated
78	T	-0.047933681	Non-glycosylated
79	S	-0.23103798	Non-glycosylated
80	S	-0.081418022	Non-glycosylated
81	S	-0.088031568	Non-glycosylated
100	S	-0.23905449	Non-glycosylated
110	T	0.0088164932	Potential Glycosylated
117	S	-0.23487916	Non-glycosylated
121	S	-0.36093834	Non-glycosylated
123	T	0.058422284	Potential Glycosylated
142	T	0.21134668	Potential Glycosylated
170	T	0.16090342	Potential Glycosylated
174	T	0.16183771	Potential Glycosylated
182	T	-0.044324336	Non-glycosylated
195	S	-0.1446803	Non-glycosylated
196	T	0.057675723	Potential Glycosylated
202	T	-0.17220422	Non-glycosylated
208	S	-0.31585482	Non-glycosylated
220	T	0.012210485	Potential Glycosylated
237	T	-0.028408959	Non-glycosylated
241	T	-0.17600358	Non-glycosylated
244	S	-0.08593959	Non-glycosylated
250	S	-0.10705108	Non-glycosylated
254	T	0.1392236	Potential Glycosylated
258	T	0.41428013	Potential Glycosylated
265	T	0.56073719	Potential Glycosylated
266	T	0.45153701	Potential Glycosylated
272	S	0.30832147	Potential Glycosylated
276	T	0.34431126	Potential Glycosylated

Supplementary Table S10. Potential N-linked glycosylated sites for Birch tree
(*Betula verrucosa*) pollen allergen Bet v 6.0102

>Bet v 6.0102 Length = 308

MAHKSKILIIIGGTGYIGKFIVEASAKSGHPTFALVRESTVSDPVKGKLVKFKGLGVTLLHGDLYDHESLVKAFK
QVDVVISTVGHLQLADQVKIIAAIKEAGNIKRFFPSEFGNDVDRVHAVEPAKTAFATKAEIRRKTEAEGIPYTYV
SSNFFAGYFLPTLAQPGLTSPPREKVVIFGDGNARAVFNKEDDIGTYTIRAVDDPRTLNKIVYIKPAKNIYSFNE
IVALWEKKIGKTLEKIYVPEEKLLKDIQESPIPINVILAINHSV FVKGDHTNFEIEASFGEASELYPDVKYTTV
EEYLQQFV

Position	Residue	Score	Prediction
104	NIK	-1.1760148	Non-glycosylated
115	NDV	-0.94422164	Non-glycosylated
153	NFF	-1.5861993	Non-glycosylated
183	NAR	-0.64990465	Non-glycosylated
189	NKE	-0.87076082	Non-glycosylated
209	NKI	-1.1692657	Non-glycosylated
219	NIY	-1.1035546	Non-glycosylated
224	NEI	-1.0168352	Non-glycosylated
260	NVI	-1.0123152	Non-glycosylated
266	NHS	1.0247376	Potential Glycosylated
277	NFE	-0.80990731	Non-glycosylated

Supplementary Table S11. Potential O-linked glycosylated sites for Birch tree

(*Betula verrucosa*) pollen allergen Bet v 6.0102

>Bet v 6.0102 Length = 308

MAHKSKILIIGGTGYIGKFIVEASAKSGHPTFALVRESTVSDPVKGKLVEKFKGLGVTLLHGDLYDHESLVKAFK
QVDVVISTVGHLQLADQVKIIAAIKEAGNIKRFFPSEFGNDVDRVHAVEPAKTAFATKAEIRRKTEAEGIPYTYV
SSNFFAGYFLPTLAQPGLTSPPREKVVFISGDGNARAVFNKEDDIGTYTIRAVDDPRTLNKIVYIKPAKNIYSFNE
IVALWEKKIGKTLEKIYVPEEKLLKDIQESPIPINVILAINHSVFVKGDHTNFEIEASFGVEASELYPDVKYTTV
EEYLQQFV

Position	Residue	Score	Prediction
5	S	0.14122811	Potential Glycosylated
13	<u>T</u>	-0.25078127	Non-glycosylated
24	<u>S</u>	-0.21148143	Non-glycosylated
27	<u>S</u>	-0.092378191	Non-glycosylated
31	T	0.26433177	Potential Glycosylated
38	<u>S</u>	-0.26718354	Non-glycosylated
39	T	0.055854732	Potential Glycosylated
41	<u>S</u>	-0.2523964	Non-glycosylated
58	<u>T</u>	-0.38195778	Non-glycosylated
69	<u>S</u>	-0.65768646	Non-glycosylated
82	<u>S</u>	-0.29144806	Non-glycosylated
83	<u>T</u>	-0.30470699	Non-glycosylated
111	<u>S</u>	-0.12550795	Non-glycosylated
128	T	0.018567136	Potential Glycosylated
132	T	0.035488752	Potential Glycosylated
140	T	0.17427381	Potential Glycosylated
148	T	0.04887897	Potential Glycosylated
151	<u>S</u>	-0.13437769	Non-glycosylated
152	<u>S</u>	-0.11676069	Non-glycosylated
162	T	0.1792828	Potential Glycosylated
169	T	0.31513568	Potential Glycosylated
170	<u>S</u>	-0.31125922	Non-glycosylated
196	<u>T</u>	-0.089901638	Non-glycosylated
198	<u>T</u>	-0.136617	Non-glycosylated
207	<u>T</u>	-0.22918987	Non-glycosylated
222	<u>S</u>	-0.522473	Non-glycosylated
237	<u>T</u>	-0.32869618	Non-glycosylated
255	<u>S</u>	-0.20915751	Non-glycosylated
268	<u>S</u>	-0.38993928	Non-glycosylated
276	<u>T</u>	-0.26907811	Non-glycosylated
283	<u>S</u>	-0.30373737	Non-glycosylated
289	S	0.0041413141	Potential Glycosylated
298	<u>T</u>	-0.14032031	Non-glycosylated
299	<u>T</u>	-0.17843752	Non-glycosylated

Supplementary Table S12. Potential N-linked glycosylated sites for Birch tree (*Betula verrucosa*) pollen (BTP)-GST

>BTP-GST Length = 237

MADASVKEHLPTPLDATSNPPPIFDGTTTRLTYTCYTCPFAQRVWITRNYKGLQEIKLVPINLQNRPAWYKEKVYP
ENKVPALAHNGKVIGESLDLIKYVDINFEGPSLLPNDPAKKAFAEELVAYSDTFNKTVFTSFKGDPVKEAGPAFD
HLEKALHKFDDGPFFLGQFSAVDIVYIPFVERFQIFLLDALKYDITAGRPKLAKWIEELNKIDAYKPTKTDPKEL
VEFYKARFAAQQ

Position	Residue	Score	Prediction
19	NPP	-1.7937911	Non-glycosylated
47	NYK	-0.99838882	Non-glycosylated
61	NLQ	-0.9049043	Non-glycosylated
64	NRP	-0.94678561	Non-glycosylated
77	NKV	-1.4683284	Non-glycosylated
85	NGK	-1.0723305	Non-glycosylated
102	NFE	-1.2568	Non-glycosylated
111	NDP	-1.1561478	Non-glycosylated
130	NKT	0.98698511	Potential Glycosylated
210	NKI	-1.2237646	Non-glycosylated

Supplementary Table S13. Potential O-linked glycosylated sites for Birch tree (*Betula verrucosa*) pollen (BTP)-GST

>BTP-GST Length = 237

MADASVKEHLPTPLDATSNPPPIFDGTTTRL^YTCYTCPFAQRVWITRNYKGLQEIKLVPINLQNRPAWYKEKVYP
ENKVP^ALEHNGKVIGE^SLDLIKYVDINFEGP^SLLPN^DPAKKAFAEELVAY^SDTFNKTVF^TSFKGDPVKEAGPAFD
HLEKALHKFDDGPF^LGQFSAVDIVYIPFVERFQIFLLDALKYDI^TAGRPKLAKWIEELNKIDAYKPT^TK^TDPKEL
VEFYKARFAAQQ

Position	Residue	Score	Prediction
5	^{<u>S</u>}	0.042131906	Potential Glycosylated
12	^{<u>T</u>}	0.21530602	Potential Glycosylated
17	^{<u>T</u>}	0.56911202	Potential Glycosylated
18	^{<u>S</u>}	0.23671748	Potential Glycosylated
27	^{<u>T</u>}	0.32100015	Potential Glycosylated
28	^{<u>T</u>}	0.14205687	Potential Glycosylated
32	^{<u>T</u>}	-0.024299782	Non-glycosylated
35	^{<u>T</u>}	-0.042475791	Non-glycosylated
45	^{<u>T</u>}	-0.28813259	Non-glycosylated
92	^{<u>S</u>}	-0.57185017	Non-glycosylated
107	^{<u>S</u>}	-0.3375704	Non-glycosylated
126	^{<u>S</u>}	-0.10759881	Non-glycosylated
128	^{<u>T</u>}	0.0021241698	Potential Glycosylated
132	^{<u>T</u>}	-0.028180874	Non-glycosylated
135	^{<u>T</u>}	-0.065184541	Non-glycosylated
136	^{<u>S</u>}	-0.19531227	Non-glycosylated
170	^{<u>S</u>}	-0.44681249	Non-glycosylated
196	^{<u>T</u>}	-0.18264225	Non-glycosylated
218	^{<u>T</u>}	-0.015174259	Non-glycosylated
220	^{<u>T</u>}	-0.093677703	Non-glycosylated

Supplementary Table S14. Potential N-linked glycosylated sites for Birch tree (*Betula verrucosa*) pollen allergen Bet v 1

>Bet v 1 Length = 160

MGVFNYETETTSVIPAARLFKAFILDGDNLFKVAPQAISSVENIEGNGGPGTIKKISFPEGFPFKYVKDRVDEV
DHTNFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISNKYHTKGDHEVKAEQVKASKEMRETLRAVES
YLLAHSDAYN

Position	Residue	Score	Prediction
5	<u>N</u> YE	-0.57018822	Non-glycosylated
29	<u>N</u> LF	-1.4633798	Non-glycosylated
44	<u>N</u> IE	-1.1930401	Non-glycosylated
48	<u>N</u> GG	-1.353941	Non-glycosylated
79	<u>N</u> FK	-1.2196001	Non-glycosylated
83	<u>N</u>YS	0.81307229	Potential Glycosylated
101	<u>N</u> EI	-1.0925162	Non-glycosylated
119	<u>N</u> KY	-1.2241629	Non-glycosylated
160	<u>N</u>	-1.1303821	Non-glycosylated

Supplementary Table S15. Potential O-linked glycosylated sites for Birch tree (*Betula verrucosa*) pollen allergen Bet v 1

>Bet v 1 Length = 160

MGVFNYETTETTSVIPAARLFKAFILDGDNLFPKVAPQAISSVENIEGNGGPGTIKKISFPEGFPFKYVKDRVDEV
DHTNFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISNKYHTKGDHEVKAEQVKASKEMRETILLRAVES
YLLAHSDAYN

Position	Residue	Score	Prediction
8	T	0.21688395	Potential Glycosylated
10	T	-0.20786176	Non-glycosylated
11	T	0.061314735	Potential Glycosylated
12	S	-0.17812432	Non-glycosylated
40	S	-0.2973415	Non-glycosylated
41	S	-0.32792595	Non-glycosylated
53	T	-0.12392585	Non-glycosylated
58	S	-0.077935513	Non-glycosylated
78	T	-0.1846367	Non-glycosylated
85	S	-0.24276603	Non-glycosylated
95	T	-0.43109848	Non-glycosylated
100	S	-0.057404079	Non-glycosylated
108	T	0.019383936	Potential Glycosylated
113	S	-0.28959811	Non-glycosylated
118	S	-0.10627707	Non-glycosylated
123	T	-0.19318228	Non-glycosylated
137	S	-0.42564195	Non-glycosylated
143	T	-0.56348001	Non-glycosylated
150	S	-0.49127545	Non-glycosylated
156	S	-0.11125905	Non-glycosylated