

Occupational sensitivity to *Tenebrio molitor* Linnaeus (yellow mealworm)

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Tenebrio molitor is an abundant stored-grain pest in the northern United States. We evaluated an individual with work-related symptoms of rhinoconjunctivitis on exposure to this insect. Prick skin tests with extracts prepared from the larval, pupal, and adult-life stages were positive for the patient and for another individual with allergy to a closely related species of beetle, *Alphitobius diaperinus*. Specific IgE antibodies to the extracts were demonstrated by RAST. RAST inhibition demonstrated immunologic cross-reactivity between the life stages of *T. molitor* and also between *T. molitor* and *A. diaperinus*, as well as slight cross-reactivity with blowfly. The proteins in the extracts of each life stage were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. More than 15 protein bands were detected in each of the extracts, although the patterns of separation were different for each life stage. After immunoblotting and autoradiography, six different IgE-binding proteins were identified in the larval extract, five in the pupal extract, and seven in the adult extract, with similar IgE-binding patterns noted for the larval and adult extracts. We conclude that this patient developed IgE-mediated sensitivity to *T. molitor* antigens as the result of occupational exposure. This study confirms the fact that beetles of the Tenebrionid family are potentially significant allergens for workers exposed to grains or grain products. (J ALLERGY CLIN IMMUNOL 1990;86:182-8.)

Occupational exposure to insects is a frequent cause of allergic disease. Laboratory workers are at particular risk for sensitization to insects; in one survey, approximately 60% of laboratories in which insects were raised had at least one worker with occupational allergy caused by insect exposure.¹ Workers in commercial industries, particularly grain handlers,^{2,3} also have been reported to develop occupational allergies to insects. Bait handlers also are at risk; allergies have been reported to yellow mealworm larvae (*Tenebrio molitor*)⁴ and to bee moth larvae (*Galleria mellonella*).⁵

In this article, we describe an additional subject with occupational allergy to *T. molitor*. With immu-

Abbreviations used

<i>T. molitor</i> :	<i>Tenebrio molitor</i>
<i>A. diaperinus</i> :	<i>Alphitobius diaperinus</i>
PBS:	Phosphate-buffered saline
BSA:	Bovine serum albumin
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TBS:	Tris-buffered saline

noblotting techniques, we have studied the allergens involved in this patient's immunologic response to *T. molitor*. Furthermore, we have also evaluated the cross-reactivity of this insect with other insect species.

CASE HISTORY

The patient was a 26-year-old woman who worked as an animal handler at a local high school. Her job included feeding the animals *T. molitor* beetles that were raised in wheat bran at the center. Before starting this job, she had no symptoms suggestive of allergy. After working at the school for a few years, she developed intermittent rhinoconjunctivitis; symptoms gradually worsened to the point at which they were continuous, with no improvement at night or on weekends. Symptoms would worsen, however, for about 1 hour after each exposure to *T. molitor*. No similar effect on her symptoms was noted when the animals them-

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selves or other foodstuffs were handled. Physical examination was remarkable only for moderate nasal congestion. Skin tests performed for 19 common aeroallergens by the prick method were all negative.

Crude extracts of each of the three developmental stages of *T. molitor* were prepared by macerating several samples of each life stage in saline. Extracts were also prepared from fresh wheat bran and bran in which the insects had been raised for different lengths of time. The patient was prick skin tested with these extracts (Table I). She had positive skin tests with each life stage of *T. molitor*. Fresh bran did not cause a reaction, whereas there was a progressively larger response to bran the longer that the beetles were kept in the bran, indicating that allergens from the beetles were likely shed into the grain over time.

MATERIAL AND METHODS

Preparation of *T. molitor* extracts

Samples of each developmental stage were killed by freezing, macerated, and then defatted by soaking in acetone for 18 hours at room temperature. After siphoning the acetone, the samples were rinsed with acetone and allowed to soak in acetone for 1 additional hour. After once again siphoning, the samples were air-dried for 24 hours. Each sample was then placed in PBS, pH 7.4, at a concentration of 1:10 wt:vol. After overnight incubation with constant stirring at 4° C, the samples were centrifuged at 900 g for 15 minutes. The supernatants were filtered through a sterilizing Millipore filter (Millipore Corp., Bedford, Mass.) and then stored at -20° C until use. Protein concentrations of the extracts determined according to the method of Bradford⁶ with a commercial kit (Bio-Rad Laboratories, Richmond, Calif.) were larvae, 9.0 mg/ml; pupae, 8.3 mg/ml; and adult, 10.1 mg/ml.

Skin test materials

Serial tenfold dilutions of each of the extracts were prepared by diluting the stock extracts with PBS. These were used to prick skin test three individuals for comparison with the patient's responses.

Direct RAST measurement. Extracts from the three life stages were coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Piscataway, N.J.) at a concentration of 3 mg of protein per milliliter of swollen gel, with methods previously described.⁷ Coupling efficiency was 50% for the larval extract, 57% for the pupal extract, and 64% for the adult extract.

The direct RAST was performed by incubating 0.5 ml of 3% sorbent with 0.1 ml of serum diluted 1:5 in RAST buffer (0.05 mol/L of Na₂HPO₄, 0.004 mol/L of NaH₂PO₄, 0.2% of BSA, 0.02% of NaN₃ [wt/vol], 0.5 mol/L of NaCl, and 0.5% of Tween [wt/vol], pH 7.5) with constant rotation at room temperature for 18 hours. After three washes with RAST buffer, Phadebas ¹²⁵I-labeled antihuman IgE (Pharmacia), 20 to 30,000 cpm, was added to each tube. The tubes were rotated overnight at room temperature. After three additional washes, the bound radioactivity was determined on an automatic gamma counter (model 1197, Searle Analytic Inc., Des Plaines, Ill.). All assays were performed

TABLE I. Skin test results to crude extracts of *T. molitor* and wheat bran in an exposed individual

Extract	Skin test reactivity
Larva	3+
Pupa	3+
Adult	3+
Fresh wheat bran	—
2-week-old bran	2+
4-week-old bran	3+

—, Negative; +, <3 mm (average wheal diameter); 2+, 3 to 5 mm; 3+, >5 to 8 mm; 4+, >8 mm.

in duplicate. The average background count was subtracted from each result before data analysis. Results of the RAST assay are expressed as the ratio of radioactive binding in the serum sample to that in a negative control sample (3% sorbent plus RAST buffer; no serum added). A ratio of 2:1 or more is considered significant.⁸

RAST-inhibition measurements. To determine the specificity of IgE binding in the direct RAST, RAST inhibition was conducted according to the method described by Gleich et al.⁹ Equal volumes of sera from two insect-sensitive individuals were pooled and diluted 1:5 in RAST buffer. Diluted sera (0.1 ml), 0.5 ml of 3% sorbent, and varying quantities of the following inhibitors were allowed to react with constant rotation at room temperature for 18 hours: (1) *T. molitor* larvae, 1.0 mg/ml (protein concentration); (2) *T. molitor* pupae, 1.0 mg/ml; (3) *T. molitor* adult, 1.0 mg/ml; (4) *A. diaperinus* larvae, 1.0 mg/ml; (5) *Alphitobius diaperinus* pupae, 1.0 mg/ml; (6) *A. diaperinus* adult, 1.0 mg/ml; (7) blowfly, 1.0 mg/ml; (8) house dust mite (*Dermatophagoides farinae*), 5000 PNU/ml (Greer Laboratories, Lenoir, N.C.); and (9) wheat, 1:20 wt:vol (Greer). The remainder of the procedure was the same as for the direct RAST. The amount of radioactivity bound to the Sepharose particles was compared to amount of a control to which inhibitor had not been added. In all tests, the amount of nonspecific binding of radioactivity was taken to be the amount bound by a blank sample (3% sorbent plus RAST buffer; no serum added). Percentage inhibition was calculated as follows:¹⁰

$$\% \text{ Inhibition} = 100 - \frac{\left(\begin{array}{c} \text{cpm bound in the} \\ \text{presence of inhibitor} \end{array} - \begin{array}{c} \text{cpm bound} \\ \text{nonspecifically} \end{array} \right)}{\left(\begin{array}{c} \text{cpm bound in the} \\ \text{absence of inhibitor} \end{array} - \begin{array}{c} \text{cpm bound} \\ \text{nonspecifically} \end{array} \right)} \times 100$$

SDS-PAGE/immunoblotting. Immunoblotting was performed with methods similar to methods previously described.¹¹ Proteins in the larval, pupal, and adult extracts of *T. molitor* were first separated by SDS-PAGE with a 3.9% polyacrylamide stacking gel and a 12.5% resolving gel. The fractions were treated at 100° C for 2 minutes in the presence of SDS and 2-mercaptoethanol. Twenty-five micrograms of

TABLE II. Skin test results to *T. molitor* (nonexposed control patients)

Developmental stage	Dilution (wt : vol)	Skin test reactivity		
		<i>A. diaperinus</i> -sensitive individual	Atopic individual	Normal individual
Larva	1:10	4+	+	+
	1:100	3+	—	—
	1:1,000	2+	—	—
	1:10,000	+	—	—
Pupa	1:10	4+	+	+
	1:100	3+	—	—
	1:1,000	3+	—	—
	1:10,000	+	—	—
Adult	1:10	4+	+	+
	1:100	2+	—	—
	1:1,000	2+	—	—
	1:10,000	2+	—	—

—, Negative; +, <3 mm (average wheal diameter); 2+, 3 to 5 mm; 3+, >5 to 8 mm; 4+, >8 mm.

TABLE III. Direct RAST results to *T. molitor*

Life stage	<i>T. molitor</i> -sensitive individual	<i>A. diaperinus</i> -sensitive individual	Ratio: $\frac{\text{CPM patient}}{\text{CPM control}}$
			Nonexposed† control subjects (n = 4)
Larva	3.85	4.55	1.28 ± 0.31* (0.97 – 1.61)
Pupa	5.86	4.96	1.02 ± 0.25 (0.75 – 1.35)
Adult	5.39	5.19	1.31 ± 0.37 (0.97 – 1.77)

CPM, Counts per minute.

*Mean ± standard deviation; range in parentheses.

protein was applied to each lane of the gel. Electrophoresis was performed at 30 mA per gel with constant current until the bromphenol blue tracking dye was about 1 cm from the bottom of the gel. A portion of the gel was sliced and stained with Coomassie brilliant blue R-250, and the remaining portion was used for the immunoblotting experiments.

Proteins were transferred electrophoretically from the SDS gel to nitrocellulose. IgE binding to the transferred proteins was detected as follows: Unoccupied sites of the nitrocellulose were blocked by gently shaking the nitrocellulose with 3% BSA in 10 mmol/L of Tris HCl, 0.9% NaCl, pH 7.4 (TBS), plus 0.02% Na₂S₂O₃ for 1 hour at 40° C. The nitrocellulose was cut into strips corresponding to the sample lanes of the original gel and transferred to incubation trays. Strips were incubated overnight in 5 ml of a solution of the patient's serum diluted 1:5 in TBS plus BSA plus Na₂S₂O₃. Control strips without antigen were blocked with BSA and incubated with TBS plus BSA plus Na₂S₂O₃ only. All strips

were washed with TBS for 10 minutes, then twice for 10 minutes with TBS in 0.5% Nonidet P40 (Sigma Chemical Co., St. Louis, Mo.), and once more with TBS. They were then incubated overnight with ¹²⁵I-labeled antihuman IgE (Pharmacia). After a final set of washes, the strips were wrapped in plastic film and placed on XAR-5 x-ray film (Eastman Kodak, Rochester, N.Y.) for autoradiography at –70° C for 1 to 3 days.

RESULTS

Skin tests

Three individuals were prick skin tested with non-glycerinated tenfold dilutions of each life stage (Table II). One of the individuals had previously reported sensitivity to *A. diaperinus*,¹² a beetle that is closely related to *T. molitor*. Although this person had no prior exposure to *T. molitor*, he had significant skin

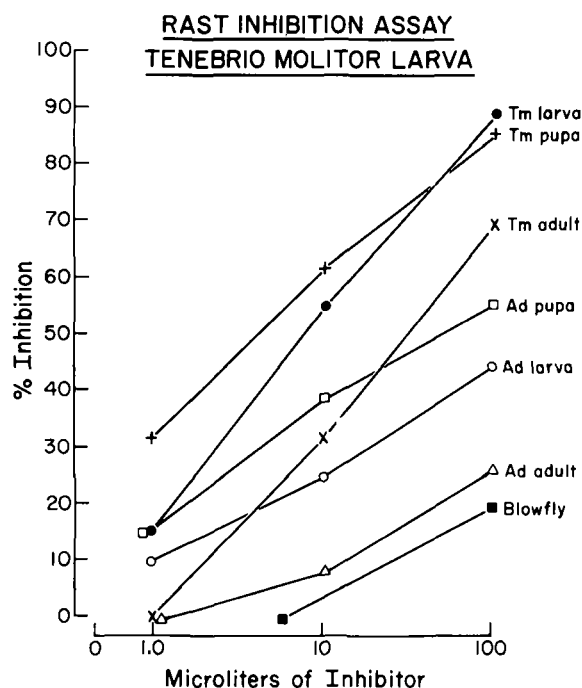


FIG. 1. RAST inhibition, *T. molitor* larva. Data plotted as percent inhibition versus amount of inhibitor added per tube; Tm, *T. molitor*; Ad, *A. diaperinus*. Protein concentration of inhibitors, 1.0 mg/ml.

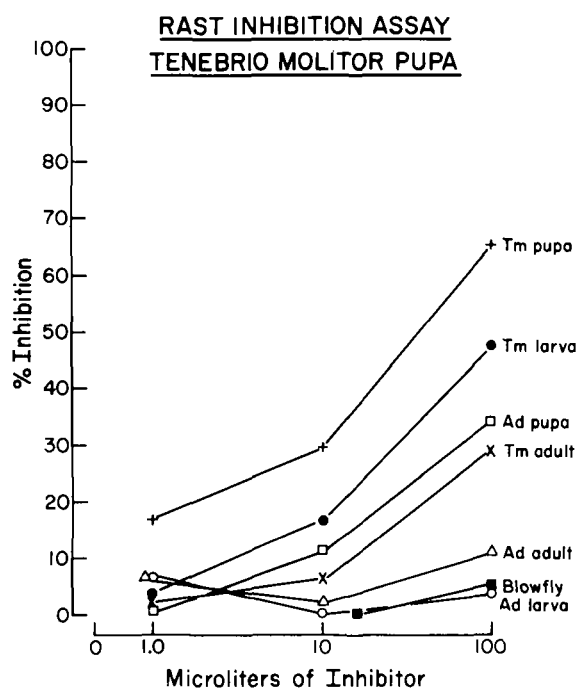


FIG. 2. RAST inhibition, *T. molitor* pupa. Data plotted as percent inhibition versus amount of inhibitor added per tube; Tm, *T. molitor*; Ad, *A. diaperinus*. Protein concentration of inhibitors, 1.0 mg/ml.

test responses to each developmental stage. The other two individuals were nonexposed atopic and normal control subjects, respectively. Both individuals had slight responses to the stock 1:10 wt:vol extracts but not to more dilute solutions, thus indicating a slight nonspecific irritant effect to the extracts at the highest protein concentrations only.

Direct RAST/RAST inhibition

RAST assays were performed with serum from the patient and also from the *A. diaperinus*-sensitive individual because of his skin test response to the *T. molitor* extracts. Results are summarized in Table III. Both insect-sensitive individuals had positive results for all three life stages of *T. molitor*. In contrast, four nonexposed control subjects had insignificant binding for all three extracts.

The results of the RAST-inhibition assays are illustrated in Figs. 1 to 3. When *T. molitor* was used as the solid-phase antigen, the RAST was significantly inhibited by *T. molitor* larva, as well as by the pupal and adult extracts (Fig. 1). Furthermore, the three life stages of *A. diaperinus* also inhibited this RAST, but generally to a lesser extent than did *T. molitor*. There was also slight inhibition with blowfly as inhibitor.

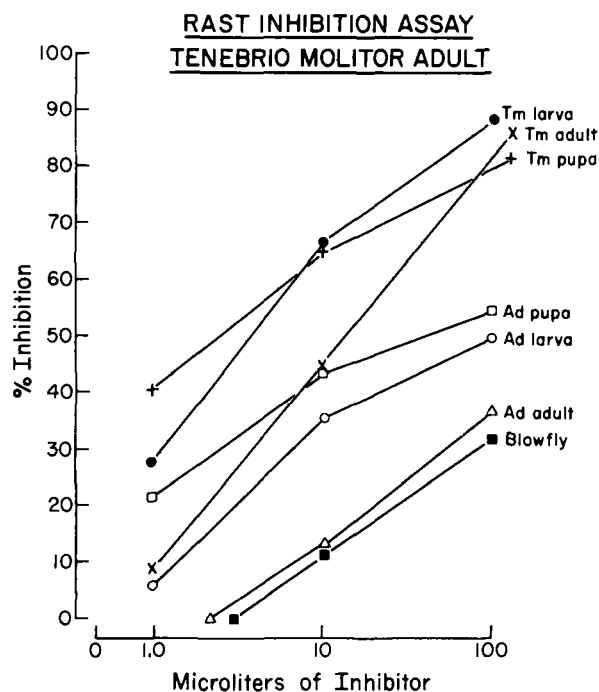


FIG. 3. RAST inhibition, *T. molitor* adult. Data plotted as percent inhibition versus amount of inhibitor added; Tm, *T. molitor*; Ad, *A. diaperinus*. Protein concentration of inhibitors, 1.0 mg/ml.

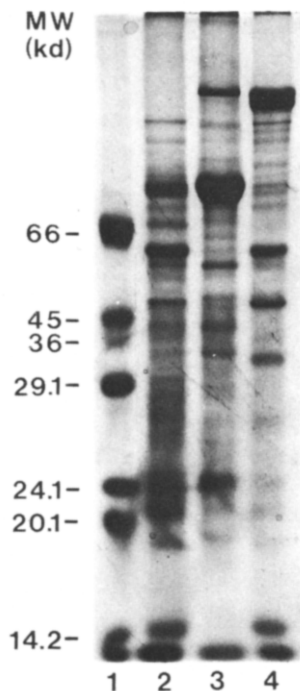


FIG. 4. SDS-PAGE of *T. molitor* extracts, 25 μ g per lane, stained with Coomassie brilliant blue. Lane 1, standard molecular weight markers; lane 2, larval extract; lane 3, pupal extract; lane 4, adult extract. Molecular weight (MW) expressed in kilodaltons.

In the *T. molitor* pupal RAST inhibition (Fig. 2), there was significant inhibition produced by the three life stages of *T. molitor* and also by *A. diaperinus* pupa. However, *A. diaperinus* larval and adult extracts and blowfly did not significantly inhibit this RAST.

The *T. molitor* adult RAST was inhibited by the three life stages of *T. molitor* and *A. diaperinus*, as well as by blowfly (Fig. 3). Neither house dust mite nor wheat caused significant inhibition for any of the three RAST-inhibition assays (data not presented).

SDS-PAGE/immunoblotting

The protein separation pattern is illustrated in Fig. 4 of the larval, pupal, and adult *T. molitor* extracts after staining with Coomassie brilliant blue. More than 15 protein bands are visible in each of the extracts. The pattern of protein staining observed, however, was different for each of the three life stages.

The autoradiograph obtained by incubating sera from each of the two insect-sensitive individuals with nitrocellulose strips containing larval, pupal, and adult *T. molitor* proteins is illustrated in Fig. 5. The IgE-binding patterns to the larval extract for the two insect-sensitive individuals were nearly identical; the finding is also true for the adult extract. Furthermore, there are similarities between the two extracts in that

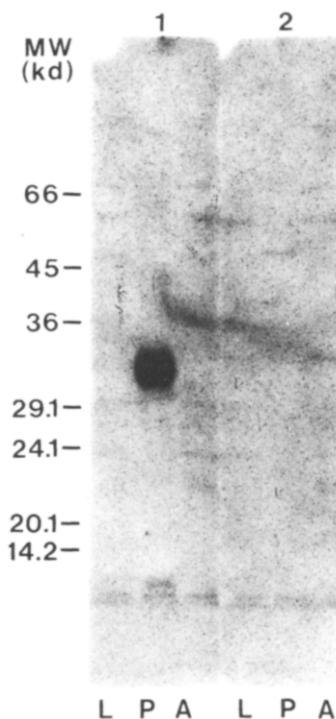


FIG. 5. Autoradiograph of *T. molitor* extracts after immunoblotting with insect-sensitive subject's sera. Column 1, *T. molitor*-sensitive individual's serum; column 2, *A. diaperinus*-sensitive individual's sera. L, larval extract; P, pupal extract; A, adult extract. Molecular weight (MW) of IgE-binding antigens in kilodaltons.

there are three IgE-binding proteins with molecular weights of approximately 67, 61, and 22 kD that were identified in both extracts. With the pupal extract, the insect-sensitive individuals had IgE binding to proteins of different molecular weights (approximately 31 and 52 kD, respectively), neither of which were identified in the larval or adult extracts. IgE binding to two or three proteins with molecular weights of <15 kD was observed for each of the three extracts; however, it is not clear if these are actually identical since they may not have been fully separated on the SDS-polyacrylamide gel. The sera from our study patient who worked directly with *T. molitor* demonstrated dense IgE binding to proteins at 32 kD in the pupal extract, which suggests this was the principal allergen to which she was sensitized. The other insect-sensitive individual who was not exposed to *T. molitor* demonstrated only faint IgE binding to proteins of this molecular weight.

DISCUSSION

T. molitor is a member of the insect order Coleoptera, which includes beetles and weevils. Occupational allergies to beetles were first described in a

TABLE IV. Partial taxonomy of the arthropods

Phylum	Arthropods				
Class	Insects			Arachnids	
Order	Diptera	Coleoptera			Acarina
Family	Calliphoridae	Tenebrionidae			Pyroglyphidae
Genus	<i>Phormia</i>	<i>Alphitobius</i>	<i>Tribolium</i>	<i>Tenebrio</i>	<i>Dermatophagoides</i>
Species	<i>regina</i> <i>meiger</i>	<i>diaperinus</i>	<i>confusum</i>	<i>molitor</i>	<i>farinae</i>
Common name	Blowfly	Lesser mealworm	Confused flour beetle	Yellow mealworm	House dust mite

museum curator exposed to dermestid beetle larvae.¹³ Since that study, there have been many other studies of occupational allergy attributed to other Coleopterans.^{2, 3, 12, 14, 15} Bernstein et al.⁴ previously evaluated four individuals with clinical sensitivity and positive skin tests to the larvae of *T. molitor*.

The patient reported in this study was exposed not only to *T. molitor* larvae but also to the pupal and adult stages of the beetle. Clinically, she experienced only rhinoconjunctivitis. The patients in the study by Bernstein et al.⁴ also had rhinoconjunctivitis; in addition, two had asthma and one individual had urticaria. Our patient had positive skin tests to all three developmental stages of *T. molitor*. In addition, the presence of specific IgE antibodies to the life stages was demonstrated by RAST and immunoblotting studies. There appear to be similar IgE-binding proteins in the larval and adult forms of *T. molitor*, as observed in the autoradiographs, whereas the pupal proteins appear to be more unique. This may be due to the fact that the proteins that are likely allergens change in the course of the development of the insect from the larval to the adult stages.¹⁶

Cross-reactivity between *T. molitor* and the closely related beetle, *A. diaperinus*, was suggested by the fact that a subject with demonstrated sensitivity to *A. diaperinus*¹² had positive RAST results to *T. molitor* (Table III) even though he had no known prior exposure to *T. molitor*. Cross-reactivity was confirmed by RAST-inhibition assays (Figs. 1 to 3), which demonstrated significant inhibition of the *T. molitor* RAST assays by the *A. diaperinus* extracts for each developmental stage. Slight cross-reactivity between blowfly (Diptera order) and the *T. molitor* larvae and adult stages was also demonstrated. This is in contrast to the study of Bernstein et al.⁴ in which no cross-reactivity was found between *T. molitor* larvae and larvae of two other insects of the order Diptera or

larvae from a moth species (Lepidoptera order). Neither the study by Bernstein et al.⁴ nor our study found cross-reactivity between *T. molitor* and house dust mite.

The pattern of cross-reactivity demonstrated is not surprising when the taxonomy of arthropods is examined (Table IV).^{17, 18} *T. molitor* and house dust mite are members of two separate classes of arthropods, insects and arachnids, and are therefore only distantly related. Blowflies are insects but belong to a different order (Diptera versus Coleoptera). The source of the proteins responsible for the slight cross-reactivity found between these insects is unknown, although it may be due to proteins in the exoskeleton, since the integument of the insect is generally presumed to contain the substances causing inhalant allergies.¹⁹

T. molitor and *A. diaperinus* both belong to the Tenebrionid family of Coleoptera,¹⁸ although they belong to different tribes (Table IV). Because of this close taxonomic relationship, it is not surprising that there is a high degree of cross-reactivity between the two insects. Our study does not clarify whether or not such cross-reactivity extends to other families of Coleoptera; to clarify the cross-reactivity would be a massive undertaking because there are approximately 154 families of Coleoptera with 277,000 species worldwide, of which 26,076 species are found in the United States alone.¹⁹

T. molitor is a cosmopolitan insect, being found abundantly in the northern United States where it is one of the largest insects infesting stored cereal products.²⁰ Thus, although the patients reported here and by Bernstein et al.⁴ had uncommon occupational exposures to *T. molitor*, potentially large numbers of workers in grain-handling occupations are at risk for being sensitized to *T. molitor*. Similarly, *A. diaperinus* is a stored-grain pest found in grain and cereal products commonly associated with poultry feed.²⁰ Both

insects appear to be potent sensitizing agents. We have additionally demonstrated that there is a high degree of cross-reactivity between these two Tenebrionid beetles. In addition to these two beetles, there are a number of other beetles in this family, many of which are also stored-grain pests.²⁰ One beetle of particular note is *Tribolium confusum*, the confused flour beetle. This insect belongs to the same tribe as *A. diaperinus* (Table IV). It could be postulated that this insect would therefore also be a potentially significant allergen. The importance of this is that *Tribolium confusum* is undoubtedly the most abundant insect pest of four mills in the United States and is found wherever grain or grain products are stored.²⁰ Awareness of the potentially allergenic nature of these abundant stored-grain pests is of importance to the clinician, since such allergies may play a role in many cases of asthma usually attributed to grains in grain-dust asthma or baker's asthma.

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