ORIGINAL ARTICLE

Investigating Sensitization to Novel Foods: A Real-Life Prevalence Study of IgE-Mediated Reactivity to Cricket, Locust, and Mealworm in Insect Food–Naïve Allergic Individuals

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Abstract

Background: With the global population on the rise, edible insects are considered a potential solution to food security, although concerns about risks such as anaphylaxis have been expressed.
Methods: We tested 2014 participants using the ALEX (Allergen Explorer) test, version 2 (ALEX^2, Macroarray Diagnostics) and extracts of 3 novel foods: *Acheta domesticus, Locusta migratoria*, and *Tenebrio molitor*. IgE-mediated sensitization status was investigated in participants who had never knowingly consumed these insects. Data were recorded using an electronic database.
Results: A total of 195 individuals (9.7% of all participants) were sensitized to insects. Tropomyosin was corecognized by 34%, and 18.5% of results were positive for arginine kinase. Reactivity to sarcoplasmic calcium-binding protein, troponin C, paramyosin, or myosin light chain proteins was recorded in less than 5% of the population, whereas 108 individuals (55.4%) did not react to invertebrate panallergens. A further 33 individuals (16.9%) exhibited monosensitization exclusively to insects. Multivariate analysis revealed an inverse association between reactivity to arachnids and sensitization to insect allergens, while a direct association was identified between Mollusca and Blattoidea and reactivity to tropomyosin. Furthermore, reactivity to myosin light chain protein correlated with sensitization to *A domesticus* and *L migratoria*, and reactivity to troponin C with sensitization to *A domesticus* and *T molitor*.
Conclusion: IgE-mediated sensitization to edible insect extract was observed in individuals with no prior exposure to these foods. Mites were unlikely to be primary sensitizers owing to their inverse association with insect reactivity. Conversely, the direct association between sensitization to insect extract and reactivity to mollusk and cockroach extract suggests their potential as primary sensitizers in these participants. A positive association was consistently observed for tropomyosin, with reactivity to all studied insects, thus supporting its role as a primary sensitizer.

Key words: Edible insects. Novel foods. *Acheta domesticus*. *Locusta migratoria*. *Tenebrio molitor*. IgE-mediated sensitization. Tropomyosin. Arginine kinase.

Resumen

Antecedentes: Con el aumento de la población mundial, los insectos comestibles se consideran una solución potencial para la seguridad alimentaria, aunque existe preocupación por riesgos como la anafilaxia.
Métodos: 2.014 participantes se sometieron a pruebas con el Allergy Explorer-ALEX^2 incluyendo extractos de tres nuevos alimentos: *Acheta Domesticus* (Ad), *Locusta migratoria* (Lm) y *Tenebrio molitor* (Tm). Se investigó la sensibilización mediada por IgE en participantes que no eran conscientes de haber consumido previamente estos insectos. Los datos se registraron en una base de datos electrónica.
Resultados: 195 individuos (9,7% de todos los participantes) estaban sensibilizados a los insectos. La tropomiosina fue co-reconocida por el 34%, y el 18,5% fueron positivos para las arginina quinasas. La reactividad a proteina sarcoplásmica ligadora de calcio (sarcoplasmic-CB), troponina-C, paramiosina, o y la cadera ligera de miosina se encontró en menos del 5% de la población, mientras que 108 individuos (55,4%) no mostraron ninguna reactividad a panalérgenos de invertebrados. Además, 33 individuos (16,9%) mostraron monosensibilización exclusivamente a insectos. El análisis multivariante reveló una asociación inversa entre la reactividad a arácnidos y la sensibilización a alérgenos de insectos, mientras que la reactividad a *Mollusca*, *Blattoidea* y tropomiosina mostró una relación directa. Además, la reactividad a la cadena ligera de miosina se correlacionó con la sensibilización a Ad y Lm, y la troponina-C con la sensibilización a Ad y Tm.

Conclusión: Se observó sensibilización IgE a extractos de insectos comestibles en individuos sin exposición previa a estos como alimentos. Los ácaros mostraron una baja probabilidad de ser sensibilizadores primarios debido a su asociación inversa con la reactividad a insectos. Por el contrario, la asociación directa de la sensibilización a insectos con la reactividad a extractos de moluscos y cucarachas sugiere su potencial como sensibilizadores primarios en estos participantes. La tropomiosina mostró sistemáticamente una asociación positiva con la reactividad a todos los insectos estudiados, lo que respalda su papel como sensibilizador primario.

Palabras clave: Insectos comestibles. Nuevos alimentos. *Acheta domesticus*. *Locusta migratoria*. *Tenebrio molitor*. Sensibilización mediada por IgE. Tropomiosina. Arginina quinasa.

Summary box

- **What do we know about this topic?**
  Edible insects are promoted as sustainable food sources, although concerns remain about allergic reactions, particularly IgE-mediated sensitization and cross-reactivity with known invertebrate allergens such as mites, mollusks, and crustaceans.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  This study shows that sensitization to edible insects can occur without prior ingestion, implicating mollusks and cockroaches as possible primary sensitizers and identifying tropomyosin as a key allergen. These findings may inform future risk assessment and diagnostic strategies.

Introduction

With the world's population projected to reach 9-11 billion by 2050, the challenge of food supply becomes increasingly daunting [1]. As traditional food resources face scarcity and difficulties in production, alternative and sustainable food sources are gaining attention. Edible insects, a rich source of protein and essential nutrients, have emerged as a potential solution to address global food security concerns [2].

Currently, more than 2 billion people around the world include insects as a regular part of their diet, consuming over 2000 different edible species [3]. Edible insects have garnered recognition for their significant nutritional value, minimal environmental impact, and highly efficient production process. In comparison to traditional livestock, they demand fewer resources, such as water and land, making them sustainable and promising food sources for the future [3].

In Europe, insects are considered a novel food because their historical consumption within the European Union has been limited [4]. However, many people are unaware that insects are already part of the European diet. It is estimated that approximately half a kilogram of insects is inadvertently consumed per person annually, as they are integrated during the production of various foods, such as cereals and tomatoes [5]. Certain countries in the European Union, such as the Netherlands and Belgium, have recognized the potential of insect-based products and have been selling them for several years [6]. Italy also recently joined this trend by introducing products made from proteins derived from *Tenebrio molitor* onto the market.

Despite the numerous benefits of edible insects, their consumption is not without risks. Literature reports include case studies [7,8] and systematic reviews [9,10] that document possible adverse reactions, including anaphylaxis [11-13], which are triggered by the ingestion of insects. Given the expanding interest in insect-based diets, understanding the prevalence and nature of such reactions becomes crucial.

Many of the allergenic components identified to date in the meat of edible insects are represented by panallergens already described in arthropods (mites and crustaceans), mollusks, and nematodes, such as tropomyosin, arginine kinase, troponin C, sarcoplasmic calcium-binding protein, myosin light chain protein, triosephosphate isomerase, and paramyosin [9,14]. However, additional molecules specific to insects have been identified, such as chemosensory proteins, odorant or pheromone-binding proteins, and hexamerin [15-16]. Consequently, such allergens may represent a significant cause of adverse reactions, even severe ones, eventually labeled as idiopathic in Western populations, who are not accustomed to consuming such protein sources.

This study surveyed the IgE-mediated sensitization status of 3 insects currently available for in vitro detection of IgE reactivity, namely, cricket (*Acheta domesticus*) [17], locust (*Locusta migratoria*), and mealworm (*T molitor*) [18], in persons who had never knowingly consumed edible insects before.

Methods

Study Design

We conducted a single-center cross-sectional observational clinical survey on sensitization to all 3 species in a population that had never consciously consumed insect proteins as part of their diet.

We also investigated the association between this reactivity and the presence of specific IgE antibodies against other insects, arachnids, crustaceans, and mollusks.

Finally, we assessed the presence of symptoms related to environmental or food allergies in these individuals.

Setting

The study involved 2014 unselected participants born in Central or Southern Italy and attending the outpatient Allergy Unit of IDI-IRCCS, Rome with a history of adverse reactions to foods, allergic rhinitis, bronchial asthma, and/or atopic eczema.

The IDI-IRCCS serves as a national reference center for dermatological diseases.

Data were collected between January 2021 and August 2023. Demographic information and clinical data were recorded using a customized electronic database.

Participants

The main eligibility criterion for the study was the presence of IgE-mediated reactivity that could be assessed by the array used to evaluate participants with a clinical history suggestive of allergy-mediated disease. The subgroup used for data analysis was characterized by sensitization to at least 1 of the insect extracts spotted on the IgE microarray (*A domesticus*, *L migratoria*, or *T molitor*).

We included all participants who reacted to molecules or extracts from invertebrates as a comparison and to identify potential molecular similarities for various types of insect, as follows: Coleoptera, specifically *T molitor*; Blattoidea, specifically *Blattella germanica* and *Periplaneta americana*; Hymenoptera, specifically *Apis mellifera*, *Vespula vulgaris*, *Dolichovespula maculata*, *Polistes dominulus*, and *Solenopsis richteri*; Ixodida, specifically *Argas reflexus*; Orthoptera, including Gryllidea, specifically *A domesticus*, and Acrididea, specifically *L molitor*; Crustacea (ie, *Chionoecetes* species, *Crangon crangon*, *Homarus gammarus*, *Litopaenaeus setiferus*, *Pandalus borealis*, and *Penaeus monodon*); Mollusca (ie, *Loligo* species, *Mytilus edulis*, *Ostrea edulis*, *Pecten species*, and *Ruditapes* species); Chelicerata (ie, Astigmata including *Acarus siro*, *Blomia tropicalis*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Glycyphagus domesticus*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentia*); and Nematoda (*Anisakis simplex*).

Variables

The first variables taken into consideration were age and sex. Additionally, we classified reactivities based on the recognition of panallergens available for evaluation of IgE whose homologous components had already been demonstrated in insect meats (ie, tropomyosin, myosin light chain protein, arginine kinase, troponin C, and paramyosin). Finally, we assessed cross-reactivity in participants with reactivity to the insects examined and to molecules or extracts derived from other insects, nematodes, crustaceans, or arachnids.

Given the observational nature of the study, no randomization procedure was implemented during enrollment.

Data Sources/Measurement

Serum IgE reactivity was analyzed using the ALEX (Allergen Explorer) test, version 2 (ALEX^2, Macroarray Diagnostics), a CE-certified platform in which different allergens and extracts are spotted onto a nitrocellulose membrane in a cartridge chip. The insect extracts used were cricket, migratory locust, and mealworm.

The chip was incubated with 0.5 mL of a 1:5 dilution of the patient's serum containing a CCD inhibitor under shaking. After 2 hours of incubation, the chips were washed 3 times, and a pretitrated dilution of antihuman IgE labeled with alkaline phosphatase was added and incubated for 30 minutes. After another cycle of extensive washing, the enzyme substrate was added, and after 8 minutes, the reaction was stopped by the addition of 100 µL of ALEX Stop Solution. The membranes were dried, and the intensity of the color reaction for each allergen spot was measured using a charge-coupled device camera. The images were digitalized using the dedicated software, and a report listing the allergens and components and their score in kU_A/L was generated. Finally, systematic variations in signal levels between lots were normalized by heterologous calibration against an IgE reference curve. A curve fit was calculated, and the resulting equation was applied to transform arbitrary intensity units into quantitative units. Lot-specific calibration parameters were encoded in the barcode. The measurement range of ALEX-specific IgE is 0.3-50 kU_A/L, according to the manufacturer's guidelines.

The molecular content of the insect extracts under study was indirectly assessed in vitro using extracts of *B germanica* and *D pteronyssinus* (both obtained from ROXALL ARISTEGUI ITALIA) and ELISA inhibition tests using sera from 10 well-characterized participants allergic to *A domestica*, *L migratoria*, and *T molitor*.

The specific IgE levels in these sera were reevaluated using ALEX^2. In brief, 100 µL of each serum was incubated overnight with an equal volume of phosphate-buffered saline (control) or both *B germanica* and *D pteronyssinus*. The inhibited samples were then processed as previously described.

Bias

The diagnosis of food allergy was not confirmed through blinded or open oral food challenges.

Quantitative Variables

Quantitative measurements of specific IgE to the insects under study were compared by sex and age group.

Statistical Analysis

All data were analyzed using IBM SPSS Statistics for Windows, Version 29.0 (IBM Corp.). The TD-Synergy Laboratory Information System (Siemens Healthcare) was used to search for and collect demographic data (age and sex), clinical data, and laboratory data for participants who attended the outpatient allergy clinic and underwent specific IgE testing. In the univariate analysis, the Mann-Whitney test (2 groups) was first used to compare continuous IgE values in males, females, and individuals with and without clinical symptoms. Subsequently, each variable of interest was dichotomized as negative or positive to examine the proportion of participants with symptoms in the 2 resulting groups.

The Pearson χ^2 test or Fisher exact test (used for 2-by-2 contingency tables with fewer than 50 cases) was used to determine whether paired observations on 2 variables expressed in a contingency table were independent of each other.

We performed multiple logistic regression for the clinical variables with dichotomous scores (present, absent) to determine whether the association between clinical symptoms and reactivity to various allergens was present after simultaneously adjusting for the other variables of interest.

We produced Venn diagrams to provide a visual representation of the distribution of the different molecules in panallergen families (VennMaster 0.38) [19].

Ethical Issues

The research was conducted ethically following the World Medical Association Declaration of Helsinki. The study was approved by the Ethics Committee of IDI-IRCCS (IDI-IRCCS CE, 495-17). Data were collected anonymously, utilizing only information obtained from routine specialist surveys. Recruited participants provided their informed consent for the use of their clinical data in an anonymous format.

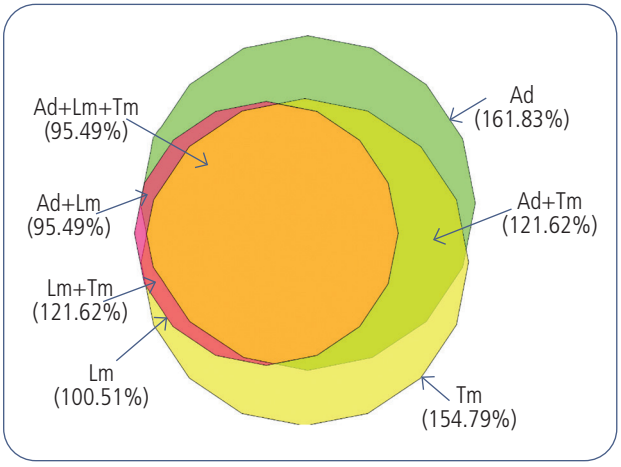

Figure 1. The Venn diagram illustrates the corecognition relationships of the 3 insect extracts examined, namely house crickets (*Acheta domesticus*, Ad = 161 [83%], pale green), migratory locusts (*Locusta migratoria*, Lm = 100 [51%], pale purple), and mealworms (*Tenebrio molitor*, Tm = 154 [79%], yellow). The Venn diagram also shows the number and proportion of the respective population in the case of multiple reactions to multiple insect species.

Results

We included 2014 consecutive individuals with various allergic conditions, including respiratory diseases, food-related allergies, and atopic dermatitis, who underwent proteomic evaluation using the ALEX^2 test. Of these, 195 were reactive to at least 1 of the extracts derived from house crickets (*A domesticus*, 161 [83%]), migratory locusts (*L migratoria*, 100 [51%]), or mealworms (*T molitor*, 154 [79%]).

The distribution of the study population is shown in Table 1.

The Venn diagram in Figure 1 illustrates the corecognition relationships of the 3 extracts. Forty-six per cent of participants reacted to all 3 extracts, 15% reacted only to *T molitor*, 3% were sensitized only to *L migratoria*, and 18% were sensitized to *A domesticus* in the absence of IgE-mediated recognition of the other 2 extracts.

Sex was associated with reactivity to insects, particularly *A domesticus* and *L migratoria*. Average values were notably higher for males, as was the frequency of positive responses (in females, 21.4% vs 14.4% for *A domesticus* and 14.4% vs 8.1% for *L migratoria*; $P<.01$).

Interestingly, none of the individuals who tested positive for insects had knowingly consumed edible insects in the past. While some had experienced allergic reactions to mollusks

Table 1. Distribution of the Study Population.[a]

			Acheta domesticus		*Locusta migratoria*		*Tenebrio molitor*
A	Mean (SD) age, y		35 (19)		34 (19)		35 (19)
	M/F		85/76		57/43		76/78
	sIgE		161/195 (83%)		100/195 (51%)		154/195 (79%)
B		Ad/Lm	95/100 (95%)	Lm/Ad	95/161 (59%)	Tm/Ad	121/161 (75.2%)
		Ad/Tm	121/154 (78.6%)	Lm/Tm	94/154 (61%)	Tm/Lm	94/100 (95%)
C	OAS		45/161 (28%)		30/100 (30%)		48/154 (31%)
	FA		27/161 (16%)		14/100 (14%)		25/154 (16%)
	AD		34/161 (21%)		22/100 (22%)		34/154 (22%)
	RS		83/161 (52%)		49/100 (49%)		87/154 (56%)
	BA		41/161 (25%)		23/100 (23%)		39/154 (25%)

Abbreviations: Ad, *Achetus domesticus*; AD, atopic dermatitis; BA, bronchial asthma; FA, food allergy; Lm, *Locusta migratoria*; OAS, oral allergy syndrome; RS, rhinitis; Tm, *Tenebrio molitor*.

[a]A, Distribution of age, sex, and the prevalence of IgE-mediated reactivity to *Acheta domesticus*, *Locusta migratoria*, and *Tenebrio molitor*. B, Cosensitization to multiple species is indicated with absolute number and percentage of reactivity within each individual insect species combination. C, Prevalence of clinical symptoms reported in the medical history of patients in the 3 subsets.

(21.3% and 3.2% reported moderate and severe reactions, respectively) or crustaceans (9.6% and 3.2% reported moderate and severe reactions, respectively), 41% of the tested individuals had not reported any adverse reactions after consuming these foods.

Additional investigations into the reactivity profiles of arthropod-derived molecules showed that approximately 40% to 60% of the population demonstrated sensitivity to allergenic components found in crustaceans, mollusks, and nematodes. These components included tropomyosin, arginine kinase, myosin light chain protein, troponin C, and sarcoplasmic calcium-binding protein (Table 2).

When we further examined the participants who reacted to tropomyosin (104 individuals, 52 females) or arginine kinase (68 participants, 39 females), only a small percentage also reacted to insect extracts, with significant variability in molecular recognition from patient to patient (Table 3). Generally, the fraction of individuals sensitized to insects was higher among tropomyosin reactors (48%-88%) than among arginine kinase reactors, where those positive to arginine kinase were also reactive to insects in 40%-53% of cases.

Remarkably, more than half of the insect-reactive individuals (108 [55.4%]) displayed no sensitization to any of the molecules included in the panel tested (Table 2).

We further examined this subset of participants by analyzing their recognition profile for molecular components and extracts from other invertebrates assessed in the test, including Astigmata (mite), Blattoidea, Crustacea, Mollusca, and Hymenoptera.

Participants who tested negative for tropomyosin, arginine kinase, myosin light chain protein, troponin C, sarcoplasmic calcium-binding protein, and paramyosin reacted to mite-derived molecules in more than 50% of cases, and 20%-30% were cosensitized to Hymenoptera. Interestingly, about 30% of individuals reactive to cricket and mealworm and less than 20% of those reactive to locusts were not cosensitized to any of the extracts or molecules from mites, cockroaches, or other invertebrates. As a result, 33 participants (16.9%) were

Table 2. Participants With IgE-Mediated Reactivity to Panallergens.[a]

	Acheta domesticus (n=161)	*Locusta migratoria* (n=100)	*Tenebrio molitor* (n=154)	Overall (N=195)
Arginine kinase	30 (19%)	26 (26%)	31 (20%)	36 (18%)
Tropomyosin	49 (30%)	46 (46%)	63 (41%)	67 (34%)
Myosin light chain	8 (5%)	7 (7%)	7 (5%)	9 (5%)
Sarcoplasmic CBP	3 (2%)	3 (3%)	3 (2%)	3 (2%)
Troponin C	13 (8%)	10 (10%)	12 (8%)	13 (7%)
Paramyosin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
None	95 (59%)	40 (40%)	76 (49%)	108 (55.5%)

Abbreviation: CPB, calcium-binding protein.

[a]"None" indicates that a participant did not have IgE-mediated reactivity to any of the panallergens present in the chip. Reactivity to arginine kinase indicates the presence of specific IgE antibodies targeting at least 1 of the arginine kinases present in the array (Pen m 2, Bla g 9, or Der p 20). Similarly, for tropomyosin, the presence of IgE antibodies against at least 1 of Ani s 3, Blo t 10, Der p 10, Pen m 1, and Per a 7 is considered reactive. Pen m 3 has been considered a marker of reactivity to myosin light chain, Pen m 4 to sarcoplasmic CBP, Cra c 6 to troponin C, and Der p 11 to paramyosin.

Table 3. Reactivity to the Individual Tropomyosin and Arginine Kinase Molecules Present in the Chip.[a]

	Acheta domesticus		*Locusta migratoria*		*Tenebrio molitor*	
Tropomyosin						
Ani s 3 (N=63)	41	65.1%	42	66.7%	52	82.5%
Blo t 10 (N=67)	42	62.7%	43	64.2%	53	79.1%
Der p 10 (N=81)	40	49.4%	39	48.1%	52	64.2%
Pen m 1 (N=50)	36	72.0%	38	76.0%	44	88.0%
Per a 7 (N=58)	38	65.5%	41	70.7%	51	87.9%
Arginine kinase						
Bla g 9 (N=52)	24	46.2%	22	42.3%	26	50.0%
Der p 20 (N=57)	25	43.9%	23	40.4%	25	43.9%
Pen m 2 (N=43)	22	51.2%	20	46.5%	23	53.5%

[a]Only the subset of participants with IgE-mediated reactivity to tropomyosin or arginine kinase were considered. The percentages are calculated as a proportion of each insect allergy within each component of the tropomyosin or arginine kinase panallergens (the number of positive patients is shown in parenthesis).

sensitized only to the 3 categories of insects evaluated in this study (Figure 2).

The IgE-mediated reactivity to arachnids was inversely associated with IgE recognition for at least 1 insect among the 3 examined (OR, 0.54; 95%CI, 0.3-0.8; *P*=.001). Conversely, recognition of extracts or molecules from mollusks, crustaceans, cockroaches, and Hymenoptera was consistently and directly associated with reactivity to crickets, locusts, and mealworms (Table 4, left-hand side).

Using multiple logistic regression analysis, we included all examined allergenic extracts or molecules, along with age and sex, in the model. The results confirmed an inverse relationship between reactivity to Astigmata (mite) and sensitization to *A domesticus* or *T molitor*. Moreover, even after adjusting for sex and age, a direct association persisted for Mollusca, Blattoidea, and tropomyosin. Additionally, myosin light chain protein was directly associated with *A domesticus* and *L migratoria*, tropomyosin C with *A domesticus* and *T molitor*, and Hymenoptera with *L migratoria* (Table 4, right-hand side).

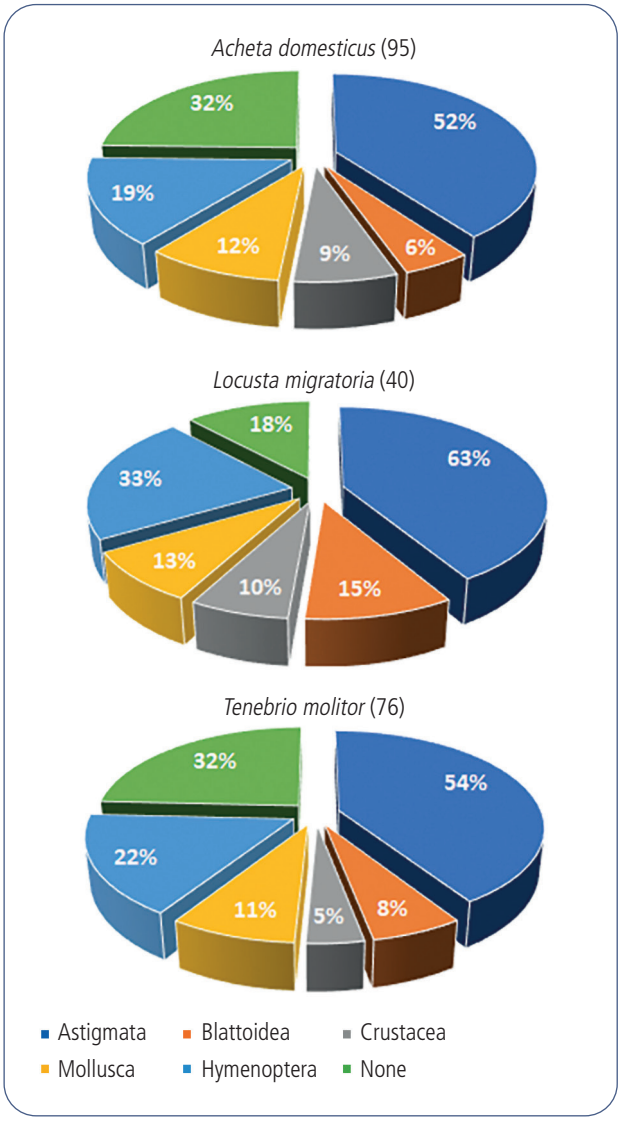

Figure 2. The figure shows the prevalence of corecognition of molecules or extracts derived from other invertebrates in individuals characterized by IgE-mediated reactivity to insects but not to tropomyosin, arginine kinase, myosin light chain, troponin C, sarcoplasmic calcium-binding protein, and paramyosin. The label 'none' indicates the fraction of participants reactive only to *Acheta domesticus*, *Locusta migratoria*, or *Tenebrio molitor*.

Inhibition experiments carried out with 10 selected sera adsorbed with cockroach and *D pteronyssinus* extracts showed that the mite extract was able to completely inhibit IgE-mediated reactivity to insects in 6 cases and nearly completely (with a single exception) in the remaining samples (Supplementary Table). The *B germanica* extract was less efficacious than mites, especially in the case of *T molitor*.

However, in the 3 participants who did not react to any of the panallergens present in the array, the signal was abolished entirely, as observed for *D pteronyssinus*. Interestingly, focusing on the 5 samples that were reactive to tropomyosin and arginine kinase, once again, the mite extract demonstrated a greater inhibitory capacity for tropomyosin than for the cockroach extract. The *B germanica* extract inhibited the signal of Ani s 3 from *Anisakis* in 4 out of 5 samples but did not effectively inhibit the other molecules. In the case of arginine kinase, only Der p 20 was effectively inhibited in 1 instance, while in all the other experiments, very limited inhibition of the signal was recorded for both mite and cockroach extract.

Discussion

A growing number of reports have recently discussed the negative effects caused by consuming or merely coming into contact with edible insects [7,12]. A study focusing on the Chinese scientific literature between 1980 and 2007 revealed that reactions were severe (eg, anaphylaxis) in 16% of cases after exposure to grasshoppers, locusts, or larvae [13]. While this matter is currently of limited significance for the European population, it is reasonable to consider that it might become more important and urgent, particularly considering recent decisions that allow the use of insect-derived proteins as food sources within the European Union [4].

In our research, we observed sensitization to extracts of edible insects, with a higher prevalence among males. The origin of this sensitization, in the absence of prior exposure, might be attributed to the inadvertent ingestion of insect proteins found in various foods [5,20]. Furthermore, the inverse correlation between sensitization to mites (both extracts and specific molecules) and sensitization to certain edible insects (*A domesticus* and *L migratoria*) suggests that mites are unlikely to be the primary sensitizers in these cases.

In contrast, the positive correlation between insect sensitization and the IgE-mediated recognition of molecular components from mollusks, cockroaches, and even Hymenoptera (in the case of reactivity to locusts) suggests that these allergenic sources may share common molecular elements with the insects studied in our investigation. In this context, potentially allergenic proteins present in *T molitor* were already assessed in 2 separate groups: one comprising individuals allergic to crustaceans, and the other consisting of a cohort allergic to dust mites [18]. The authors not only confirmed the

presence of known proteins such as tropomyosin or arginine kinase, but also identified other specific insect proteins, such as apolipoprotein-III, larval cuticular protein, and the 12-kDa hemolymph protein. Additionally, a bioinformatic and proteomic analysis by the same group enabled characterization of further potential allergens in insects. Some of these allergens are highly prevalent in mollusks and are shared with Blattoidea, such as cockroach allergen–like protein [8,15].

Table 4. Associations Between Insect Reactivity and Corecognition of Molecules, Including Both Species-Specific and Panallergenic Compounds From Other Invertebrates.

	Acheta domestica					
	ORc	**95%CI**	***P* Value**	**OR_{adj}**	**95%CI**	***P* Value**
Arachnida	0.540	(0.4-0.8)	.001	0.328	(0.2-0.5)	.000
Mollusca	5.057	(3.3-7.8)	.000	1.810	(1.0-3.1)	.034
Crustacea	5.019	(3.4-7.3)	.000			
Blattodea	6.549	(4.3-9.9)	.000	2.533	(1.3-4.9)	.006
Hymenoptera	2.419	(1.6-3.6)	.000			
Tropomyosin	15.429	(8.1-29.4)	.000	6.713	(2.8-15.9)	.000
AK	5.600	(3.0-10.5)	.000			
MLC	9.935	(3.0-33.4)	.000	5.696	(1.4-22.5)	.013
SCB	7.234	(1.2-43.6)	.012			
TnC	13.334	(4.7-38.0)	.000	7.897	(2.2-26.9)	.001
	Locusta Migratoria					
	ORc	**95%CI**	***P* Value**	**OR_{adj}**	**95%CI**	***P* Value**
Arachnida	1.343	(0.8-2.3)	.289			
Mollusca	6.867	(4.3-11.1)	.000	2.107	(1.0-4.2)	.036
Crustacea	10.632	(6.8-16.7)	.000			
Blattodea	16.703	(10.4-26.8)	.000	6.016	(3.2-11.2)	.000
Hymenoptera	3.514	(2.3-5.5)	.000	2.394	(1.3-4.2)	.003
Tropomyosin	41.524	(20.7-83.5)	.000	7.001	(2.9-17.0)	.001
AK	8.717	(4.6-16.6)	.000			
MLC	12.344	(3.8-39.7)	.000	10.810	(2.4-49.2)	.002
SCB	12.727	(2.1-77.1)	.000			
TnC	11.347	(4.4-29.5)	.000			
	Tenebrio molitor					
	ORc	**95%CI**	***P* Value**	**OR_{adj}**	**95%CI**	***P* Value**
Arachnida	0.781	(0.5-1.2)	.231	0.430	(0.3-0.7)	.000
Mollusca	5.718	(3.7-8.9)	.000	1.879	(1.0-3.4)	.035
Crustacea	6.392	(4.3-9.4)	.000			
Blattodea	13.575	(8.8-20.9)	.000	6.249	(3.6-10.7)	.000
Hymenoptera	2.218	(1.5-3.3)	.000			
Tropomyosin	51.000	(21.2-122.5)	.000	12.308	(4.5-33.5)	.000
AK	6.593	(3.5-12.3)	.000			
MLC	7.295	(2.3-23.3)	.000			
SCB	7.639	(1.3-46.1)	.009			
TnC	10.775	(4.0-29.2)	.000	5.882	(1.6-21.3)	.007

Abbreviations: AK, arginine kinase; MLC, myosin light chain protein; SCB, sarcoplasmis calcium-binding protein; TnC, tropomysin C.
Left, crude OR (ORc).
Right, adjusted OR (ORadj) after adjustment for age and sex after multiple logistic regression analysis. The missing data for ORadj indicates the lack of statistical significance.

Of note, only a subset of participants (40%-60%, depending on the specific insect under consideration) were cosensitized to molecules that are already known and can be tested in vitro, such as tropomyosin, arginine kinase, troponin C, myosin light chain protein, and sarcoplasmic calcium-binding protein. Consequently, certain participants reacted to insect extracts, indicating their ability to recognize molecular components that are currently not detectable in vitro with existing resources. This highlights the continued significance of allergenic extracts in modern allergy diagnostics. Despite advancements in knowledge and the availability of molecules for in vitro evaluation of allergic participants, the use of allergenic extracts is still relevant and far from outdated. When dealing with new sources of sensitization, the initial step to identify and subsequently study or classify participants is based on allergenic extracts.

Tropomyosin consistently exhibited a positive association with reactivity to *A domesticus*, *L migratoria*, and *T molitor*. Importantly, not all patients who reacted to tropomyosins were simultaneously positive to insect extracts, and sometimes they were positive to certain insects but not to others, indicating significant variability in antigen recognition. However, it should be noted that cross-reactivity for some edible insect species can vary [21]. Regarding *T molitor*, our findings revealed a significant association with troponin C, consistent with previous literature [8]. Limited cross-reactivity between *T molitor* and *A domesticus* arginine kinase was also evident [22]. Nevertheless, it is crucial to avoid solely relying on structural identity to predict cross-reactivity, as confirmation through further tests, including food challenges, is essential [17].

Inhibition experiments indicate that both mites and cockroach contain molecules able to elicit an IgE-mediated reaction against insects, albeit with different efficiencies. Considering the molecular component of each extract, tropomyosin, and to a lesser extent arginine kinase, might be considered common mite allergens in sensitization to insects, while cockroach contains a larger fraction of unidentified constituents.

The clinical significance of these sensitizations should also be considered. Less than 50% of cases had previously experienced reactions, mostly related to mollusks, with crustaceans being involved in less than 10% of the patients examined. A study involving 15 shrimp-allergic participants demonstrated that those who were cosensitized to tropomyosin or arginine kinase reacted to double-blind placebo-controlled food challenges with *T molitor*, leading to moderate-to-severe symptoms [23]. Generally, individuals with allergies to crustaceans and/or mollusks should be made aware of the possibility of cross-reaction with edible insects [23,24]. Similarly, participants who are allergic to dust mites and keep reptile pets at home should also exercise caution, as these animals are often fed live insects such as grasshoppers, crickets, and locusts, [17].

It is important to emphasize the added value of using a technology that involves chelation (albeit imperfect) of the signal generated by CCD recognition, as many arthropod allergens are glycosylated, with the result that the presence of IgE against CCDs can be a nonsignificant cause of cross-reactivity.

Limitations

The clinical significance of the insect-specific sensitization patterns in patient data was not evaluated via food challenges. This selective approach might not have impacted the subset of participants who consistently tolerate foods containing panallergens, such as tropomyosin or arginine kinase.

Generalizability

The study population is from a temperate, western country. Therefore, our observations should be understood as applicable to the climate, dietary habits, and food choices of this population and may not necessarily be equally applicable to other contexts and more diverse settings.

Conclusion

The findings presented in this study raise significant questions about the implications of sensitization to edible insects. Although the exact cause of sensitization remains uncertain, it is plausible to consider that inadvertent ingestion of insect proteins may be a contributing factor. The possibility of inhalant dust mite allergy being linked to the subsequent development of tropomyosin-mediated food allergy, as observed in pollen fruit syndrome, has been a subject of debate for years [24]. The shared molecular recognition between insects and specific allergenic sources highlights the need for further investigation to understand potential cross-reactivity. Additionally, the growing interest in edible insects as a sustainable protein source demands a thorough assessment of the risks associated with their allergenicity to safeguard public health [2,4,6,25]. It is worth noting that food processing methods, such as enzymatic hydrolysis and high-temperature treatments, can modify the allergenicity of insect proteins, potentially reducing their allergenic potential [17]. As this issue gains prominence, future research and regulatory efforts should focus on establishing standardized testing methods to identify allergenic components in edible insects [4]. Proper labeling and consumer awareness campaigns can also play a crucial role in mitigating potential risks for sensitized individuals.

In conclusion, sensitization to edible insects poses a potential health concern that warrants further exploration. While the current prevalence is low, the increasing acceptance of insects as food sources necessitates proactive measures to address the allergenicity associated with these novel dietary choices.

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Conflicts of Interest

Enrico Scala has received fees for consultancy and participation in speakers' bureaus from Stallergenes and Thermo Fisher Scientific. The remaining authors declare that they have no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available for reasons of privacy or ethical restrictions.

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