

Is mealworm or shrimp allergy indicative for food allergy to insects?

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Abbreviations used:

BAT basophil activation test,
DBPCFC double blind placebo controlled food challenge
LC-MS/MS liquid chromatography-tandem mass spectrometry
LCP larval cuticle protein
PBST PBS containing 1 % Tween 20,
SCP sarcoplasmic calcium-binding protein,
SPT skin prick test

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Abstract.

Scope: The growing world population is a key driver for the exploration of sustainable protein sources to ensure food security. Mealworm and other insects are promising candidates. Previously we found that shrimp allergic patients are at risk for mealworm allergy, and that mealworm can induce a primary allergy [3].

This study set out to investigate the allergenic potential of edible insects, suggested for human consumption by agencies such as WHO/FAO, in both the shrimp (potentially cross-reactive) and primary mealworm allergic population. The following insects were studied: mealworm, house cricket, giant mealworm, lesser mealworm, African grasshopper, large wax moth and black soldier fly.

Methods and results: 15 shrimp (mealworm sensitized or allergic) patients and four primary mealworm allergic subjects, who participated in previous studies, were included. All shrimp allergic patients were sensitized to multiple insects with similar response profiles for all insects tested. Primary mealworm allergic patients, showed IgE binding to proteins from only a few insects on immunoblot, although basophil activation test was positive for all tested insects.

Conclusion: Shrimp allergic patients are most likely at risk of food allergy to mealworm and other insects. Primary mealworm allergy does not mean subjects are likely to react to all insects.

Introduction

In order to preserve agricultural land, energy and water and reduce CO₂ footprint, changes to our current systems of food production must be made. Therefore, on recommendation of the FAO, governments are sanctioning investigations aimed at determining if certain insects can be consumed as a new sustainable protein candidate for feed and food [1, 2]. In two recent studies we showed

that the majority of shrimp food allergic patients also had mealworm food allergy [3]. In addition we found that primary mealworm allergy can develop in professional and hobby insect breeders [4]. Prevalence of food allergy ranges in Europe up to 5.7% depending on age, with shellfish among the ten most prevalent foods [5]. Prevalence of insect food allergy in Europe is not known. At present insects are not commonly consumed in Western countries. There are some reports on food allergy to insects in Asia, which are especially focused on the pupal stage of the silkworm (*Bombyx mori*), which is regularly consumed in Asia. As a result of the cultural influences of traditional Chinese food and medicine, Chinese people often eat oil-fried, water-boiled or ground pupa powder of the silkworm and anaphylaxis has been described upon ingestion [6]. In China, it was estimated from literature (1980 -2007) that around 17 % of food related anaphylaxis was caused by eating insects (locust, grasshopper and silkworm) [7]. Since the atopic profiles of these patients are not clear, it might be possible that anaphylaxis was due to cross-reactivity with shellfish and not to primary sensitization by insects.’ Food allergy to mealworm was reported previously in the US by Frye et al. [8]. Frye et al., presented a case of anaphylaxis in a patient after ingestion of mealworm. In a previous study we demonstrated that shrimp allergic patients were food allergic to mealworm in a double blind placebo controlled food challenge (DBPCFC). This allergy was based on cross- or co-sensitization to multiple mealworm allergens such as tropomyosin and arginine kinase [3]. In insects (e.g. cockroach and cricket) and arthropods (e.g. shrimp and crab) homologous proteins such as tropomyosin and arginine kinase have been described, which may contribute to cross-reactivity between shrimp, mealworm and other insect species [9, 10]. This could suggest that shrimp and mealworm allergy might be indicative for allergy to other insects. In our previous study we also showed that primary mealworm sensitized subjects were not allergic to shrimp. The study suggested that primary sensitization for mealworm was not caused by tropomyosin and arginine kinase, but that other proteins, such as larval cuticle protein (LCP) might play a role [4]. This assertion is at

conflict with the shrimp cross-reactivity theory and might indicate that primary mealworm sensitization is not indicative for allergy to other insects.

Before insects can become a substantial part of the diet, potential risks of food allergic reactions should be explored. In this paper the risk of food allergy to insects other than mealworm is addressed. Seven insects from 4 different orders (Coleoptera, Lepidoptera, Diptera and Orthoptera) and different life stages (larvae and adult) were investigated. These insects are described in the EFSA report as possible novel food or feed [11]. IgE binding (which we will refer to as sensitization) and functional IgE binding to *Tenebrio molitor*; mealworm (larvae), *Acheta domesticus*; house cricket (bug), *Zophobas morio*; giant mealworm (larvae), *Alphitobius diaperinus*; lesser mealworm (larvae), *Locusta migratoria*; African grasshopper (bug), which we will call grasshopper, *Galleria mellonella*; large wax moth (larvae) and *Hermetia illucens*; black soldier fly (larvae) was tested in blood from patients with shrimp allergy or a primary mealworm allergy from our previous studies [3, 4]. Proteins were identified, using LC-MS-MS, in the different insect extracts for comparative analysis.

Materials and methods

Study population

15 shrimp allergic patients and four primary mealworm allergic subjects showing sensitization (IgE binding in ImmunoCAP an BAT) to mealworm from our previous studies, were included in the study [3, 4]. 13 out of 15 shrimp allergic patients had a food allergy to mealworm as indicated by a positive DBPCFC. Two primary mealworm allergic subjects had a positive DBPCFC to mealworm and the other two had an inhalant allergy to mealworm.

All subjects gave written informed consent before participation. The study was approved by the local ethics committee (NL43731.041.13).

Specific IgE

ImmunoCAP mealworm was specifically produced for this project by Thermo Fisher Scientific, Uppsala, Sweden and was tested according to the manufacturer's recommendations. IgE is expressed in kU/L. Tests were considered positive with a value of 0.35 or higher.

Insect extracts

Fresh Yellow mealworms (*Tenebrio molitor*), giant mealworms (*Zophobas morio*), lesser mealworms (*Alphitobius diaperinus*), large wax moths (*Galleria mellonella*) and black soldier flies (*Hermetia illucens*) all in final larval stage, and crickets (*Acheta domesticus*) and grasshoppers (*Locusta migratoria migratorioides*) in adult form were kindly provided by Dutch insect farm Kreca (Ermelo, the Netherlands). Dutch shrimp (*Crangon crangon*) were bought from a local store.

Five grams of insects or boiled shrimp were extracted using a sequential protein extraction method [12]. First the insects or shrimp were mixed with 25 mL ice-cold Tris buffer (20 mM Tris buffer pH 7.6 containing 1 mM phenylthiocarbamide (Sigma Aldrich) and Halt Protease Inhibitor Cocktail (Thermo Scientific). Subsequently the insects or shrimp were disrupted, using an ultraturrax (3 x 10 sec) under continuous cooling. The ultraturrax was washed with 5 ml cold Tris buffer and the wash liquid was added to the sample suspension. After centrifugation (30 min, 15 000 x g at 4 °C), the supernatant was recovered. The insoluble residue was washed once with 5 mL Tris buffer. The 30 mL and 5 mL supernatant were combined. 25 mL was used for sample cleanup and concentration using TCA precipitation. Secondly, the remaining pellet was extracted overnight at 4 °C with 30 mL urea buffer (6 M urea in 20 mM Tris buffer pH 7.6 containing 1 mM phenylthiocarbamide and Halt Protease Inhibitor Cocktail). The sample was subsequently centrifuged and the supernatant was collected. The pellet was washed once more with 5 mL urea buffer, centrifuged and the supernatant was combined with the 30 ml urea supernatant. 25 mL of the extract was TCA precipitated. Tris and urea extracts were combined (1:1).

Immunoblot and dot blot

For SDS-PAGE, the Criterion system with an Any kD Ready Gel® Tris-HCl gel (Bio-Rad, Hercules, CA, USA) was used according to the manufacturer's instructions. Insect extracts (5 µg) were loaded on the gel, under reducing conditions (Laemmli buffer) together with 5 µg shrimp control. After separation, proteins were transferred to a polyvinylidene difluoride membrane using the Criterion Blotter system (Bio-Rad) according to the manufacturer's instructions.

The membrane was blocked overnight with 5% dried milk powder in phosphate buffered saline with 0.1% Tween 20 (PBST) and incubated overnight with serum from a shrimp allergic patient or primary mealworm allergic subject (1:50) in 1 % dried milk powder in PBST. After thorough washing, the membranes were incubated for 1 hour with HRP-labeled Goat anti human IgE (KPL, Gaithersburg, MD, USA) 1:50.000 in PBST. After washing, the bands were visualized using a chemi-luminescent peroxidase substrate kit ECL (Sigma) according to the manufacturer's instructions. Control blots were performed to exclude a-specific binding and binding of IgG. Blots were scanned using the Chemidoc XRS+ image scanner with Imagemag software (Bio-Rad).

Dot blots were performed on a PVDF membrane, using a Bio-Dot Microfiltration Apparatus (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Insect extracts (5 µg of protein) were loaded on the membrane, under reducing conditions (Laemmli buffer) together with 5 µg shrimp control. Reducing conditions were used on dot blot to keep the binding potential similar to the proteins on Western blot. After loading the protein on the membrane, the same procedure described for the immunoblot was performed.

Basophil activation test (BAT)

BAT's were performed with blood from shrimp allergic patients and primary mealworm allergic subjects from our previous studies [3, 4]. Five shrimp allergic patients were excluded from the BAT because they were non-responders, showing only IgE binding to freeze dried mealworm or spontaneous release in previous BAT. One shrimp allergic patient was not able to participate as a

result of emigration. BAT's were performed as described previously by Meulenbroek et al. [13] with minor modifications. Cells were incubated with a dilution series ($1:10^7$ to $1:10^2$) of insect extracts (5 mg/mL). Mealworm or shrimp extract (5 mg/mL) was used as positive control. CD63, CD123 and CD203c expression was analyzed by flow cytometry using FACSCanto II and FACSDiva software (BD Bioscience, USA). The results were expressed as a percentage of CD63+ basophils. The BAT was considered positive when the percentage of CD63+ cells was at least 5% and no spontaneous expression of CD63 on the cells was measured. Individuals with basophil response of 0-5% CD63, after anti-Fc ϵ RI stimulation, i.e. the positive control, were regarded as non-responders. In total 13 BATs were approved.

Nano LC-MS/MS analysis and protein identification

Proteins from the Tris/urea mixtures of each insect (20 μ g) were digested and analyzed according to Verhoeckx et al. [14], with small changes. Chromatography was performed on an Easy LC 1000 nanoscale liquid chromatography (nanoLC) system (Thermo Fisher Scientific, Odense, Denmark). The analytical nanoLC column was a pulled fused silica capillary, 75 μ m i.d., in-house packed to a length of 10 cm with 3 μ m C18 silica particles from Dr. Maisch (Entringen, Germany). Tryptic peptides (125 ng) were loaded at 500 nL/min directly onto the analytical column. A binary gradient was used for peptide elution. Mobile phase A was 0.1% formic acid, 2% acetonitrile, whereas mobile phase B was 0.1% formic acid, 80% acetonitrile. Gradient elution was achieved at 350 nL/min flow rate, and ramped from 8% B to 35% B in 60 min, and from 30% B to 100% B in additional 8 min. After 5 min at 100% B, the column was re-equilibrated at 0% B for 2 min before the following injection. MS detection was performed on a quadrupole-orbitrap mass spectrometer Q-Exactive (Thermo Fisher Scientific, Bremen, Germany) operating in positive ion mode, with nanoelectrospray (nESI) potential at 1800 V applied on the column front-end via a tee piece. Data-dependent acquisition was performed by using a top-12 method with resolution (FWHM), AGC target and maximum injection time (ms) for full MS and MS/MS of, respectively, 70,000/17,500, $10^6/10^5$, 50/60. Mass window

for precursor ion isolation was 1.6 m/z, whereas normalized collision energy was 25. Ion threshold for triggering MS/MS events was 2e4. Dynamic exclusion was 30 s. Data was processed using Proteome Discoverer 1.3 (Thermo Fisher Scientific, Bremen, Germany), using Sequest as search engine, and the Swiss Prot database accessed on September 2016 as sequence database (8,109,403 sequences for Metazoa taxonomy). The following search parameters were used: MS tolerance 15 ppm; MS/MS tolerance 0.02 Da; fixed modifications carbamidomethyl cysteine; enzyme trypsin; max. missed cleavages 1. Search results were filtered by q-values using Percolator integrated in Proteome Discoverer, to achieve a peptide-level FDR of less than 1%. Minimum peptide count was 2.

Analysis

Descriptive analyses were performed using SPSS Inc, Chicago, version 21.0.

Results

Shrimp and primary mealworm allergic patients

The 15 shrimp allergic patients, of whom 13 had a food allergy to mealworm, had a median age of 38 years (range 19-69) and 47% was male. Sensitization to shrimp was shown by a positive ImmunoCAP (> 0.35 kU/L) with a median of 5.5 kU/L (range 0.37 to 53.3 kU/L). The majority had inhalant allergies to HDM (11/15) and pollen (11/15). 8 had atopic dermatitis and 9 patients had one or more other food allergies. None of the patients had knowingly consumed mealworm or other insects [3]. Four primary mealworm allergic subjects regularly exposed to some of the tested insects were also studied. In addition to mealworm, cricket was consumed by subject 1, 3 and 4, grasshopper by subject 2, 3 and 4 and lesser mealworm by subject 1, 2 and 4. Wax moth was consumed only by subject 4 and giant mealworm only by subject 3. The super worm and black soldier fly had never been consumed by any of the primary mealworm allergic subjects. Oral allergy symptoms were reported for grasshopper in subject 2 and 3, and cricket in subject 4; however this was not confirmed with a food challenge.

Sensitization to all insects in shrimp allergic patients

Sensitization to mealworm was shown by a positive ImmunoCAP to mealworm (> 0.35 kU/L) in all shrimp allergic patients with a median of 5.8 kU/L (range 0.64 to 19.1 kU/L), see Table 1. IgE binding on dot blot to extracts of different insects, was found for all patients. This IgE binding was comparable to that of mealworm extract, except for two patients. These patients showed only IgE binding to proteins of a few insects, as shown in Table 1. Basophil reactivity to all insect species was highly similar, see Table 2. The BAT results corroborated the dot blot findings, and showed to be somewhat more sensitive than CAP.

Cross-reactive proteins were identified using immunoblot. Different protein profiles were recognized by the shrimp allergic patients. The majority (9/15) recognized a band at approximately 40 kDa. Identification of this band in mealworm previously showed that it contained both tropomyosin and arginine kinase. Patient 12 showed also IgE binding to a 50 kDa proteins and patients 4, 8, and 10 mainly recognized bands with a molecular weight > 100 kDa, including a band at 200 kDa. Patients 9 and 11, who had no food allergy to mealworm, recognized proteins with different molecular weights compared to the other 13 patients. Figure 1 shows two representative immunoblot's of patients 2, 4, 9 and 12.

Insect sensitization of primary mealworm allergic subjects

The primary mealworm allergic patients were all sensitized to mealworm and subjects 3 and 4 had a food allergy to mealworm. Only subject 2 showed minor sensitization to shrimp but had no food allergy to shrimp, which was tested in a food challenge [4]. Although all subjects were sensitized to some common inhalant allergens (tree and grass pollen and animal dander), only subject 4 had mild rhinoconjunctivitis to HDM and birch pollen. Subjects 1 and 2 had atopic dermatitis and none had any other food allergy [4]. In contrast to the shrimp allergic group, primary mealworm allergic subjects did not show sensitization to all tested insect extracts on dot blot (Table 1). However, sensitization

was shown in the BAT to all insects, with differences in reactivity. Subjects 2, 3 and 4, who had experienced clinical symptoms after eating grasshopper and cricket, showed a positive BAT for these insects (Table 2). Dot blot, immunoblot and basophil reactivity were different for all insects. All primary mealworm allergic subjects recognized proteins between 10-200 kDa depending on the insect tested. Protein binding was mainly seen for black soldier fly, giant mealworm, cricket and wax moth, which confirms the dot blot data. Striking is that IgE from these subjects did not recognize bands with an MW of tropomyosin or arginine kinase. See Figure 1 for two representative immunoblot's of the food allergic subjects 3 and 4.

Nano LC-MS/MS analysis

Proteins in the different insect extracts were identified and analyzed using LC-MS to clarify the sensitization results to the different insects. It can be concluded from Table 3 that both tropomyosin and arginine kinase were present in all insect extracts. A wide range of other proteins and putative allergens (e.g., myosin light chain and triosephosphate isomerase) were also identified in the different insect species (Table 3). The previously described new allergen, mealworm larval cuticle protein (LCP) was also present in the giant mealworm and cricket extract, but was not found in the other insect species tested.

Discussion

Shrimp allergic patients with food allergy to mealworm showed IgE reactivity (blot and BAT) to all insect extracts. The main IgE binding proteins were tropomyosin and/or arginine kinase (9/13). Binding to other proteins with a MW of ~50 kDa and MW > 100 KDa was also seen. Primary mealworm allergic subjects showed sensitization to some tested insect extracts. No clear similarities between insect sensitization patterns on the immunoblot were seen for the primary mealworm allergic subjects. Furthermore, basophil reactivity to the insect extracts tested was different for these subjects.

Accepted Article

That IgE from shrimp allergic patients binds to proteins from different insects is not surprising because crustaceans and insects both belong to the same phylum (Arthropoda). Within the clade Pancrustacea, shrimp belong to the sub-phylum crustacea and insects belong to the sub-phylum Hexapoda [15]. As a result of this phylogenetic relation, homology between proteins of shrimp and different insects can be expected and has been previously documented. Sequence identity between arginine kinase from shrimp (*Litopenaeus Vannamei*) and silkworm (*Bombyx mori*) was reported to be 83% [16] and for tropomyosin from house fly and Dutch shrimp 76% and up to 86% between cockroach and non-biting midge [17, 18]. High sequence homology is indicative for cross-reactivity, but must be confirmed by additional tests such as IgE binding (immunoblot), functional IgE binding (basophil activation test or skin prick test) or by a food challenge - the gold standard in food allergy research. Inhibition tests are needed to determine if this IgE binding is due to cross-reactivity or co-sensitization. However, this was not the purpose of this study. Only a few reports show cross reactivity between shrimp and insects. Srinroch et al. used pooled serum from 16 prawn allergic patients to show cross reactivity, on immunoblot, with arginine kinase from de field cricket (*Gryllus bimaculatus*) and Lanchester's freshwater prawn (*Macrobrachium lanchesteri*) [19]. Another paper showed cross-reactivity, also using immunoblotting, between arginine kinase of Bombay locust (*Patanga succincta*) and IgE from pooled prawn allergic patient sera (n=16) [20]. Cross reactivity is also supported by the simultaneous IgE reactivity to different tropomyosins (e.g. cockroach, HDM, anasakis) on ImmunoCAP ISAC, a diagnostic tool used in food allergy diagnosis. To our knowledge, this is the first study to investigate cross-reactivity between IgE from shrimp and mealworm allergic patients with house cricket, giant mealworm, lesser mealworm, African grasshopper, large wax moth and black soldier fly.

A considerable number of arthropod allergens (e.g. tropomyosin, arginine kinase, myosin light chain and triosephosphate isomerase) were identified in the insect extracts using LC-MS/MS, which could also be involved in the cross-reactivity. Tropomyosin and arginine kinase were identified in all insect

extracts, and most patients showed reactivity to these allergens. This supports the possibility of cross-reactivity to insects and confirms the comparable IgE binding and BAT reactivity results for the shrimp allergic patients. We therefore speculate that tropomyosin and arginine kinase were the most dominant allergens responsible for cross reactivity between shrimp and insects, but cross-reactivity to the other allergens cannot be excluded. In contrast to the shrimp allergic group, where similar reactivity's were seen between the different insect extracts, primary mealworm sensitized subjects showed variability in the degree and percentage of sensitization to the different insects. They showed lack of sensitization to some tested insect extracts (e.g. grass hopper, black soldier fly) in immunoblot and variable activation intensities in the BAT. Primary mealworm allergic subjects hardly recognized the pan allergens tropomyosin and arginine kinase, which is in line with our previous study where we showed that mealworm larval cuticle proteins instead of tropomyosin and arginine kinase seem to play a principal role in primary mealworm allergy [4].

Comparison of sequences from insect cuticle proteins was previously performed by Andersen et al. [21]. Some homology was found between cuticle proteins from different insect orders, while most have quite dissimilar sequences [21]. This is strengthened by the fact that LCP was only identified in two other insects in our study. A common peptide sequence was found in giant mealworm and cricket extracts, which was also documented for their clades by Andersen et al. [21]. This could explain the possible allergic reaction one of our primary mealworm allergic subjects experienced when consuming cricket and not with other insects. The low homology of inter-species larval cuticle proteins, the different protein binding profiles seen on immunoblot, and variation in BAT, might suggest primary sensitization to the different insects could be caused by different proteins. This indicates mealworm allergy is not indicative for insect allergy and suggests the possibility of species-specific insect allergy when primarily sensitized to insect-specific proteins. Species specific insect allergy has been described previously for housefly (*Musca domestica*) and cockroach [22, 23].

Binding of IgE from a person solely allergic to housefly was inhibited by housefly in ELISA, but only

mildly by the closely related lesser housefly. No inhibition was seen with blowfly (*Lucilia* spp.), fruit fly (*Drosophila* spp.), horsefly (*Haematopota pluvialis*) and mosquito (*Culex pipiens*) [22]. Specific insect allergy was also described by Lopata et al, who demonstrated mono-sensitivity to American cockroach (*Periplaneta Americana*) or German cockroach (*Blattella Germanica*) in 17 out of 38 subjects [23]. Siracusa et al. showed inhibition neither from wax moth (*Galleria mellonella*) and mealworm (*Tenebrio molitor*) on greenbottle (*Lucilla caesar*), nor from greenbottle and mealworm on wax moth, nor from greenbottle and wax moth on mealworm [24]. These results are in line with our data, where the primary sensitized subjects can consume many other insects without symptoms. Because of their quite homogenous reaction to all tested insect extracts, combined with the allergic reaction to mealworm in 87% of our previously studied shrimp allergic patients [3], we expect that shrimp allergic patients could likely have a clinical reaction when eating other insects. The clinical relevance of mealworm sensitization in shrimp allergic patients was high (87%). Information on predictive values of insect sensitization and food allergy are scarce. Some information can be found on insect sensitization and inhalant allergies. Siracusa et al. found sensitization in 31.6% of 76 workers with live fish bait (e.g. mealworm and wax moth) to at least one insect. Work related asthma and rhinitis was seen in 29% of those workers [25]. Another study investigating occupational allergy to grasshopper (*Locusta migratoria*) [26] could not differentiate between symptomatic and asymptomatic patients, based on sensitization results. From eight sensitized subjects that worked in a breeding facility, five had clinical symptoms when working in the grasshopper facility. From the four primary mealworm sensitized subjects in our study, three developed respiratory symptoms when working with mealworm [4]. No other research has been performed studying food allergy to insects. Therefore it is difficult to draw any conclusions on the clinical relevance of the insect sensitization in primary mealworm allergic subjects. DBPCFC is needed to translate sensitization to clinical outcome.

Based on the information presented in this paper we conclude that shrimp allergic patients are probably at risk not only for food allergy to mealworm but also to other insects. Given the variability in sensitization to other insects, primary mealworm allergic subjects are suspected not to react to all insects.

Author contributions:

HB. Has carried out patient studies and *in vitro* work, interpreted the results and wrote the manuscript, AK. Has supervised the study and helped interpreting the results and prepare the manuscript, GJ. Prepared the extracts and helped writing the manuscript, MG. Performed the LC-MS analyses and helped writing the manuscript SHJ provided the *in vitro* work and helped write the manuscript, GH. Has supervised the study and helped interpreting results and prepare the manuscript, KV. Has supervised the study and helped interpreting results and writing the manuscript.

References:

1. FAO/WHO. Foods derived from modern biotechnology. Foods derived from modern biotechnology; Rome, 2009.
2. FAO, FAO Forestry Paper: Edible insects: future prospects for food and feed security, FAO, Rome 2013.
3. Broekman, H.C., Verhoeckx, K.C., den Hartog Jager, C.F., Kruizinga, A.G., et al., Majority of shrimp allergic patients are allergic to mealworm. *J Allergy Clin Immunol.* 2016. 137(4):1261-3.
4. Broekman, H.C., Knulst, A.C., den Hartog Jager, C.F., van Bilsen, J.H.M., et al., Primary respiratory and food allergy to mealworm. *J Allergy Clin Immunol.* 2017 Mar 6. pii: S0091-6749(17)30340-8

5. Nwaru, B.I., Hickstein, L., Panesar, S.S., Roberts, G., et al., Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy*. 2014;69(8):992-1007. Epub 2014/05/13.
6. Ji, K.M., Zhan, Z.K., Chen, J.J., Liu, Z.G., Anaphylactic shock caused by silkworm pupa consumption in China. *Allergy*, 2008. 63(10): 1407-1408.
7. Ji, K.M., Chen J., Li M., Liu Z., et al., Anaphylactic shock and lethal anaphylaxis caused by food consumption in China. *Trends in food science & Technology*, 2009. 20: 227-231
8. Freye, H.B., Esch, R.E., Litwin, C.M., Sorkin, L., Anaphylaxis to the ingestion and inhalation of *Tenebrio molitor* (mealworm) and *Zophobas morio* (superworm). *Allergy Asthma Proc*, 1996. 17(4): 215-9.
9. Binder, M., Mahler, V., Hayek, B., Sperr, W.R., et al., Molecular and immunological characterization of arginine kinase from the Indianmeal moth, *Plodia interpunctella*, a novel cross-reactive invertebrate pan-allergen. *Journal of Immunology*, 2001. 167(9): p. 5470-7.
10. Reese, G., Ayuso, R., and Lehrer, S.B., Tropomyosin: an invertebrate pan-allergen. *Int Arch Allergy Immunol*, 1999. 119(4): p. 247-58.
11. EFSA Scientific Committee, Risk profile related to production and consumption of insects as food and feed. *EFSA Journal* 2015;13(10):4257
12. Broekman, H.C., Knulst, A.C., den Hartog Jager, S.F., Monteleone, F., et al., Effect of thermal processing on mealworm allergenicity. *Mol Nutr Food Res*. 2015. 59(9):1855-64.
13. Meulenbroek, L. A., de Jong, R. J., den Hartog Jager, C. F., Monsuur, H. N., et al., IgG antibodies in food allergy influence allergen-antibody complex formation and binding to B cells: a role for complement receptors. *J. Immunol*. 2013,191, 3526–3533.
14. Verhoeckx, K.C., van Broekhoven, S., den Hartog Jager, C.F., Gaspari, M., et al., House dust mite (Der p 10) and crustacean allergic patients may react to food containing Yellow mealworm proteins. *Food Chem Toxicol*, 2014. 65: p. 364-73.

15. Regier, J.C., Shultz, J. W., Zwick, A., Hussey, A., et al., Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. *Nature*, 2010,463 (7284): 1079–1084
16. Liu, Z., Xia, L., Wu, Y., Xia, Q., et al., Identification and characterization of an arginine kinase as a major allergen from silkworm (*Bombyx mori*) larvae. *Int Arch Allergy Immunol*, 2009. 150(1): 8-14.
17. Romero, M.R., Claydon, A.J., Fitches, E.C., Wakefield, M.E., et al., Sequence homology of the fly proteins tropomyosin, arginine kinase and myosin light chain with known allergens in invertebrates. *Journal of insects as food and feed*, 2016; 2(2): 69-81.
18. Jeong,K.,Y., Yum, H-J., Lee, I.,Y., Ree, H.,I., et al., Molecular Cloning and Characterization of Tropomyosin, a Major Allergen of *Chironomus kiiensis*, a Dominant Species of Nonbiting Midges in Korea. *Clin Diagn Lab Immunol*. 2004 Mar; 11(2): 320–324.
19. Srinroch, C., Srisomsap, C., Chokchaichamnankit, D., Punyarit, P., Phiriyangkul, P., Identification of novel allergen in edible insect, *Gryllus bimaculatus* and its cross-reactivity with *Macrobrachium* spp. allergens. *Food Chemistry*, 2015; 184:160-166.
20. Phiriyangkul, P., Srinroch, C., Srisomsap, C., Chokchaichamnankit, D., Punyarit, P., Effect of food thermal processing on allergenicity proteins in Bombay Locust (*Patanga Succincta*). *International Journal of Food Engineering* 2015; 1 (1) 23-28
21. Andersen, S.,O., Rafn, K., Roepstorff, P., Sequence studies of proteins from larval and pupal cuticle of the Yellow mealworm. *Insect Biochem.Molec.Biol*, 1997; 27(2): 121-131.
22. Focke, M., Hemmer, W., Wöhr, S., Götz, M., et al., Specific sensitization to the common housefly (*Musca domestica*) not related to insect panallergy. *Allergy*. 2003 May;58(5):448-51.
23. Lopata, A.L., Horner, W.E., Menon, P.K., Oliver, J., et al., Cockroach allergenic activity: analysis of commercial cockroach and dust extracts. *J Allergy Clin Immunol* 1991; 88: 895-901.

24. Siracusa, A., Bettini, P., Bacocoli, R., Severini, C., et al., Asthma caused by live fish bait. J Allergy Clin Immunol, 1994. 93(2): 424-30.
25. Siracusa, A., Marcucci, F., Spinozzi, F., Marabini, A., et al., Prevalence of occupational allergy due to live fish bait. Clin Exp Allergy 2003; 33:507-510
26. Lopata, A.L., Fenemore, B., Jeebhay, M.F., Gäde, G., Potter, P.C., Occupational allergy in laboratory workers caused by the African migratory grasshopper *Locusta migratoria*. Allergy. 2005;60 :200-205.

Figure 1. Figure 1. Representative immunoblots of four shrimp allergic patients 2, 4, 9 and 12 (A, B, C and D) and two primary mealworm allergic subjects 3 and 4 (E and F) with tris/urea insect extracts. The immunoblots show IgE binding to multiple insects with similar response profiles for all insects tested (shrimp allergic patients) and IgE binding to proteins from only a few insect (primary mealworm allergic subjects).

Figure 1a Coomassie stained gel of the insect extracts used in immunoblot

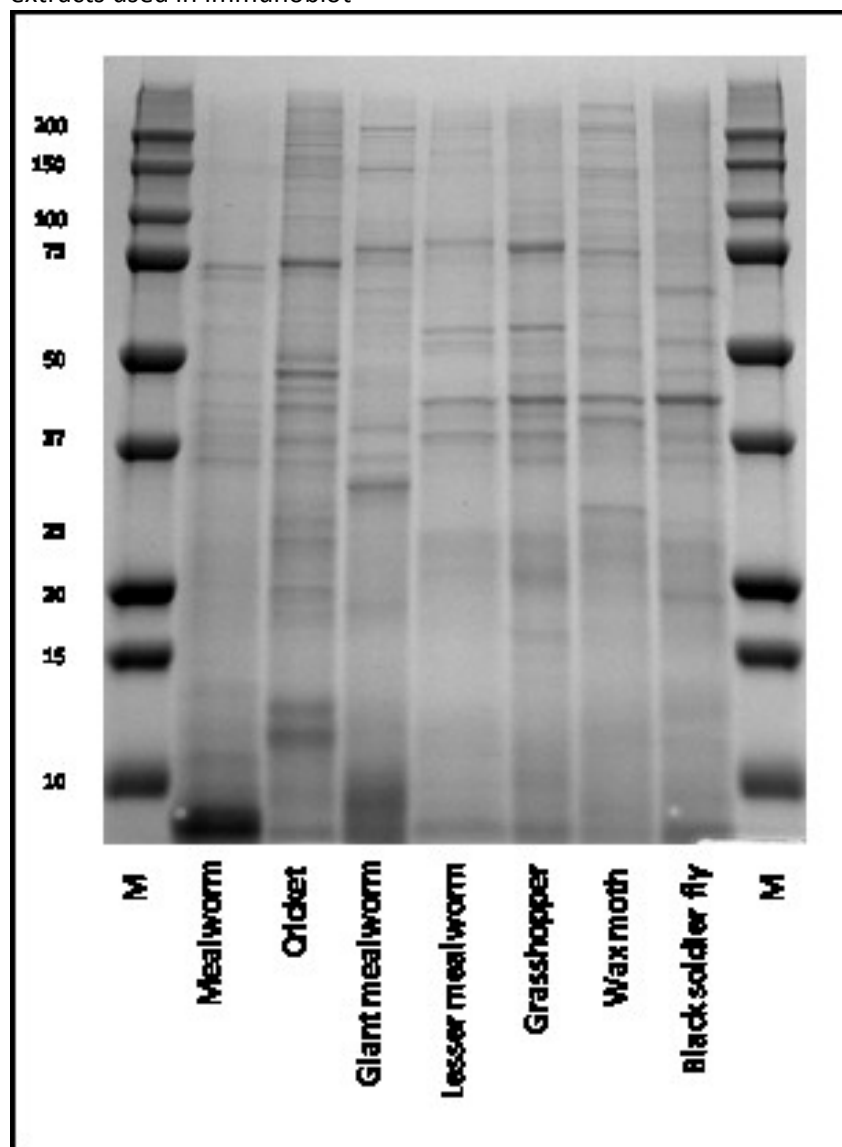
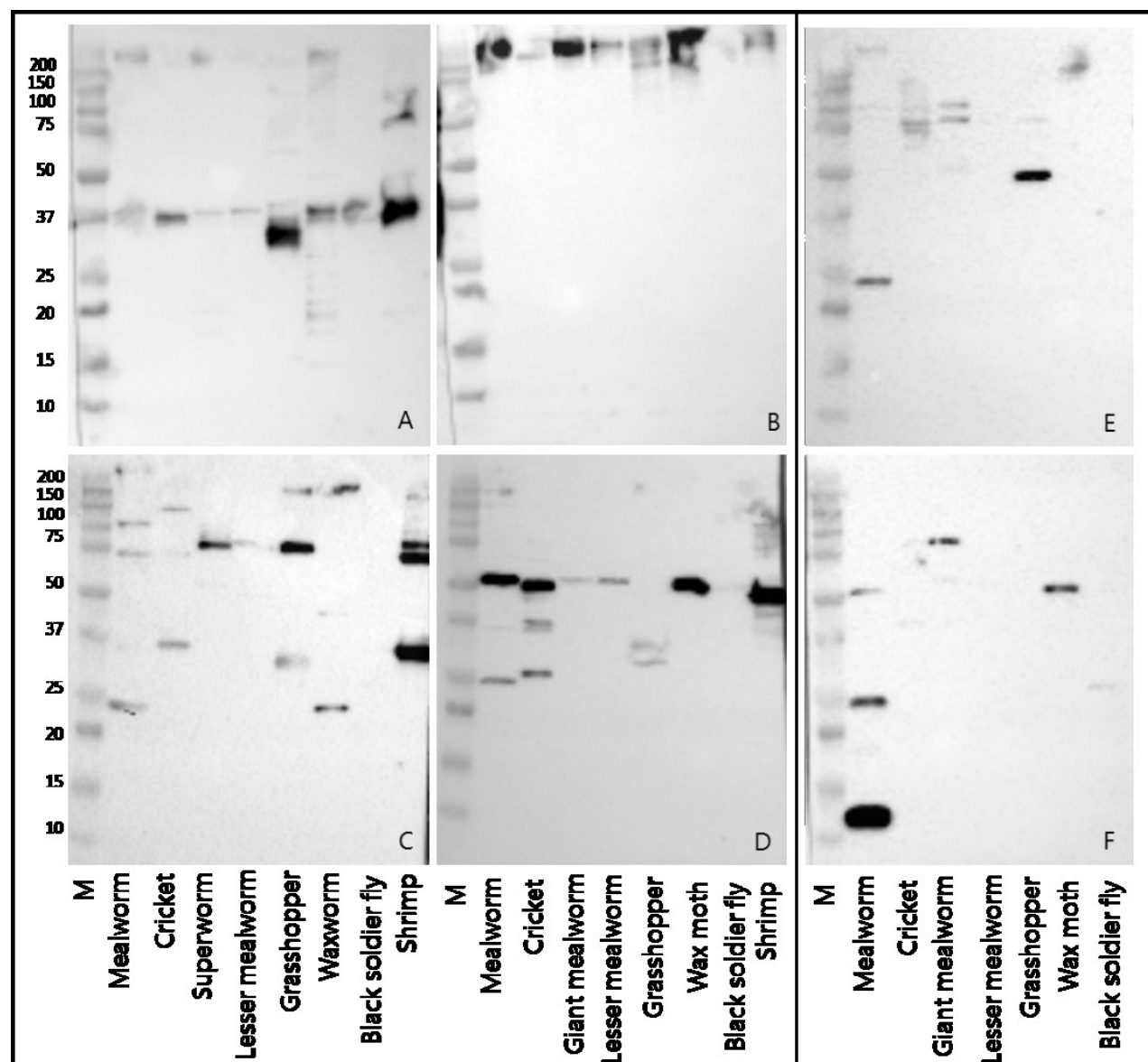


Figure 1b



Graphical Abstract

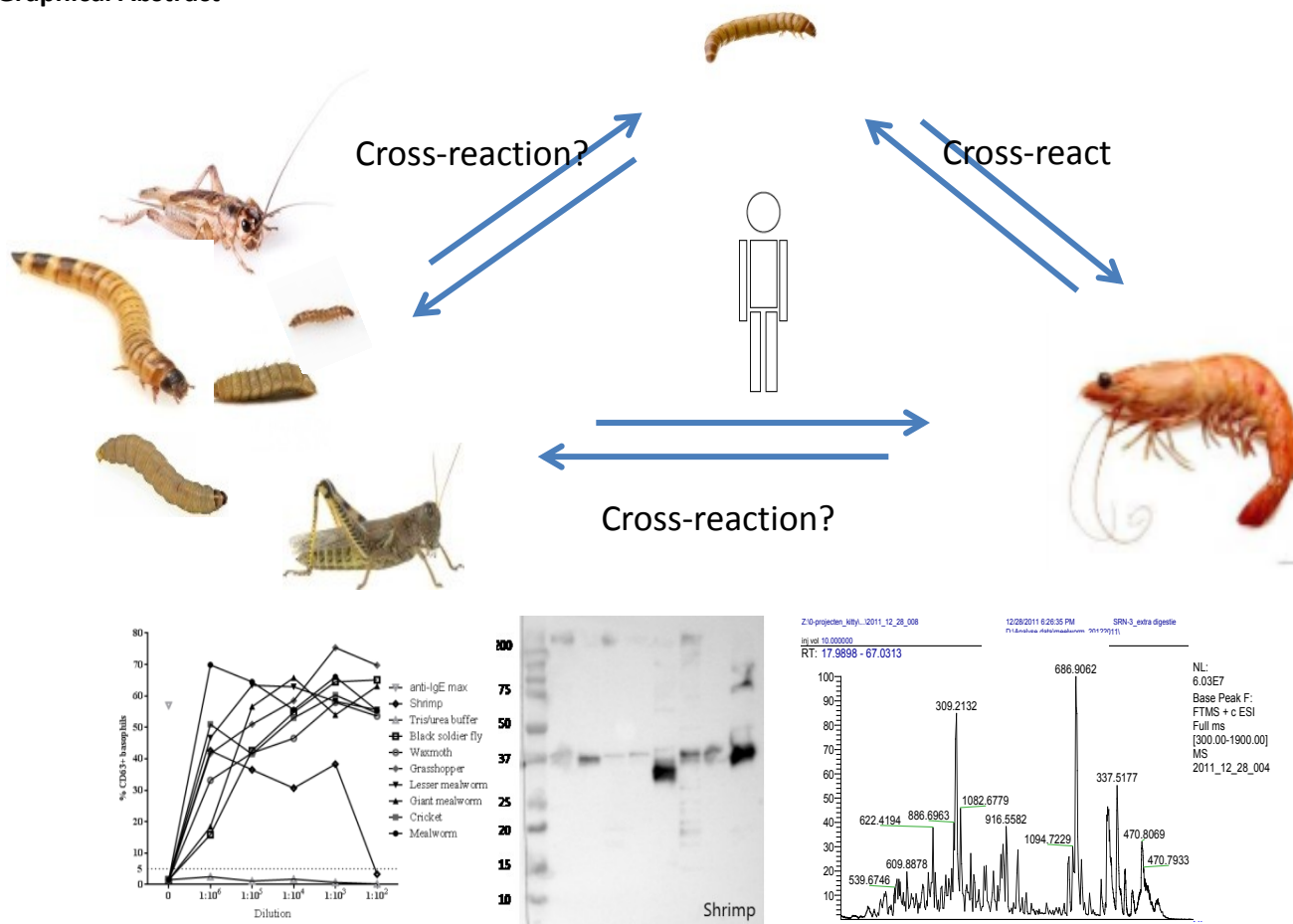


Table 1. IgE binding profile of 15 shrimp allergic patients and 4 primary mealworm allergic subjects. Mealworm Cap IgE values are expressed in kU/L. Immuno dot blot's with insect extracts from mealworm, cricket, giant mealworm, lesser mealworm, grasshopper, wax moth and black soldier fly are expressed as + or -, where, + indicates IgE binding – no IgE binding.

Shrimp Patient	CAP mealworm	Dot mealworm	Dot cricket	Dot giant mealworm	Dot lesser mealworm	Dot grasshopper	Dot wax moth	Dot black soldier fly
1	0.64	+	+	+	+	+	+	+
2	19.1	+	+	+	+	+	+	+
3	18.9	+	+	+	+	+	+	+
4	15.0	+	+	+	+	+	+	+
5	7.78	+	+	+	+	+	+	+
6	5.83	+	+	+	+	+	+	+
7	3.97	+	+	+	+	+	+	+
8	16.8	+	-	+	+	+	-	-
9	1.0	+	+	+	+	+	+	-
10	10.3	+	+	+	+	+	+	+
11	0.81	+	+	-	-	+	-	-
12	3.49	+	+	+	+	+	+	+
13	4.76	+	+	+	+	-	-	-
14	3.50	+	+	+	+	+	+	+
15	12.7	+	+	+	+	+	+	+
Mealworm Subject	CAP mealworm	Dot mealworm	Dot cricket	Dot giant mealworm	Dot lesser mealworm	Dot grasshopper	Dot wax moth	Dot black soldier fly
1	0.75*	+	-	-	-	-	-	-
2	2.32*	+	+	+	+	-	-	-
3	2.26*	+	+	-	-	-	+	-
4	14.6*	+	+	-	-	-	+	-

* previously published [4]

Table 2. Functional IgE binding in basophil activation test of 15 shrimp allergic patients and 4 primary mealworm allergic subjects to different insects (mealworm, cricket, giant mealworm, lesser mealworm, grasshopper, wax moth and black soldier fly). The results are expressed as a percentage of CD63+ basophils. The grey color codes represents the ratio of maximum % of CD63 up-regulation with respect to mealworm.

Shrimp Patient	@IgE	Mealworm	Cricket	giant mealworm	lesser mealworm	Grasshopper	wax moth	black soldier fly
4	67.6	64.9	68.3	60.8	48.0	76.6	60.7	41.6
5	28.5	25.4	45.3	45.2	41.1	71.3	41.0	59.2
6	55.7	60.1	63.5	48.0	54.7	69.9	65.8	23.2
7	49.3	30.5	50.5	32.5	30.8	56.4	44.7	66.1
9	74.6	41.4	36.4	41.6	48.7	49.5	34.9	43.3
10	41.7	51	48.5	35.2	54.7	62.1	60.7	63.9
12	26.6	5.6	19.8	3.2	2.9	38.2	9.3	9.8
13	39.5	51.2	40.7	44.4	41.6	42.9	42.5	53.0
15	57	69.9	60.3	65.8	63.4	75.4	58	65.1
Mealworm Subject	@IgE	mealworm	cricket	giant mealworm	lesser mealworm	grasshopper	wax moth	black soldier fly
1	54.9	77.1	57.0	71.6	71.7	62.5	64.8	67.2
2	26.9	58.9	10.9	34.8	17.8	58.7	16.1	43.9
3	6.8	27.8	14.4	16.6	8.9	13.6	5.1	12.3
4	18.8	28.9	51.8	5.4	21.9	18.8	20.2	41.2

<0.25	0.25-0.5	0.5-0.75	0.75-1.25	1.25<
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Table 3. Proteins homologous to known allergens identified by LC-MS/MS in the Tris/Urea extracts. Identification was based on homology with metazoan proteins in the Swiss Prot database. Proteins were assigned as allergen by the IUIS allergen nomenclature subcommittee

Allergen	mealworm	giant mealworm	lesser mealworm	cricket	grasshopper	wax moth	black soldier fly
Myosin light chain	x	x	x	x	x	x	x
Tropomyosin	x	x	x	x	x	x	x
Arginine kinase	x	x	x	x	x	x	x
Paramyosin	x	x	x	x	x	x	-
Troponin T	x	x	-	x	x	x	x
GAPDH	x	x	x	x	-	x	x
Triosephosphate isomerase	x	x	x	-	-	-	x
Apolipophorin	x	-	-	x	x	x	-
Profilin	x	-	x	x	x	-	-
Troponin C	-	x	x	-	x	-	-
Glutathione S-transferase	x	x	-	-	x	-	-
Hexamerin	x	-	-	-	x	x	-
Fatty acid binding protein	-	-	-	-	x	x	-
Hemolymph	x	-	-	-	-	x	-
Larval cuticle protein A1A	x	x	-	-	-	-	-
Larval cuticle protein A2B	x	-	-	x	-	-	-
Larval cuticle protein A3A	x						