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TOXIC MOTHS: SOURCE OF A TRULY SAFE DELICACY

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ABSTRACT.—A field survey of local traditional food habits in northern Italy revealed that children in Carnia have traditionally eaten sweet ingluvies (the crop) from day flying moths of the genus *Zygaena* and its mimic, *Syntomis*. These moths are brightly colored, and all species of *Zygaena* contain cyanogenic glucosides, which release toxic hydrogen cyanide upon degradation. The presence of cyanogenic glucosides in larvae and imagos (adults) as well as in ingluvies of *Zygaena* and *Syntomis* moths was investigated using liquid chromatography-mass spectrometry. The sugar content of the ingluvies was determined using gas chromatography and high-performance anion-exchange chromatography. The ingluvies contained very low quantities of cyanogenic glucosides but high quantities of various sugars. We conclude that over time children have become acquainted with the use of *Zygaena* as a convenient, supplementary source of sugar in early summer when *Zygaena* is very common and easy to catch by hand. Because the ingluvies have a very low cyanogenic content, children can include this resource as a seasonal delicacy at minimum risk.

Key words: Edible insects, Zygaena, Syntomis, cyanogenic glucosides.

RESUMEN.—Una encuesta sobre los hábitos alimenticios de la región italiana de Carnia detectó que entre los niños era tradicional chupar el abdomen de las polillas diurnas del género *Zygaena* y su especie mimética *Syntomis* para obtener el líquido dulce de su interior. Ambas mariposas poseen en sus alas manchas similares con colores brillantes. Estudios anteriores revelaron que las especies del género *Zygaena* contienen glucósidos cianogénicos, que liberan cianuro una vez que estos glucósidos son degradados. En este trabajo se ha analizado la presencia

de glucósidos cianogénicos en las larvas, imagos y en el líquido del abdómen de las polillas de los géneros *Zygaena* y de *Syntomis* por cromatografía líquida de alta definición y por espectrofotometría de masas. El contenido de azúcares se determinó mediante cromatografía gaseosa y por cromatografía de intercambio iónico. Se determinó que el abdómen de *Zygaena* posee un bajo contenido en glucósidos cianogénicos pero un alto contenido en glucosa, fructosa y sacarosa mientras que los de *Syntomis* mostraron bajo contenido en azúcares y ningún glucósido cianogénico. Los niños de la región de Carnia han adoptado un alimento rico en azúcares y con bajo riesgo de toxicidad, que además es abundante y de fácil acceso ya que estas polillas son fáciles de capturar.

RÉSUMÉ.—Une enquête des habitudes alimentaires traditionnelles locales faite dans le nord de l'Italie a montré que les enfants de Carnia ont traditionnellement consommé les excréments sucrées provenant de certains lépidoptères (Zygaena et Syntomis). Ces papillons sont intensément colorés et toutes les espèces de Zygaena contiennent des glucosides cyanogéniques qui, lorsque décomposés, relâchent des hydrocyanides toxiques. La présence de ces composés toxiques dans les larves et imagos ainsi que dans les excréments de Zygaena et Syntomis a été étudiée par chromatographie en phase liquide à haute performance couplée à un spectromètre de masse. Le contenu en sucre des excréments a été mesuré par chromatographie en phase gazeuse et chromatographie échangeuse d'ions. Les excréments contiennent très peu de glucosides cyanogéniques mais possèdent une grande quantité de sucres différents. Ainsi, avec le temps, les enfants ont eu connaissance de l'utilisation de Zygaena en tant que source de sucre supplémentaire et commode, surtout en début d'été alors que les Zygaena sont très communs et faciles à attraper à la main. De plus, étant donné que les excréments contiennent peu de glucosides cyanogéniques, les enfants peuvent avoir recours à cette délicieuse ressource saisonnière sans encourir des risques de toxicité.

INTRODUCTION

Many insects have evolved poisonous or toxic compounds as an adaptive means to prevent predation. Their bright colors alert predators to their toxicity. Non-toxic insects often mimic the coloration and behavior of toxic insects as their strategy for avoiding predation. The colorful Zygaena moths (Figure 1) contain the cyanogenic glucosides (CNglcs) linamarin and lotaustralin, which degrade into toxic hydrogen cyanide. Some researchers claim this toxicity is an efficient defense against predators (Harborne 1988; Rothschild 1973; Sbordoni et al. 1979; Wray et al. 1983; Zagrobelny et al. 2004). Larvae of Zygaena are able to sequester CNglcs from their food plants as well as to synthesize their own CNglcs if the level obtained by sequestration is not adequate (Nahrstedt 1988; Zagrobelny et al. 2007a). In addition, Zygaena larvae can release defense droplets rich in CNglcs when attacked by predators. Syntomis moths, such as Syntomis phaegea (Figure 1), have developed the Zygaena ephialtes color pattern to escape from predators (Bullini et al. 1969; Harborne 1988; Rothschild 1973; Sbordoni et al. 1979). Zygaena and its mimic Syntomis are able to produce additional warning odor compounds such as pyrazines (Rothschild et al. 1984), a common trait of many insect species. Because of their rich chemical defense arsenal, Zygaena moths can perch safely on

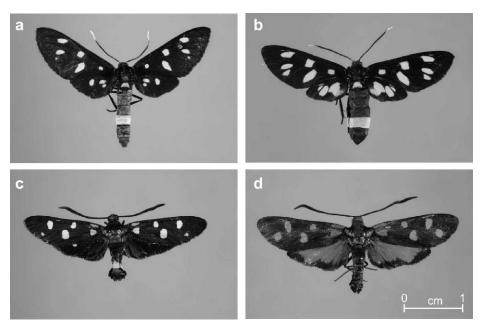


FIGURE 1.—Colorful "edible" butterfly species from Carnia, Friuli, northeastern Italy. *Syntomis phaegea* (a) male and (b) female, (c) *Zygaena ephialtes* and (d) *Zygaena transalpina* (Photo L. Dreon, identification Paolo Mazzei).

flowers when feeding on nectar or awaiting mates. They react slowly to predators and are easily collected even by hand.

While interviewing elderly informants about traditional non-conventional wild food consumption in the Carnia region of northeastern Italy, we discovered that during childhood in the 1940s to the 1960s, they and their friends commonly ate the sweet ingluvies, the crop or swollen pocket of the gut (called el miel locally), from day flying moths of the toxic Zygaena-Syntomis complex (called Pavea locally; Figure 2). These moths were abundant on flowers in traditional hay meadows from June through August (Dreon and Paoletti 2009; Paoletti and Dreon 2005). We independently interviewed several people in villages in the Pordenone province of northeastern Italy who also reported Zygaena/Syntomis as the only moths used for food. A total of seven different species of Zygaena (Z. charon, Z. filipendulae, Z. lonicerae loti, Z. osterodensis, Z. transalpina, and Z. ephialtes) are known to live in this region (Quaia 2000). The informants specified that only the ingluvie (Figure 2), not the entire moth, was eaten. These observations prompted us to assess the toxicity of both ingluvies and other parts of the moths, and to investigate the possible nutritional benefits to children from this unconventional food.

A wide variety of invertebrates (i.e., insects, earthworms, spiders, crustaceans, and bivalves) form part of the diet of human populations in Asia, South America, Africa and Oceania (for an extensive review see De Foliart 2007, 2005; Paoletti 2005). Insects, especially the larvae, are rich in proteins, fatty acids, sugars and other important nutrients (Bukkens 2005). Insects can constitute an important source of supplementary food during times of food shortage and as



FIGURE 2.—(a) *Zygaena lonicerae* above and *Zygaena transalpina* below perching on flower of *Cardus nutans*. (b) Ingluvie and egg mass in *Zygaena transalpina* female imago torn apart.

part of an everyday diet, especially when insects are abundant, easy to catch, and nutritionally rich. In some regions, particular insect species are considered a true delicacy (De Foliart 2007, 2005; Paoletti 2005). Urban populations in Asia eat more species of insects, prepared as a greater variety of dishes, than people living in the countryside (Yhoung-Aree and Viwatpanich 2005). In Western societies, the use of invertebrates as food is limited to a very few species of macroinvertebrates, snails, prawns, crabs, and a few species of bivalves, and there are few reports of the consumption of insects (Paoletti 2005). Worldwide, accounts of people eating adult moths are extremely rare. A recent comprehensive review on the subject (Paoletti 2005) found only one reported case, the Bogong moth, *Agrotis infusa*, eaten by Australian Aborigines (Yen 2005). To our knowledge, the research presented here provides the first documentation of the consumption of moths by people not only in northeastern Italy, but in all of Europe, and provides data on one of the few cases of insects eaten by Europeans.

MATERIALS AND METHODS

Informants aged 52 to 86 years (six males and three females) were independently interviewed on different occasions and later asked to collect their familiar edible moths with the researchers (ALD and MGP). The informants all lived in villages of the Pordenone province: Arba, Tramonti di Sotto, Polcenigo, Vivaro, Erto e Casso and Cimolais. During the summers of 2006 and 2007, larvae and imagos (fully developed adults) of *Zygaena transalpina* and *Syntomis phaegea*, the imagos of *Z. ephialtes* and the plants larvae feed on were collected at Tramonti di Mezzo, Fanna, and Chievolis villages in the Pordenone province (ranging from 250 to 600 meters asl). We also collected insects with a similar color pattern to the *Zygaena-Syntomis* complex (*Lygaeus* sp., *Graphosoma* sp., *Cercopis vulnerata*, *Coccinella septempunctata*, *Cryptocephalus* sp.). The insects and their food plants were stored at 4°C for 3 to 6 hours during transportation. Ingluvies were extracted from imagos with a dissection forceps, and all samples were frozen in liquid nitrogen and kept at -80° C. Some specimens were eaten in the field soon after capture to test for the presence and flavor of the ingluvies.

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The frozen samples were crushed using a mortar and pestle and were subsequently passed through an Anopore 0.45 μ m filter (Whatman). Metabolites including CNglcs were extracted from the samples using a 1 ml 55% MeOH/ 0.1% formic acid (vol/vol) solution. All insect extractions were diluted by a factor of 10 while ingluvie extractions were not diluted. Analytical liquid chromatography - mass spectrometry (LC-MS) was carried out using an Agilent 1100 Series LC linked to a Bruker HCT-*Plus* ion trap mass spectrometer. Separation was carried out using a Synergi 2 μ m Fusion-RP column (20 × 2 mm, flow rate 0.2 ml min⁻¹). The mobile phases were: A, 0.1% (vol/vol) formic acid in water with 50 μ M sodium chloride; B, 0.1% (vol/vol) formic acid in acetonitrile. The gradient program was: 0 to 2 min, isocratic 1% (vol/vol) B; 2 to 10 min, linear gradient 1 to 40% (vol/vol) B; 10 to 15 min, linear gradient 40 to 100% (vol/vol) B; 15 to 17 min, isocratic 100% (vol/vol) B; 17 to 17.08 min, linear gradient 100 to 1% (vol/vol) B; 17.08 to 20 min, isocratic 1% (vol/vol) B.

The spectrometer was run in electrospray mode and positive ions were observed. Mass spectral data were analyzed with native DataAnalysis software (Bruker Daltonik). Total amounts of CNglcs were estimated from an internal standard of the CNglc amygdalin. Linamarin (retention time = 1.5 min) and lotaustralin (retention time = 4.0 min) were monitored as their sodium adducts. This analytical system does not separate individual low mass sugars (glucose, fructose, sucrose), which co-elute at retention time = 0.8 min.

Seven ingluvies from Zygaena transalpina were analyzed for the presence of sugars with gas chromatography equipment adopting the protocols normally used to study honey composition. Analysis was carried out at the National Institute for Apiculture research unit of beeculture and sericulture (CRA-Unità di ricerca di apicoltura e bachicoltura) in Bologna, Italy, following the UNI protocol (UNI 11027;2003; Bogdanov et al. 1997). The average weight of ingluvies was 50 mg, and individual ingluvies were analyzed after the addition of 200 μ l of distilled water. The solution was dried and any sugars with free aldehydes or ketone groups, such as glucose and fructose, were converted to their oximes by adding an oxime reagent. These were silvlated and the derivatives were separated and quantified by gas chromatography using mannitol as the internal standard. The gas chromatograph (Fisons GC 8000 series) was fitted with an SE 52 capillary column and a flame ionization detector. The fused silica capillary column measured 25 m in length, 0.32 mm in diameter, and 0.1–0.15 μ m in film thickness. Samples of 0.6 µl were injected and separated using a 4 ml/min flow rate of hydrogen. One ingluvie from Z. transalpina and three ingluvies from S. phaegea were also analyzed for neutral sugars using high-performance anionexchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a Dionex DX-500 system fitted with a CarboPac PA1 column. Samples of 30 µl were injected and separated using a 1 ml/min flow rate of 0.1 M NaOH.

RESULTS

Informants reported that catching and eating *Zygaena* moths was an enjoyable custom, traditionally taught by the oldest relatives in the household to young family members. They indicated that during the summer, a child could

	Zygaena transalpina							Syntomis phaegea					
	Sample Average						Sample		Average				
Sugar (mg)	1	2	3	4	5	6	7	D	N=8	А	В	С	N=3
Glucose	6.5	*	14.5	18.6	40.3	84.9	-	28.3	32.2	1.1	3.1	0.2	1.5
Fructose	6.6	*	6.6	13.8	22.6	81.5	14.8	25.7	24.5	1.1	2.5	0.3	1.3
Trehalose	*	-	-	-	*	*	-	-	-	-	-	-	-
Maltose	-	-	-	*	-	*	-	-	-	-	-	-	-
Saccharose	-	-	-	-	-	*	-	-	-	-	-	-	-

TABLE 1.—Sugar content of ingluvies from Zygaena transalpina and Syntomis phaegea.

Note. Ingluvies 1–7 from *Z. transalpina* analyzed by gas chromatography. Ingluvies from *Z. transalpina* (D) and *S. phaegea* (A, B, C) analyzed by anion exchange chromatography, Values are mg; ingluvies have an average weight of 50 mg. (*) Present in trace amount, (-) not detectable.

collect and eat from 5 to 20 moth ingluvies on a day trip to the hay meadows around their villages. To verify the information from informants, we tasted 10 ingluvies of *Z. transalpina* and *Z. ephialtes* and some from the mimic *S. phaegea*. Both species of *Zygaena* had ingluvies with a sweet, delicate flavor. In contrast, although most male and female *S. phaegea* we caught did not have large ingluvies, the few we found did not taste sweet to us.

Using gas chromatography, we analyzed the sugar content of *Zygaena transalpina* ingluvies. This analysis indicated the presence of large amounts of fructose and glucose in the majority of the samples as well as the presence of minor amounts of trehalose, saccharose and maltose in some samples (Table 1). Anion exchange chromatography gave similar results (Table 1, Figure 3). The ingluvies from *S. phaegea* analyzed with anion exchange chromatography contained about 5% of the sugars present in *Z. transalpina* ingluvies (Figure 3). The difference in the composition and amounts of sugars is possibly related to the species of flowers visited by the adult moths before capture.

To assess whether ingluvies from the Zygaena-Syntomis complex were cyanogenic, samples of adults and ingluvies from Z. transalpina, Z. ephialtes, and S. phaegea were tested for CNglcs using LC-MS analyses (Figures 4 and 5). Only very small amounts of CNglcs were present in ingluvies compared to whole adults and some ingluvies entirely lacked cyanogenic compounds (Figure 4). S. phaegea was acyanogenic, and we could not detect any other toxic compounds in this insect with our LC-MS procedure. However, Rothschild et al. (1984) previously reported that Syntomis spp. contain pyrazines as deterrent compounds, and the ingluvies of *S. phaegea* that we collected tasted bad to us, so this moth is not completely unprotected. This study confirms, as reported in Zagrobelny et al. (2007a), that the CNglc content of individual larvae and imagos varies. This may reflect that the larvae had eaten food plants with different levels of CNglcs or that we collected larvae from different instar stages (Zagrobelny et al. 2004; Zagrobelny et al. 2007a). To assess the access to cyanogenic compounds by the Zygaena species studied, the food plant of the larvae of many Zygaena species (Lotus corniculatus L.) was collected at the same locations as the moths. The plant samples were all cyanogenic, and the level of CNglcs was comparable to those previously reported by Zagrobelny et al.

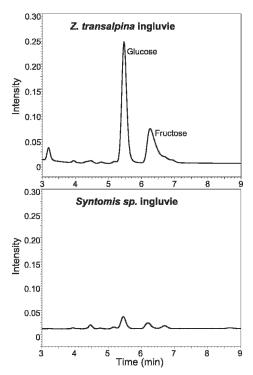


FIGURE 3.—Neutral sugars from *Zygaena transalpina* and *Syntomis phaegea* ingluvies analyzed with anion exchange chromatography.

(2007a). We detected no cyanogenic compounds in the food plants (*Cirsium* sp., *Knautia* sp., *Valeriana* sp., *Centaurea* sp.) visited by adult *Zygaena* and *Syntomis* moths. Previous studies found no significant difference in the content of CNglcs in virgin male and female imagos of *Z. filipendulae* (Zagrobelny et al. 2007b). However, due to transfer of a nuptial gift containing CNglcs during mating, mated female imagos had a higher content of CNglcs than mated males (Zagrobelny et al. 2007b). We do not know whether the adults collected in this study were virgin or had mated, but we infer that the virginity status does not affect the sugar content of the ingluvies because all specimens analyzed showed similar sugar levels (Table 1). Analyses of other insect species with a red-black coloration similar to *Zygaena* (*Lygaeus* sp.) showed that they were all acyanogenic.

DISCUSSION

About 20% of plants eaten by humans contain cyanogenic compounds (Bak et al. 2006; Jones 1998). When plant tissue containing CNglcs is digested, the CNglcs are brought into contact with degrading enzymes which cause the release of toxic hydrogen cyanide, glucose, and an aldehyde or ketone (Nahrstedt 1996; Vetter 2000). If the amount of cyanide ingested is small in relation to body weight

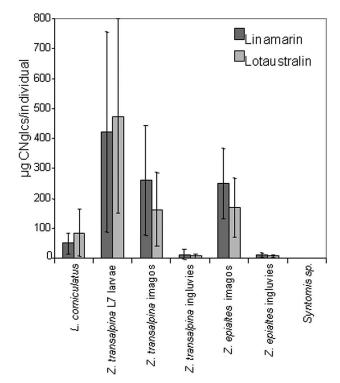


FIGURE 4.—The average content and standard deviation of linamarin and lotaustralin in *Zygaena transalpina* and *Zygaena ephialtes* larvae, imagos, and ingluvies. The CNglc content of imagos and ingluvies from *Syntomis phaegea* and of the food plant *Lotus corniculatus*.

and is diluted over time, humans as well as other mammals can detoxify hydrogen cyanide using the rhodanese enzyme (thiosulfate cyanide sulphur transferase). The presence of an adequate amount of sulfur-containing amino acids in the body is essential for this detoxification process. Chronic cyanide poisoning, therefore, most frequently occurs in populations suffering from a diet low in proteins composed of sulfur-containing amino acids. One example comes from the Sub-Saharan countries of Africa where cassava, which contains CNglcs, constitutes the major staple crop. When traditional processing methods that remove the toxic constituents of cassava are not carried out (e.g., due to lack of water, famine or war) acute or chronic cyanide intoxication is observed (Jones 1998; Jørgensen et al. 2005; Rosling et al. 1992).

Humans eating cyanogenic insects also need to detoxify the emitted hydrogen cyanide. To assess the potential danger from consuming toxic hydrogen cyanide with the moths, we estimated the number of *Zygaena* ingluvies and imagos that children would have to consume to receive a minimum deadly dose of hydrogen cyanide. We used the human LD_{50} (the median lethal dose) of hydrogen cyanide: 1.5–3.5 mg/kg (Jones 1998), which corresponds to 25 mg for a child weighing 15–20 kg. From the average weight of ingluvies (50 mg) and whole moths (100 mg) and their hydrogen cyanide content (Table 2), we estimate

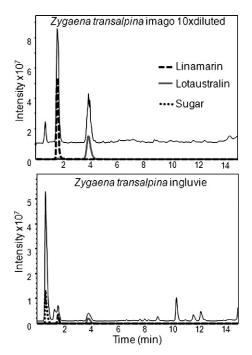


FIGURE 5.—Linamarin, lotaustralin and sugar content in a Zygaena transalpina female and her ingluvie as monitored by LC-MS. Total ion traces (solid black), overlaid with selected m/z ion traces for linamarin (dash), lotaustralin (gray) and sugar (dot).

TABLE 2.—Cyanide content	of Zygaena and	d other edible	products	and th	ne minimum
amount a child would have t	o eat to obtain	a fatal dose.			

	Cyanide content (mg/kg fresh weight)	Minimum amount (g) fatal to a childª
Zygaena species and source		
Z. filipendulae imagos	200-2500	10
Z. transalpina imagos	300-1500	17
Z. transalpina L7 larvae	200-1500	17
Z. transalpina ingluvies	0–100	250
Z. ephialtes imagos	150-1500	17
Z. ephialtes ingluvies	0–100	250
Other products		
White Clover ^b	17–157	160
Lotus corniculatus/japonicus	0-220	114
Cassava (average data) ^c	300	83
Bitter almonds ^d	1875	13
Marzipan ^b	5–7	5000

^a Human LD₅₀ dose of hydrogen cyanide: 1.5-3.5 mg/kg; minimum LD₅₀ dose for a child of 15-20 kg

^b Nama Bjarnholt, personal communication.
^c Kirsten Jørgensen, personal communication.
^d Raquel Sanchez Pérez, personal communication.

that children would have to eat a minimum of 5000 ingluvies or 170 whole moths in a short time period to receive a minimum deadly dose. The average human adult, weighing 75 kg, would have to eat at least 20,000 ingluvies. Since children typically catch only 5 to 20 moths in a day, they would not eat enough to reach a deadly dose. Furthermore, a child would most likely begin to feel dizzy and intoxicated after eating just 20 moths, and an adult, after eating 100, if they could catch that many *Zygaena* moths in one day. If they only ate the ingluvies, they would ingest hardly any hydrogen cyanide at all.

The tiny amounts of CNglcs detected in the ingluvies in the present study (Figure 4) could come from the scales from insect wings and other small insect parts that were caught in the ingluvie droplet when the insect was torn apart. The droplet itself may actually be completely acyanogenic. However, because this type of contamination is likely to occur whenever a person eats the ingluvie droplet, this estimate of the CNglcs ingested with ingluvies is probably realistic. The small insect parts ingested with the ingluvie would probably also provide the enzymes for the degradation of CNglcs to HCN, even if the ingluvies do not contain such enzymes themselves. *Syntomis* moths look very much like *Zygaena* and have probably been collected and eaten by humans, even though the ingluvies from these moths do not taste sweet. This is probably a minor problem compared to the risk of consuming the cyanogenic parts of *Zygaena*.

There may be other health benefits from eating *Zygaena* ingluvies. Cyanogenic plants of the genus *Rhodiola* are used for medicinal purposes, partly due to a class of secondary metabolites called rhodiocyanosides (Yoshikawa et al. 1995). *Rhodiola* plants also contain the CNglcs linamarin and lotaustralin, as do *Zygaena* moths. In low doses, lotaustralin possesses an antihistamine effect (Yoshikawa et al. 1997). Therefore, the children in the Carnia region may have experienced multiple benefits from eating *Zygaena*.

Two questions remain. Why do Zygaena imagos have such a nutritious acyanogenic compartment? And why would humans risk exposure to toxic hydrogen cyanide releasing compounds when eating these ingluvies to gain a minor amount of sugar? All other examined tissues in Zygaena are cyanogenic (Zagrobelny et al. 2007b). Humans may, in fact, be one of the few predators that can actively tear apart the entire moth in a manner that permits separating this nutritious acyanogenic compartment while other predators would eat the entire insect. For the insect, we hypothesize that the ingluvie may play a role as an energy storage compartment where sugars from ingested nectars can be stored. Humans were probably first motivated to eat the ingluvies due to their high sugar content. Children are attracted to sweet flavors, an evolutionary behavior that promotes the ingestion of energetic food. They are also very curious, putting anything into their mouths. This innate behavior may well lead children to experiment with novel nutritional sources in their local environments, discoveries that can then benefit the whole community in times of food scarcity. In the tropics, children are prone to eat several types of animals caught in the wild, sometimes raw. This practice has been observed in many different places and cultures for example in Vietnam, Venezuela, and in the Alto Orinoco basin (Cerda et al. 2005; Paoletti and Dreon 2005; Paoletti and Dufour 2005). This local habit probably reaches far back in time and may explain why local traditional

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foods often incorporate many different components. One such example is *pistç*, a traditional mix of about 50 to 60 edible plants, collected in the wild and eaten cooked together (Paoletti et al. 1995). Foods collected in the wild have always been an important supplementary resource in rural areas especially in times of food scarcity, a quite common experience in the mountainous regions of northeastern Italy. The habit of eating *Zygaena* ingluvies could also encompass other species of *Zygaena* than those examined here since a total of seven different species exist within this region (Quaia 2000). *Z. filipendulae* imagos from Denmark also contain sweet tasting ingluvies. Populations of *Zygaena* species are known to fluctuate dramatically from year to year (Esch and Naumann 1998), and children would probably have eaten any species containing sweet ingluvies that they came across.

Many investigations of the uses of non-conventional foods have been carried out on other continents, but in the course of this study we discovered that nonconventional foods have also been used among European cultures until very recent times. In fact, *Zygaena* were not the only insects eaten in northern Italy (Dreon and Paoletti 2009). The use of *Zygaena* as food is an old tradition, perhaps even thousands of years old, but it probably disappeared in recent years due to children's easy access to other sugary foods. Since the informants in this study lived quite far apart (200–300 km), the habit of eating *Zygaena* could have existed in a much broader area than immediately apparent from this study. It could even have been a custom in all of Europe, since *Zygaena* species are widespread and common across the continent (Naumann 1999). Maybe it is time to start looking more thoroughly into our own past and our surrounding landscape for traditional, unconventional foods.

NOTE

¹ Personal communications with Nanna Bjarnholt, Kirsten Jørgensen, and Raquel Sanchez Pérez, Plant Biochemistry Laboratory, Department of Plant Biology and Biotechnology, University of Copenhagen, conversations June 2007.

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