Zur Arbeit

Die vorliegende Arbeit erläutert die wissenschaftlichen Grundlagen des ‚Prinzips der Homologen Gruppen'. Dieses Prinzip wird erstmalig in der Zulassung biologischer Allergie-Arzneimittel eingeführt werden. Die relevante ‚Note for Guidance on Allergen Products' (CPMP/BWP/243/96) von 1996 wurde gerade grundlegend überarbeitet und wird auf die vorliegende Arbeit verweisen.

Aktuell hat die revidierte Fassung der ‚Note for Guidance on Allergen Products' die Zustimmung der ‚Biologics Working Party' erhalten und wird in Kürze zur Kommentierung der Öffentlichkeit vorgelegt werden.

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The Principle of Homologous Groups in Regulatory Affairs of Allergen Products – A Proposal

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Abstract

Among other legal regulations the ‘Note for Guidance on Allergen Products CPMP/BWP/243/96’ released by the ‘European Agency for Evaluation of Medicinal Products (EMEA)’ provides regulatory instructions regarding the quality of allergen extracts for diagnostic or therapeutic purposes. The current revision of this guideline intends to transform the so-called ‘principle of taxonomic families’ to the ‘principle of homologous groups’. According to this concept the data of one allergen extract demonstrating stability, efficacy and safety can, to a limited extent, be extrapolated to other allergen extracts belonging to the same homologous groups.

The present work proposes the formation of homologous groups for pollen species and animal-derived materials on the basis of similar biochemical composition and homology/cross-reactivity of allergens or allergen sources. Some tree pollen species could be assigned to three different homologous groups, some weed pollen species to one homologous group and numerous grass pollen species to one homologous group on condition that data rely on single defined representative species. A homologous group for mites is limited to the *Dermatophagoides* species and the grouping of vertebrate-derived materials such as dander could be possible under restrictions.

The criteria for formation of the proposed homologous groups are illustrated in detail to provide an opportunity for extending existing homologous groups by further species in case of new insights in allergens and cross-reactivity of allergen sources. In this way, the concept of homologous groups could serve as a dynamic tool in the regulation of allergen products.

Introduction

In the field of marketing authorisation of industrially manufactured allergen products in Europe, the legal requirements on quality are laid down in the European Pharmacopoeia [1]. More detailed instructions on this issue concerning allergen extracts for *in vivo* diagnosis and immunotherapy can be found in the ‘Note for Guidance on Allergen Products’ [2], which is valid in the European countries (EU and EFTA exclusive of Switzerland). This guideline has been prepared by the ‘Biotechnology Working Party (BWP)’ and the present version was released in 1996 by the ‘Committee for Proprietary Medicinal Products (CPMP)’ of the ‘European Agency for Evaluation of Medicinal Products (EMEA)’ in London.

One topic of this Note for Guidance lays down the practice that under certain conditions data of one allergen extract can be extrapolated to other allergen sources of the same taxonomic family. A prerequisite for the take-over of data from one extract to other extracts or mixtures thereof is that the allergen products run through identical manufacturing processes and form final products of identical formulation. Mixtures of allergen sources of different families are excluded. This concept of extrapolation of data within a taxonomic family is functional with respect to the great variety of different allergen sources and the numerous possible mixtures thereof on the one hand, and their obvious biochemical resemblance on the other hand. For example, pollen have a very similar biochemical composition with similar water content, and their low proteolytic enzymatic activity ensures the stability of mixtures of extracts. Last but not least, neither the long-term experience with this principle during the last years nor scientific literature disproves the practice of this principle.

Over the last decade allergy research generated new in-depth information on allergenic molecules in allergen sources and, especially driven by recombinant DNA technology, the protein and cDNA sequences of the majority of clinically relevant allergens have been identified. It turned out that allergens belonging to identical protein families are present in different allergen sources and that these homologous allergens often show similar biochemical properties and cross-reactivity. The presence of allergens with high sequence identity as well as with cross-reactivity among different allergen sources may not necessarily be restricted to allergen sources of one taxonomic family. The classification of allergen sources in one

homologous group on the basis of homology and cross-reactivity of allergens is more specific and satisfactory than mere taxonomic relationship. Therefore, it has been suggested by regulators to change the ‘concept of taxonomic families’ into the new ‘concept of homologous groups’ [3]. The Note for Guidance is currently under thorough revision in many topics and one objective is to ‘re-define and elaborate in detail the concept of taxonomic allergen families’ [4].

We tried to bring the suggested ‘concept of homologous groups’ to life and therefore evaluated the scientific literature (as at April 2006) with respect to the questions of cross-reactivity and homology of allergen sources. Hence, we established a list of homologous groups which may serve as a proposal for further discussions on this issue. Allergen sources have been included in the same homologous group on the basis of two criteria: The allergen sources have comparable physicochemical and biological properties i.e. they derive from comparable tissues and therefore are alike in overall composition of proteins, carbohydrates, lipids, enzymes and water content. This criterion ensures that for example grains and pollen from cereal species are distinguished as different allergen sources because they differ in kind and content of allergens. The allergens found in wheat (*Triticum aestivum*) grains (Tri a 14, 18, 19, 25, 26) are not present in wheat pollen (Tri a 1, 2, 3, 4, 5, 12, 13) but the pollen allergens of wheat are homologous to pollen allergens in several grass species (see table 7) [5, 6]. The second criterion applied for the formation of homologous groups has been cross-reactivity between the allergen sources shown by cross-reactivity of single allergens or the whole extracts. Cross-reactivity mainly has been shown on the basis of patients’ sera supported by studies with allergen-specific polyclonal animals’ sera or monoclonal antibodies in some cases. In most cases, cross-reactivity is linked with homology of allergens belonging to identical protein families with a high degree of sequence identities on protein or cDNA level.

We reviewed the scientific literature in the fields of pollen from trees, grasses and weeds, of mites, insect venoms and vertebrates and could establish six homologous groups among mites and pollen from trees, grasses and weeds. It was not possible to group any allergen sources from insect venoms and vertebrates due to insufficient homology. In each homologous group we suggest one or more species to be possible representative allergen source due to sufficient scientific information on

characterised allergens and cross-reactivity with other species. In regulatory practice, equally to the ‘concept of taxonomic family’, the required studies can be performed with a representative species of one homologous group which finally can serve as surrogate for all species within this group on two conditions: The final extracts of one homologous group firstly have to be of identical formulation and secondly have to be run through identical production processes. In several homologous groups more than one species are qualified as representative allergen source because of equivalent substantial levels of allergen characterisation.

With particular interest regarding marketing authorisation applications, new scientific knowledge on the homology and cross-reactivity of allergen sources would allow to add further species to one homologous group if the extracts fulfil the four specified terms illustrated in the preceding paragraphs: (1) Comparable physicochemical and biological properties of the source materials, (2) cross-reactivity/structural homology of allergens, (3) identical formulation of the final products, (4) identical production process of the allergen extracts. Providing in this article in detail the scientific basis for group formation as well as the criteria and the decisions made, manufacturers and regulatory authorities will be enabled to use the ‘concept of homologous groups’ in a reasoned manner and as a dynamic tool which can be adapted to the scientific progress.

Tree pollen

Three groups of homologous tree species are suggested as noted in table 1.

Table 1: Suggestion for homologous groups of tree species

The 'birch group'	
Betula verrucosa = B. pendula = B. alba*	European white birch
Alnus glutinosa	Alder
Carpinus betulus	Hornbeam
Corylus avellana	Hazel
Quercus alba	Oak
Representative allergen source: Birch	
* Correct taxonomic name according to NCBI taxonomic database [7]	
The group of *Oleaceae*	
Olea europaea	Olive
Fraxinus excelsior	Ash
Ligustrum vulgare	Privet
Syringa vulgaris	Lilac
Representative allergen sources: Olive or Ash	
The group of *Cypressaceae*	
Juniperus sp.	Cedar
Cupressus sp.	Cypress
Representative allergen sources: Cedar or Cypress	

The 'birch group'

The 'birch group' consists of five tree species belonging to the same order of *Fagales*, four species are members of the taxonomic family *Betulaceae*, while oak

(*Quercus alba*) belongs to the *Fagaceae* family, according to the 'National Center for Biotechnology Information (NCBI)' taxonomy database (see table 1) [7]. Table 4 illustrates that birch is the best characterized species within this group, with six allergens identified and listed in the official 'International Union of Immunological Societies (IUIS)' list of allergens, including numerous isoforms and variants of Bet v 1 [6]. In all other species in this group, a Bet v 1-homologous major allergen is present as well as the Bet v 2 homologue profilin. The sequence identities of Bet v 1 homologues within this group are considerably high. For example, three Car b 1 cDNA clones from hornbeam have a deduced amino acid sequence identity of 73% to 75% to Bet v 1 [8]. Besides from similar sequential features birch pollen allergens and their homologues in other tree pollen are also congruent in their importance as minor or major allergens (see table 4). Additionally, cross-reactivity studies performed partially at the level of human IgE antibodies confirm the obvious relatedness of allergens from these tree species [8-15]. For example, the Bet v 1 and Bet v 2 homologues of alder, hazel, hornbeam and oak showed comparable reactivity to IgE antibodies of tree pollen-allergic subjects. By preabsorption of 102 sera with a combination of recombinant rBet v 1 and rBet v 2 the binding of IgE to whole tree pollen extracts was inhibited to a large extent (to oak: 72%, to hornbeam: 77%, to hazel: 80% and to alder: 88%) [10]. These data underline the predominance of the Bet v 1- and Bet v 2-homologous allergens in the mentioned *Fagales* extracts and illustrate why these species can be summarised within one group, although their spectrum of identified allergens may still be incomplete. The predominance of whole birch pollen extract has been confirmed by inhibition studies. Birch pollen extract completely inhibited human IgE-binding to alder, hornbeam and oak whereas the reverse inhibition of IgE-binding to birch pollen extract by the mentioned extracts was incomplete [15]. The presence of luminal-binding protein Cor a 10 which was described as minor allergen in hazel pollen has not been shown for birch or other *Fagales* species [16]. In the single reference [16] on this type of allergen a probable cross-reactivity to a 70 kDa band in birch extract was indicated which thus does not disprove the suggested group formation. Furthermore, the relevance of this protein family as allergens appears to be very limited.

Concluding from these results and since birch pollen is a very strong immunogen in Central Europe, birch is recommended to be the representative allergen source

within this group. Extrapolation of data has to be based on studies with birch pollen extracts.

The *Oleaceae* group

The second group of homologous tree pollen species comprises four members of the olive family (*Oleaceae*): olive, ash, lilac and privet. Extracts of olive, ash, lilac and privet showed comparable IgE-binding patterns in western blotting when probed with pooled serum of pollen-allergic subjects with positive skin prick test to olive pollen extract [17]. The major allergen Ole e 1 is a 20 kDa trypsin inhibitor and its homologues Fra e 1, Syr v 1 and Lig v 1 are present as major allergens in all *Oleaceae* of this group (table 5) [17]. The minor olive allergen Ole e 3, a calcium-binding protein/polcalcin, is a pan-allergenic structure and homologues have been described in ash and lilac [18-20]. The beta-1,3-glucanase Ole e 9 is a major allergen, and although respective allergens have not been identified in the remaining *Oleaceae* species, cross-reactive structures have been detected in ash, lilac and privet [21-23]. According to one reference [24], a 11 kDa allergen named Ole e 10 by the authors, has been identified as glycosyl transferase. In ash, lilac and privet homologues have not been identified until now, but the existence of a cross-reactive structure can be concluded, since extracts of these pollen were able to inhibit binding of IgE to Ole e 10 [24]. In addition to the relatedness of the allergens, cross-reactivity of the extracts has widely been demonstrated with patients' sera [19, 25, 26]: Extracts of *Oleaceae* mutually inhibited IgE-binding [19, 25], but olive pollen extract was predominant, as in inhibition studies it had the highest IgE-binding capacity of all tested *Oleaceae* [26]. Therefore, olive is recommended as representative allergen source within the group of *Oleaceae*.

Olive trees are not present in Central and Northern Europe and consequently olive pollen is not an allergizing pollen species in allergic subjects from these regions. From a scientific and practical point of view, it would not make sense to require studies based on olive tree pollen for applying the principle of homologous groups to extracts of other *Oleaceae* species which are intended for the markets in Central and Northern Europe. Therefore, we suggest ash to be another possible representative allergen source within the homologous group of *Oleaceae* because it is the second best-characterised species of the *Oleaceae* family.

The *Cupressaceae* group

A third group of homologous tree species includes the *Juniperus* and *Cupressus* species of the cypress family (*Cupressaceae*). Each species of both genera can function as representative allergen source for extrapolation of data. The allergens of the cypress family (table 6) show a high degree of amino acid sequence identity: For example, the major allergen Jun a 1 shares 97% amino acid sequence identity with Jun v 1 and 95% identity with the major allergens Cup a 1 and Cup s 1. The allergens Jun a 3 and Cup a 3, which are thaumatin-like proteins, share 95% amino acid sequence identity with each other [27-30]. Therefore, the grouping of members of trees from the cypress family is justified by this remarkably high degree of sequence identity and further supported by a few cross-reactivity studies [31].

Non-grouped tree species

Several tree pollen species could not be assigned to one of the above mentioned groups (table 1) because of the absence of scientific information on allergen extracts or on identified allergens. As a consequence, the tree species *Acer sp*. (maple), *Populus sp*. (poplar), *Robinia pseudoacacia* (locust tree), *Salix sp*. (willow), and *Ulmus sp*. (elm) have not been included in one of the groups. First results with extracts of the tree species *Fagus sylvatica* (European beech) in skin tests indicated that beech, belonging to the order *Fagales*, is a possible candidate for the ‘birch group’, but at the present time the information is not substantial enough to justify this classification [32]. Likewise, allergens of *Tilia sp*. (linden) have not been identified to date, and results with *Tilia* pollen extracts showing cross-reactivity with *Platanus sp*. pollen but not with olive pollen were considered insufficient for grouping [33]. Concerning *Platanus* species (plane tree), several allergens have been identified and characterised, but cross-reactivity studies with other tree pollen species did not sufficiently demonstrate an immunological relationship of the allergens, that would allow a group formation [9, 34, 35].

In case of the tree species *Cryptomeria japonica* (Japanese cedar) of the *Cupressaceae* family, high amino acid sequence identities are shared between its allergens Cry j 1 and Cry j 2 and Jun a 1 and Jun a 2, respectively, of *Juniperus ashei* (mountain cedar). Even so, it is recommended not to include this species in the homologous group of *Cupressaceae* (table 1) since *Cryptomeria japonica* trees are rarely present in continental Europe and Northern America and therefore are without

clinical relevance in these regions. Additionally, the different genetic backgrounds of the Asian, the European, and the American populations may cause different sensitization patterns to cypress tree pollen in allergic subjects. Since it has not been demonstrated that the clinical relevance of homologous allergens of various cypress trees in different populations is comparable, the inclusion of *Cryptomeria japonica* in the homologous group of *Cupressaceae* may not be justified to date.

Pollen of grass species and cereal species

One group of sweet grasses of the *Poaceae* (*Gramineae*) family, subfamily of *Pooideae* is suggested in table 2.

Table 2: Sweet grasses of the *Poaceae* (*Gramineae*) family, subfamily of *Pooideae*

Anthoxanthum odoratum	Sweet vernal grass
Avena sativa	Oat
Dactylis glomerata	Orchard grass/Cocksfoot
Festuca sp.	Meadow fescue
Holcus lanatus	Velvet grass/Yorkshire fog
Hordeum vulgare	Barley
Lolium perenne	Perennial ryegrass
Phleum pratense	Timothy grass
Poa pratensis	Kentucky bluegrass
Secale cereale	Cultivated rye
Triticum aestivum	Cultivated wheat
Representative allergen sources: Timothy grass, Orchard grass or Kentucky bluegrass	
Additional grass species belonging to the homologous group of *Pooideae* with reservations	
Agropyron sp.	Couch grass, Crested wheatgrass
Agrostis sp.	Bent grass
Alopecurus pratensis	Meadow foxtail
Arrhenatherum elatius	False oat
Bromus sp.	Brome grass
Representative allergen sources: Timothy grass or Kentucky bluegrass	

As table 7 illustrates numerous allergens belonging to eleven allergen families have been identified in pollen of various grass species. For example, in *Phleum pratense* as well as in *Lolium perenne* ten pollen allergens with molecular weights ranging from 6 kDa to 60 kDa have been described up to now. Three allergen families are suitable as criterion for homologous group formation of grass species: Firstly, group 1 grass pollen allergens comprise 26-31 kDa glycoproteins and belong to the protein family of expansins. This protein family has members in all the grass species listed in table 7 and a prevalence of IgE recognition in 90% to 95% of grass pollen-allergic subjects [36-39]. Secondly, group 5 grass pollen allergens are major allergens with sensitization frequencies of 65-68% [36] and 80-90% [38, 40] among grass pollen-allergic subjects. They have been identified in many members of the *Pooideae* subfamily (and in all the members listed in table 7) but not outside this taxon. Thirdly, the group 2 grass pollen allergens consist of non-glycosylated proteins of 10 kDa to 13 kDa with 85% to 95% protein sequence identity between species. They are major or minor allergens as IgE-binding to the individual members is found in 40% to 60% of grass pollen-allergic subjects [40]. Allergens of this family have been described for all grass species in table 7 except for *Festuca* sp.

For other allergen families such as group 3 grass pollen allergens, group 6 grass pollen allergens or group 11 grass pollen allergens insufficient information is available for several grass species to substantially support group formation but there are no contradictory data. The group 12 grass pollen allergens are profilins which are highly conserved pan-allergenic proteins ubiquitously found throughout the plant kingdom and thus are not useful as markers for defining homologous groups. Similarly, the group 4 grass pollen allergens are not a very distinct criterion for the formation of homologous groups, despite the high sensitization rate of up to 80% among grass pollen-sensitized individuals. Since it is likely that the IgE response to group 4 grass pollen allergens is in part directed to the glycan structures of the glycoprotein (10% to 15% of their MW account for carbohydrates) IgE-binding and cross-reactivity studies may be impaired by a considerable rate of false-positive results [36].

Group formation on the basis of homologous structures is supported by IgE-binding results with patients' sera. Andersson and Lidholm [36] performed ImmunoCAP

measurements in 143 grass pollen-sensitized individuals and found very similar IgE-binding capacity for *Phleum pratense* and all grass species listed in table 7 with the exception of *Hordeum vulgare* which was not tested. Van Ree *et al*. [37] determined RAST (radioallergosorbent test) values in 209 sera from grass pollen-sensitized individuals for 8 of the 11 grass species in table 7 (not tested were *Avena sativa*, *Hordeum vulgare* and *Triticum aestivum*). Highest responses were observed to *Poa pratensis* followed by *Festuca rubra*, *Phleum pratense*, *Dactylis glomerata*, *Lolium perenne*, *Holcus lanatus*, *Anthoxanthum odoratum* and *Secale cereale* indicating similar IgE-binding properties among the mentioned grass species, and implying at least a partial cross-reactivity.

Several cross-reactivity studies with grass pollen allergens showing that the majority of grass species are highly cross-reactive are further indicators for the suggested group formation [37, 38, 41-53] reviews: [36, 40, 54-58]. For example, RAST inhibition tests demonstrated that extract of *Phleum pratense* pollen was able to inhibit binding of IgE antibodies of subjects with grass-positive skin tests to extracts of *Poa pratensis*, *Lolium perenne*, *Agrostis alba*, *Festuca sp.*, *Anthoxanthum odoratum*, *Agropyron sp.* and *Bromus sp.*. Similarly, extracts of *Agropyron sp.* were able to inhibit the binding to the listed extracts of grass species [52].

Five additional grass species of the *Pooideae* subfamily (*Agropyron sp.*, *Agrostis sp*., *Alopecurus pratensis*, *Arrhenatherum elatius* and *Bromus sp*.) are separately listed in table 8. Allergenic structures in these species have been identified by IgE-binding or using group-specific monoclonal or polyclonal antibodies but detailed characterization of individual allergens has rarely been performed [41, 44, 46-48, 52, 59, 60]. These species could be assigned to the homologous group of *Pooideae* if *Phleum pratense* or *Poa pratensis* is chosen as representative allergen owing to the fact that cross-reactivity among its members has been shown [52].

For the homologous group of *Pooideae*, *Dactylis glomerata* and *Poa pratensis* especially qualify as representative reference allergens due to the high degree of IgE cross-reactivity found between these grasses and other species, as does *Phleum pratense*, the best studied allergenic grass species which contains members of all relevant grass pollen allergen families.

We did not group the grass species *Cynodon dactylon* (Bermuda grass) within the homologous group of grass species because the cross-reactivity of *Cynodon dactylon* with other grass species is not substantial. In pollen extracts of *Cynodon dactylon* immunologically active members of group 2 and group 5 grass pollen allergens were absent and cross-reactivity to group 4 grass pollen allergens could not be shown [42, 46, 55]. Furthermore, *Cynodon*-positive patients' sera were only in part detected by other grass pollen extracts such as *Dactylis glomerata* and *Poa pratensis* [37]. Therefore, the inclusion of *Cynodon dactylon* in the homologous group of grass species should be justified.

Weed pollen

We suggest to form one group of weed species (table 3).

Table 3: Suggestion for a homologous group of weed species

Ambrosia artemisiifolia, Ambrosia trifida	Ragweed
Artemisia vulgaris	Mugwort
Parietaria judaica, Parietaria officinalis	Pellitory
Representative allergen sources: Ragweed or Mugwort, but not Pellitory	

In *Ambrosia* and *Artemisia,* allergens belonging to the same protein families are pectate lyases with the major allergen Amb a 1 (ragweed) and the minor allergen Art v 6 (mugwort), the lipid transfer proteins represented by the minor allergens Amb a 6 and Art v 3, and the calcium-binding proteins/polcalcins Amb a 9 and Art v 5, which are both minor allergens as well. Cross-reactivity between pollen extracts of the weeds *Ambrosia* (ragweed) and *Artemisia* (mugwort) was shown by ragweed extract inhibiting 80% of the IgE-binding to mugwort extract with sera from mugwort-allergic subjects [61]. Concluding from these data, the listing of *Ambrosia* and *Artemisia* as homologous weeds in one group appears to be justified.

In *Parietaria* (pellitory) the spectrum of identified pollen allergens is incomplete in comparison to that of *Ambrosia* and *Artemisia* (table 9), but the most important and best characterized allergens of *Parietaria*, Par j/o/m 1, are lipid transfer proteins and therefore belong to the same protein family as the allergens Amb a 6 from ragweed and Art v 3 from mugwort [62]. Moreover, weed pollen-allergic subjects are often co-sensitized to all three weeds *Ambrosia*, *Artemisia* and *Parietaria*. Even in regions where *Parietaria* is a rare weed the sensitization of weed-allergic subjects to *Parietaria* is highly associated (93% to 100%) with concomitant sensitization to ragweed and mugwort [63]. Therefore, we propose the inclusion of *Ambrosia*, *Artemisia* and *Parietaria* in one group of homologous weed species with the restriction of *Ambrosia* or *Artemisia* but not *Parietaria* being representative allergen source for all three weeds.

To date the *Plantago* species (plantain) cannot be assigned to any homologous group because of insufficient information on plantain allergens and their cross-reactivity [64, 65]. The only identified pollen allergen of *Plantago lanceolata* in the IUIS list, Pla l 1, is a member of the Ole e 1 family with an amino acid sequence identity of 40% with Ole e 1, but natural nPla a 1 is to a negligible degree cross-reactive to nOle e 1 [66].

House dust mites

The broadest and most complex spectrum of allergens among allergen sources discussed in this article has been identified in the house dust mite genus *Dermatophagoides* (table 10). In the two common species *D. farinae* (American house dust mite) and *D. pteronyssinus* (European house dust mite) to date 19 allergens have been identified with molecular weights ranging from 14 kDa to 177 kDa whereof the considerable number of 15 allergens is present as homologues in both species. Consequently, the grouping of these two mite species solely on the basis of presence of related allergens appears to be justified and contradictory results from cross-reactivity studies are not expected.

We did not assign the following species of mites to the homologous group of house dust mites because of the absence of identified allergens as well as of insufficient information on cross-reactivity of allergen extracts: *Acarus siro* (flour mite), *Glycyphagus domesticus* (house mite), *Lepidoglyphus destructor* (house mite), *Tyrophagus putrescentiae* (storage mite) and *Thyreophagus entomophagus* (flour mite).

Insect venoms

Until now, exclusively the insect venoms of honey bee (*Apis mellifera*) and yellow jacket species (*Vespula*) are relevant as diagnostic and therapeutic extracts and double sensitization to both venoms is rather frequent [67-69]. Within groups of *Vespula*-sensitized subjects according to RAST results the rate of subjects also sensitized to honey bee venom ranges from 45% (278/611) to 89% (41/46) and, correspondingly, in honey bee-positive subjects co-sensitization to *Vespula* species ranges from 45% (278/622) to 84% (41/49) [67, 68]. Reasons for double sensitization to honey bee and yellow jacket could be independent sensitization to both insect venoms or cross-reactivity between the two venom extracts. According to studies of Reisman *et al.* and Straumann *et al.* 17/25 and 12/22 double-positive sera, respectively, showed cross-reactivity in inhibition studies, thus assuming cross-reactivity as the predominant reason for double sensitization to honey bee venom and yellow jacket venom [70, 71]. However, latest data of inhibition studies with glycan-rich pollen extracts and synthetic neoglycoproteins revealed cross-reactive carbohydrate determinants (CCD) as a major cause of double sensitization to extracts of honey bee venom and yellow jacket venom [69, 72, 73]. Hemmer *et al*. showed that in 10/15 double-positive sera the cross-reactivity was solely caused by CCD [72]. Jappe and colleagues identified IgE-reactivity to CCD in 123/147 (75%) double-positive sera [73]. In honey bee venom glycosylated allergens currently known are phospholipase A2 (Api m 1), hyaluronidase (Api m 2) and acid phosphatase (Api m 3) [74, 75] whereof the glycan parts of phospholipase A2 and hyaluronidase were characterized as IgE-binding CCD [72, 76, 77]. In yellow jacket venom solely the major allergen hyaluronidase (Ves m/v/f/g/p/s 2) has been shown to be a glycoprotein containing CCD [72, 78] but further, yet unidentified IgE-binding glycoproteins are present in yellow jacket venom as well as in honey bee venom [72, 79]. The only homologous allergenic structure in honey bee venom and yellow jacket venom identified so far is hyaluronidase with an amino acid sequence identity of 53% between Api m 2 (Swiss-Prot Q08169) and Ves v 2 of *Vespula vulgaris* (Swiss-Prot P49370) (table 11) [80, 81]. The comparison of the three-dimensional structures of the two hyaluronidases Api m 2 and Ves v 2 showed a high resemblance but differences of the surface topology and the charge distribution on the surface were appraised as reasons for the absence of common IgE epitopes and cross-reactivity

[82, 83]. Concluding from these data, the venoms of honey bee and yellow jacket cannot form one homologous group.

However, scientific literature provides hints that honey bee venom could be homologous with bumble bee venom. Homologues of the major allergens phospholipase A2, hyaluronidase and acid phosphatase from honey bee venom are also present in bumble bee venom, as noted in table 11. The amino acid sequence identity between the phospholipases Api m 1 of honey bee venom and Bom p 1 and Bom t 1 of bumble bee venom are 54% and 53%, respectively [84]. A high percentage of 75% and 85% of double-positive sera in honey bee-allergic subjects and bumble bee-allergic subjects, respectively, could indicate cross-reactivity between the two venoms [85]. Six of seven bumble bee-allergic patients reported amelioration of their bumble bee allergy after immunotherapy with honey bee venom [86].

The vespids of the genera *Vespula* (yellow jackets), *Dolichovespula* (white face hornet and yellow hornet), *Polistes* (wasps) and *Vespa* (European hornet) seem to be candidates for another homologous group of insect venoms. Cross-reactivity was demonstrated between the two yellow jacket species *Vespula maculifrons* and *Vespula squamosa* as well as between *Vespula maculifrons* and *Vespa crabro* (European hornet) on the basis of inhibition studies with human sera [87]. A mixture of venoms of *Vespula maculifrons*, *Vespula germanica* and *Vespula vulgaris* inhibited IgE-binding to *Dolichovespula maculata* (white face hornet) and vice versa and antigenic relatedness could be possible between *Vespula species* and *Polistes species* (wasps) due to RAST correlation studies [88, 89]. A possible cross-reactivity within the vespids could among others be based on phospholipase A1B as common structure (table 11) as demonstrated by studies with phospholipases of *Vespula*, *Dolichovespula maculata* and *Polistes exclamans* and supported by a considerable amino acid sequence identity of 69% of Ves m 1 with Dol m 1.01 [81, 90, 91]. Antigen 5 (Ag5) allergens display another common and potentially cross-reactive structure within the vespids sharing amino acid sequence identities of 57% to 67% of Ves v 5 (*Vespula vulgaris*) with the Ag5 allergens of the *Dolichovespula*, *Polistes* and *Vespa* species and identities of 71% to 98% within diverse *Vespula* species [92-94].

Allergen extracts derived from vertebrates

For the establishment of a homologous group we considered the following animal species: Cat (*Felis domesticus*), dog (*Canis familiaris*), horse (*Equus caballus*), mouse (*Mus musculus*), rat (*Rattus* sp.), guinea pig (*Cavia porcellus*), hamster (*Cricetus cricetus*) and rabbit (*Oryctolagus cuniculus*).

The known allergens of these animals are present in different body compartments such as pelt, fur, dander, sudor, saliva, urine and serum showing varying types and contents of allergens [95-100]. In addition to the tissue source, the production method influences the allergen composition of an extract [101]. Consequently, the establishment of a homologous group of animals is, basically, impossible. However, allergens belonging to the same protein family and showing cross-reactivity are present in taxonomically distant animal species. One example is a 67 kDa albumin, which is present as allergen in nearly all of the above mentioned species and which was demonstrated as cross-reactive structure in cats, dogs, horses, mice, rats and guinea pigs [102-104]. Another example is the 21 kDa allergen lipocalin which is cross-reactive between cat, horse and dog [105]. The 21 kDa lipocalins of cat (Fel d 4), mouse (Mus m 1) and rat (Rat n 1) share considerable amino acid sequence identities with horse allergen Equ c 1 (70%, 49%, 51%). However, in specific cases allergen extracts from different animal species may be included in one homologous group and proof of quality for one extract can be extrapolated to the other extracts of the group. Prerequisites could be (1) identical source material used for extract preparation, (2) known presence of cross-reactive allergens, and (3) sufficient cross-reactivity shown by inhibition studies. For example, under these conditions dog and cat extracts could be grouped. As table 12 demonstrates only one allergen, the 69 kDa albumin, is present in both species and is known as a cross-reactive allergen [106]. Nevertheless, inhibition studies surprisingly showed cross-reactivity of cat and dog extracts under certain conditions [96, 106-108]. Data demonstrated cross-reactivity for cat hair and dog hair but not for cat saliva and dog saliva or cat urine and dog urine, respectively [96]. Thus, subject to the quality of individual extracts and the demonstration of the presence of homologous allergens grouping may be justified on the basis of detailed data provided by the manufacturer.

Conclusion

For allergen sources sharing a similar biochemical matrix composition, the ‘concept of taxonomic families’ could be replaced by the ‘concept of homologous groups’ forming groups of antigenically related allergen sources with well-characterised allergen sources as ‘representative allergens’. We present a proposal of homologous groups consisting of tree pollen species (‘birch group’, *Oleaceae*, *Cupressaceae*), grass and cereal pollen species (*Pooideae*), weeds and house dust mites (*Dermatophagoides* species). In most of the cases, the homologous groups reflect a close taxonomic relatedness. The concept of homologous groups is dynamic and groups could be complemented by additional species depending on the availability of new information on identified allergens, homologous allergen families and cross-reactivities.

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Table 4: Known pollen allergens in tree species of the order *Fagales*, the 'birch group'

MW	Protein family	*Betula verrucosa*	*Alnus glutinosa*	*Corylus avellana*	*Carpinus betulus*	*Quercus alba*
17 kDa	Bet v 1 family	Bet v 1 major	Aln g 1 major	Cor a 1 major	Car b 1 major	Que a 1 major
15 kDa	Profilins	Bet v 2 minor	(Aln g profilin)* minor	Cor a 2 minor	(Car b 2)* minor	(Que a 2)* minor
23 kDa	Ca^{2+}-binding proteins/ Polcalcins	Bet v 3 minor				
8 kDa	Ca^{2+}-binding proteins/ Polcalcins	Bet v 4 minor	Aln g 4 minor			
34 kDa	Isoflavone reductases	Bet v 6 minor				
18 kDa	Cyclophilins	Bet v 7 minor				
70 kDa	Luminal-binding proteins			Cor a 10 minor		

* Allergen not listed in the IUIS official list of allergens [6]; Allergen named by Allergome database [5]

Table 5: Known pollen allergens in tree species of the *Oleaceae* family

MW	Protein family	*Olea europaea*	*Fraxinus excelsior*	*Syringa vulgaris*	*Ligustrum vulgare*
16-20 kDa	Trypsin inhibitors	Ole e 1 major	Fra e 1 major	Syr v 1 major	Lig v 1 major
13-18 kDa	Profilins	Ole e 2 minor	(Fra e 2)* minor		
7-10 kDa	Ca^{2+}-binding proteins/ Polcalcins	Ole e 3 minor	(Fra e 3)* minor [19]	Syr v 3 n.d.	
32 kDa	Unknown	Ole e 4 major [109]			
16 kDa	Superoxide dismutases	Ole e 5 minor			
5 kDa	Unknown	Ole e 6 major [110]			
10 kDa	Lipid-transfer proteins	Ole e 7 minor			
21 kDa	Ca^{2+}-binding proteins	Ole e 8 minor			
46 kDa	Beta-1,3-glucanases	Ole e 9 major	(Fra e 9)* n.d.	(Syr v 9)* n.d.	(Lig v 9)* n.d.
11 kDa	Glycosyl hydrolase homologues	Ole e 10 major			

* Allergen not listed in the IUIS official list of allergens [6]; Allergen named by Allergome database [5]
n.d. No data available

Table 6: Known pollen allergens in tree species of the *Cupressaceae* family

MW	Protein family	*Juniperus sp.:* *J. virginiana, J. ashei, J. communis, J. oxycedrus*	*Cupressus sp.:* *C. arizonica, C. sempervirens*
43 kDa	Pectate lyases	Jun v 1 major Jun a 1[+] major (Jun c 1)* n.d. (Jun o 1)* n.d.	 Cup a 1 major Cup s 1 major
43 kDa	Polygalacturonases	Jun a 2 major	
30-34 kDa	Thaumatins, PR-5	Jun a 3 minor [111] Jun v 3 n.d.	Cup s 3 major (Cup a 3)* major [28]
18 kDa	Ca^{2+}-binding proteins/ Calmodulins/ Polcalcins	Jun o 4 (obsolete name Jun o 2) minor [9]	

* Allergen not listed in the IUIS official list of allergens [6]; Allergen named by Allergome database [5]
[+] Former name: Jun s 1 (*Juniperus sabinoides*)
n.d. No data available

Table 7: Known pollen allergens in grass species of the *Pooideae* subfamily

MW	Protein family	*Phleum pratense*	*Lolium perenne*	*Poa pratensis*	*Dactylis glomerata*	*Antho-xanthum odoratum*	*Avena sativa*	*Festuca sp.: F. pratensis, F. elatior L., F. rubra*	*Holcus lanatus*	*Hordeum vulgare*	*Secale cereale*	*Triticum aestivum*
26-32 kDa	Expansins/ Group 1 allergens	Phl p 1 major [36]	Lol p 1 major	Poa p 1 major	Dac g 1 major	Ant o 1 major	(Ave s 1)*	(Fes p 1)* (Fes e 1)* (Fes r 1)*	Hol l 1 major	(Hor v 1)*	(Sec c 1)*	(Tri a 1)*
10-13 kDa	Unknown/ Group 2 allergens	Phl p 2 borderline minor/ major [36]	Lol p 2 minor [36]	(Poa p 2)*	Dac g 2 minor	(Ant o 2)*	(Ave s 2)*		(Hol l 2)*	(Hor v 2)*	(Sec c 2)*	(Tri a 2)*
11 kDa	Expansins/ Group 3 allergens	(Phl p 3)*	Lol p 3 minor [36]		Dac g 3 major							(Tri a 3)*
55-60 kDa	Group 4 allergens/ Berberine bridge enzymes	Phl p 4 major	Lol p 4 major [112]	(Poa p 4)*	Dac g 4 major	(Ant o 4)*	(Ave s 4)*	Fes p 4 major (Fes e 4)*	(Hol l 4)*	(Hor v 4)*	(Sec c 4)*	(Tri a 4)*
31 kDa	Ribonucleases/ Group 5 allergens	Phl p 5 major [36]	Lol p 5 major	Poa p 5 major	Dac g 5 major	(Ant o 5)*	(Ave s 5)*	(Fes p 5)* (Fes e 5)* (Fes r 5)*	Hol l 5 major	Hor v 5 n.d.	(Sec c 5)*	(Tri a 5)*
15 kDa	P-particle associated proteins/ Poa IX/Phl p VI allergen family[#]	Phl p 6 major [113]		(Poa p 6)*		(Ant o 6)*	(Ave s 6)*					
6 kDa	Ca^{2+}-binding proteins/ Polcalcins	Phl p 7 minor [36]	(Lol p CBP)*	(Poa p CBP)*	(Dac g CBP)*	(Ant o CBP)*	(Ave s CBP)*	(Fes e CBP)*				(Tri a CBP)*
12 kDa	Cytochrome C		(Lol p 10)*+ minor [114]	(Poa p 10)*								
16 kDa	Trypsin inhibitors/ Ole e 1 family[#]	Phl p 11 minor [115]	Lol p 11 major [116]									
14 kDa	Profilins	Phl p 12 minor [36]	(Lol p 12)*	(Poa p 12)*	(Dac g 12)*	(Ant o 12)*	(Ave s 12)*			(Hor v 12)*	(Sec c 12)*	(Tri a 12)*
50-60 kDa	Polygalacturonases/ Group 13 allergens	Phl p 13 major	(Lol p 13)* major [117]	(Poa p 13)*	(Dac g 13)*	(Ant o 13)*	(Ave s 13)*	(Fes p 13)* (Fes e 13)*		(Hor v 13)*	(Sec c 13)*	(Tri a 13)*

* Allergen not listed in the IUIS official list of allergens [6]; Allergen named by Allergome database [5]
[#] According to Swiss-Prot [30]
[+] Doubtful relevance as allergen [36, 114]
n.d. No data available

Table 8: Pollen allergens in additional grass species recommended with reservations to be included in the homologous group of *Pooideae*

MW	Protein family	*Agropyron cristatum*	*Agrostis sp.: A. alba, A. capillaris*	*Alopecurus pratensis*	*Arrhenatherum elatius*	*Bromus sp.: B. arvensis, B. inermis*
28-31 kDa	Group 1 allergens		(Agr a 1)* (Agr ca 1)*	(Alo p 1)*	(Arr e 1)*	(Bro a 1)*
60 kDa	Group 4 allergens [46]		(Agr a 60 kDa)[+]			(Bro a 4)*
26-33 kDa	Group 5 allergens [41]			(Alo p 5)[++]		
12 kDa	Ca^{2+}-binding proteins/ Polcalcins	(Agr c CBP)*				(Bro i CBP)*

* Allergen not listed in the IUIS official list of allergens [6]; Allergen named by Allergome database [5]
[+] Allergen neither listed in the IUIS official list of allergens nor in Allergome database; Allergen referenced in [46]
[++] Allergen neither listed in the IUIS official list of allergens nor in Allergome database; Allergen referenced in [41, 46]
Note: The attribution ‘major’ or ‘minor’ allergen is not yet possible, because of lack of information.

Table 9: Known pollen allergens in weed species

MW	Protein family	*Ambrosia sp.:* *A. artemisiifolia,* *A. trifida,* *A. psilostachya*	*Artemisia vulgaris*	*Parietaria sp.:* *P. judaica,* *P. officinalis,* *P. mauritanica*
38-42 kDa	Polysaccharide lyase I family/ Pectate lyases	Amb a 1 major	Art v 6 minor	
38 kDa	Polysaccharide lyase I family/ Pectate lyases	Amb a 2[+] major	Art v 2 major [118]/ minor [119]	
11 kDa	Unknown (contains 1 plastocyanin-like domain)	Amb a 3 minor [120]		
5-8 kDa	Unknown	Amb a 5 minor Amb t 5 minor [121] Amb p 5 n.d.		
10-15 kDa	Lipid transfer proteins	Amb a 6 minor	Art v 3 minor	Par j 1[++] major [62] Par o 1 major [122] Par j 2[++] major/minor according to region [63, 123] (Par m 1)* major [124]
12 kDa	Unknown	Amb a 7 minor		
14 kDa	Profilins	Amb a 8 minor (Amb t Profilin)* n.d.	Art v 4 minor	Par j 3 minor
9 kDa	Ca^{2+}-binding proteins/ Polcalcins	Amb a 9 minor	Art v 5 minor	(Par j CBP)* n.d.
18 kDa	Ca^{2+}-binding proteins/ Polcalcins	Amb a 10 minor		
24-28 kDa	Plant defensin family/ PR-12		Art v 1 major	
10 kDa	Cystastin proteinase inhibitors	(Amb a Cystatin proteinase inhibitor)* n.d.		

[+] Amb a 2 shares 65% amino acid sequence identity with Amb a 1
[++] Par j 1 and Par j 2 allergens share sequence identity [123, 125]
* Allergen not listed in the IUIS official list of allergens [6]; Allergen named by Allergome database [5]
n.d. No data available

Table 10: Allergens in house dust mites of the genus *Dermatophagoides*

MW	Protein family	*Dermatophagoides pteronyssinus*	*Dermatophagoides farinae*
25 kDa	Cysteine proteases	Der p 1 major	Der f 1 major
14 kDa	Group 2 mite allergens/ NPC2 family[#]	Der p 2 major	Der f 2 major
25 kDa	Serine proteases: Trypsins	Der p 3 minor/major	Der f 3 minor/major
57 kDa	Amylases	Der p 4 minor	(Der f 4)* minor [126]
15 kDa	Unknown	Der p 5 major	(Der f 5)* minor [127]
25 kDa	Serine proteases: Chymotrypsins	Der p 6 minor [128]	Der f 6 minor [128]
25-31 kDa	Unknown	Der p 7 major	Der f 7 major
26 kDa	Glutathione S transferases	Der p 8 minor	(Der f 8)* n.d.
30 kDa	Collagenolytic serine proteases	Der p 9 major	(Der f 9)* n.d.
37 kDa	Tropomyosins	Der p 10 major	Der f 10 major
96 kDa	Paramyosins	Der p 11 major	Der f 11 major
15 kDa	Fatty acid-binding proteins	(Der p 13)* n.d.	(Der f 13)* n.d.
177 kDa	Apolipophorin-like proteins	Der p 14 major	Der f 14 major
63-105 kDa	Chitinases (95 kDa)	(Der p 15)* major [129]	Der f 15 major
55 kDa	Gelsolins/ Villins		Der f 16 minor
53 kDa	Ca^{2+}-binding proteins		Der f 17 minor
60 kDa	Chitinases (60 kDa)	(Der p 18)* major [129]	Der f 18 major
40 kDa	Arginine kinases	Der p 20 n.d.	
16 kDa	Unknown	Der p 21 n.d.	

[#] According to Swiss-Prot [30]
* Allergen not listed in the IUIS official list of allergens [6]; Allergen named by Allergome database [5]
n.d. No data available
Note: All prevalences according to Thomas *et al.* [130], if not otherwise indicated.

Table 11: Known allergens of insect venoms

MW	Protein family	*Apis mellifera*	*Vespula sp.:* *V. flavopilosa,* *V. germanica,* *V. maculifrons,* *V. pensylvanica,* *V. squamosa,* *V. vidua,* *V. vulgaris*	*Dolichovespula sp.:* *D. maculata,* *D. arenaria*	*Polistes sp.:* *P. annularis,* *P. dominulus,* *P. exclamans,* *P. fuscatus,* *P. gallicus,* *P. metricus*	*Vespa sp.:* *V. crabro,* *V. mandarinia*	*Bombus sp.:* *B. pensylvanicus,* *B. terrestris*
16 kDa	Phospholipases A2	Api m 1 major					Bom p 1 Bom t 1
34 kDa	Phospholipases A1B		Ves m 1 major Ves v 1 major Ves s 1 major (Ves g 1*)	Dol m 1	Pol a 1 Pol d 1 Pol e 1 Pol g 1	Vesp c 1 Vesp m 1	
46 kDa	Hyaluronidases	Api m 2 major	Ves m 2 major [79] Ves v 2 major [131] (Ves f 2*) (Ves g 2*) (Ves p 2*) (Ves s 2*) major [132]	Dol m 2	Pol a 2 (Pol e 2*) (Pol g 2*)	(Vesp c 2*)	(Bom p Hyaluronidase*)
49 kDa	Acid phosphatases	Api m 3 major					(Bom p Acid phosphatase*)
3 kDa	Melittin	Api m 4 minor					

MW	Protein family	*Apis mellifera*	*Vespula sp.:* *V. flavopilosa,* *V. germanica,* *V. maculifrons,* *V. pensylvanica,* *V. squamosa,* *V. vidua,* *V. vulgaris*	*Dolichovespula sp.:* *D. maculata,* *D. arenaria*	*Polistes sp.:* *P. annularis,* *P. dominulus,* *P. exclamans,* *P. fuscatus,* *P. gallicus,* *P. metricus*	*Vespa sp.:* *V. crabro,* *V. mandarinia*	*Bombus sp.:* *B. pensylvanicus,* *B. terrestris*
30-34 kDa	Serine Proteases				Pol d 4 Pol e 4 (Pol g 4*)		Bom p 4 Bom t 4
23 kDa	Unknown/ Antigen 5		Ves f 5 n.d. Ves g 5 n.d. Ves m 5 major [79] Ves p 5 n.d. Ves s 5 major [132] Ves vi 5 n.d. Ves v 5 major [131]	Dol m 5 Dol a 5	Pol a 5 Pol d 5 Pol e 5 Pol f 5 Pol g 5 Pol m 5	Vesp c 5 Vesp m 5	
8 kDa	Allergen C	Api m 6 minor					
39 kDa	CUB Serine Protease	Api m 7 major [133]					

* Allergen not listed in the IUIS official list of allergens [6]; Allergen named by Allergome database [5]
n.d. No data available

Table 12: Known allergens from dog (*Canis familiaris*) and cat (*Felis domesticus*)

MW	Protein family	*Canis familiaris*		*Felis domesticus*	
		Allergen	Origin	Allergen	Origin
23-25 kDa	Lipocalins	Can f 1 major [134]	Salivary protein		
19 kDa	Lipocalins	Can f 2 minor [134]	Salivary protein		
69 kDa	Albumins	Can f 3 minor [134]	Serum protein, Salivary protein	Fel d 2 minor [134]	Serum protein
18 kDa	Unknown	Can f 4 major [135]	Epithelial protein		
18 kDa	Secretoglobin/ Uteroglobin			Fel d 1 major [134]	Salivary protein, Epithelial protein
11 kDa	Cystatins			Fel d 3[#] minor [134]/ major [136]	Epithelial protein
21 kDa	Lipocalins			Fel d 4 major [105]	Salivary protein
400 kDa	IgA			Fel d 5w major [137]	Serum protein
800-1000 kDa	IgM			Fel d 6w borderline minor/major [137]	Serum protein
150 kDa	IgG			Fel d 7w n.d.	Serum protein

[#] Fel d 3 contains cysteine protease inhibitor motif also present in Can f 1 and Can f 2 and two of three lipocalin motifs [136]
n.d. No data available

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