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Sequestration as defense mechanism in insects: A review

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Abstract

Herbivorous insects commonly use sequestration of chemical defences from host plants to avoid predation. The ability to create and handle bioactive chemicals is a critical component in the co-evolution of plants and insects. Plants create bioactive natural chemicals to defend themselves; however some insects detoxify and/or sequester the substances, allowing new niches with fewer competitors to emerge. The cyanogenic glucosides, which are found in over 2500 plant species and are thought to play a significant role in plant defence against herbivores due to their bitter taste and release of lethal hydrogen cyanide upon tissue damage, are one of the most important bioactive components.

Keywords: Detoxify, predation, sequestration, cyanogenic glucosides and defence

1. Introduction

More than 250 insect species, including beetles (*Termonia et al.*, 2001)^[91], true bugs (Duffey and Scudder, 1972; Scudder and Duffey, 2011)^[26, 82], and sawflies (Björkman and Larsson 1991)^[7], have been found to sequester plant compounds into animal tissues for defence (Duffey, 1977; Opitz and Müller, 2009)^[27, 70]. Sequestration specific, selective uptake and accumulation of plant toxins. Selective uptake, transport, modification, storage and deployment of plant secondary chemicals in insects for own defence. Toxins typically connects the first tropic level (plants) via second tropic level (insect herbivore) to the third tropic levels (predators and parasitoids). Many sequestering species have evolved to deal with plant defences (Opitz and Müller, 2009)^[70], but they aren't always immune to these toxins (Adler *et al.*, 1995; Björkman and Larsson, 1991; Dimarco and Fordyce, 2017; Mason and Bowers, 2017; Smilanich *et al.*, 2009; Zalucki *et al.*, 2001; Zvereva and Kozlov, 2016; Zvereva *et al.*, 2017)^[3, 7, 25, 61, 86, 98, 99, 100]. Coleoptera and Lepidoptera are the most common hosts, but Heteroptera, Hymenoptera, Orthoptera, and Sternorrhyncha are also frequent hosts (Opitz and Müller, 2009)^[70]. Averting detection, avoiding attack, and tricking predators are just a few of the many defensive techniques (Ruxton *et al.*, 2004)^[81]. Selectivity of uptake can be realised by one (or) by a combination of many mechanisms. Plant poisons can be taken up and stored by a variety of insect herbivores as a form of self-defence against their natural enemies. These compounds are efficient against a wide range of predators, including invertebrates such as spiders and ants, as well as vertebrates such as birds and mammals (Trigo, 2000; Nishida, 2002; Opitz and Müller, 2009; Trigo, 2011)^[93, 67, 70, 92]. Tolerance methods that maintain plant poisons functioning are closely tied to toxin sequestration. The herbivore is thought to be able to manage the spatiotemporal dynamics of toxin buildup by using specific transporters. This pattern has also been observed in the adults of the nymphalid butterfly *Euphydryas phaeton*, which acquired defensive iridoid glycosides as larvae from two different host plants (Bowers, 1980; Bowers, 1988)^[9, 8], and the adults of the heliconiine butterfly *Heliconius erato*, whose larvae feed on four different *Passiflora* species (Hay-Roe and Nation, 2007)^[43]. Specific enzymes are also present in some herbivores, which help to increase the bioactivity of the toxins that have been sequestered. Plants in various groups (e.g., Asteraceae, Boraginaceae, and Leguminosae) produce these chemicals, which are sequestered by specialised grasshoppers, hemipterans, beetles, moths, and butterflies (Trigo, 2011; Macel, 2011; Eisner and Meinwald, 1995)^[92, 56, 28], where they have defensive and sexual communication roles. PAs are always present in N-oxide form in specialist insects (Hartman and Ober, 2008)^[38]. Plant toxin sequestration has been investigated by ecologists for decades. The recently discovered molecular pathways, when combined with transient, non-transgenic

approaches to regulate insect gene expression, will aid in understanding the role of toxin sequestration in natural food web dynamics. *Utetheisa ornatrix*, an arctiine rattlebox moth, is one of the most studied species in terms of its ecological dependence on PAs (Trigo, 2011; Eisner and Meinwald, 1995; Conner and Jordan, 2009; Eisner *et al.*, 2002; Conner and Iyengar, 2015) [92, 28, 19, 29, 18]. *U. ornatrix* can be found in the Neotropics and warm Nearctic regions (Da Costa, 2010) [21], where it eats a variety of *Crotalaria* legume species (Sourakov, 2015) [87]. These alkaloids are sequestered by *U. ornatrix* larvae and passed on to pupae and adults. Males pass PAs to females during mating, who then pass them on to the eggs. As a result, PAs defend all life stages of *U. ornatrix* from predators (Eisner and Meinwald, 1995) [28]. Sequestration of cardenolides has been investigated in terms of host plant chemistry (Brower and Moffitt, 1974; Brower *et al.*, 1982; Martin *et al.*, 1992) [11, 12, 59], compound polarity (Malcolm *et al.*, 1989; Nelson 1993; Roeske *et al.*, 1976) [57, 65, 79], toxicity to vertebrate predators (de Roode *et al.*, 2008, Gowler *et al.*, 2015; Sternberg *et al.*, 2012) [23, 34, 88]. Different cardenolide compounds have been proven to be poisonous to

monarch butterflies as well as vertebrates (Rasmann *et al.*, 2009; Petschenka *et al.*, 2018; Seiber *et al.*, 1983) [76, 74, 83], and possibly parasites (Sternberg *et al.*, 2012) [88].

1.1. Sequestered compounds include various

- Aromatic compounds
- Nitrogen-containing metabolites
 - Alkaloids, 1.1.2.2. Cyanogenic glycosides and
 - Glucosinolates

1.2 Isoprenoids

- Cardiac glycosides, 1.1.3.2. Cucurbitacins and
- Iridoid glycosides.

1.3 Plant families which are sequestering of toxic compounds in has been investigated

Aristolochiaceae, Asteraceae, Fabaceae, Apocynaceae and Plantaginaceae. Sequestration Coleoptera and Lepidoptera (predominant). Heteroptera, Hymenoptera, Orthoptera and Sternorrhyncha (frequently) (Nishida, 2002) [67].

Table 1.1: Plant allelochemicals sequestered by Lepidoptera/others, and their examples

Sl. No.	Chemical Class of Sequestrates	Representative Sequestereres	Examples
A. Terpenic Metabolites			
1.	Cardenolides (CGS)	Danainae, Arctiidae, Ctenuchidae	Milkweed butterflies, Tiger moths, <i>Ctenucha virginica</i>
2.	Grayanoides (GTS)	Geometridae	Loopers
3.	Iridoide Glycosides (IGS)	Nymphalidae, Geometridae, Sphingidae	Brush-footed butterflies, Loopers, Hawk moth
B. Nitrogen Containing Metabolites			
1.	Pyrrolizidine alkaloids (PAs)	Danainae, Arctiidae	Milkweed butterflies, Tiger moths
2.	Quinolizidine alkaloids (QAs)	Pyralidae	Snout moth
3.	Cyanogenic glycosides (CNS)	Zygaenidae	Burnet moth

Table 1.2: Compound investigated in insects

Compound	Insects	References
Benzoxazinoid glucosides	Western corn rootworm, <i>Diabrotica virgifera virgifera</i>	Robert <i>et al.</i> , 2017 [77]
Cardenolides (CGS)	Milkweed butterflies, Tiger moths, <i>Ctenucha virginica</i>	Nishida, 2002 [67] Matthew <i>et al.</i> , 2012 [62]
Pyrrolizidine alkaloids (PAs)	Milkweed butterflies, Tiger moths	Nishida, 2002 [67]
Cyclopentenyl cyanogens	Lepidopterans	Hagg <i>et al.</i> , 2014 [37]; Lechtenberg, 2011 [54]
β -cyanoalanine	<i>Clossiana euphrosyne</i>	Kebeish <i>et al.</i> , 2015 [51]
Cyclopentenyl glycine derived CNGs	Euptopia hegesia	Szewczyk and Zidorn, 2014 [90]

2. Sequestration mechanism in insects (Physiological mechanism involved in sequestration) (Petschenka and Agrawal, 2016) [73].

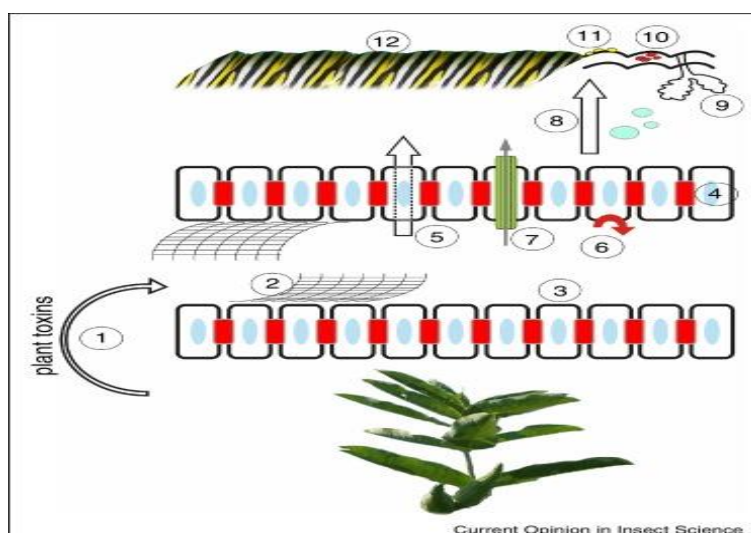


Fig 2.1: Physiological mechanism involved in sequestration

1. Plant toxins are injected.
2. Contact the peritropic membrane. The peritropic envelope may be involved in the process of sequestration as plant toxins could bind to the envelope and toxins could be retained in the gut.
3. Sequestration may be modulated by active and passive barrier functions as well as metabolism by degrading enzymes.
4. Polar toxins cannot be sequestered passively as paracellular diffusion across the mid gut epithelium and sequestration may be prohibited.
5. Non-polar (lipophilic) compounds can be sequestered passively as they can cross cell membrane.
6. Proteins like efflux carriers can mediate an active barrier to prevent toxins from reaching the body cavity.
7. One possibility for how polar toxins can be sequestered via specific carrier proteins. Within the haemolymph, Sequestered plant metabolites can be metabolized.
8. Potential binding to haemolymph proteins. Some toxins are transported across the haemolymph.
9. In to glands
10. Reservoirs (or) in to the integument were they are stored.
11. Exposed to predators in the form of droplets.
12. Protection mediated by sequestered plant toxins is often displayed by aposematic colouration.

3. Sequestration of secondary metabolites by insects and effects on higher trophic levels

3.1 Cardenolides

Many of the plant (Apocynaceae: Milk weeds) produce toxic chemicals Cardiac glycosides (or) Cardenolides that help to deter herbivore. These compounds specifically bind and inhibit the sodium and potassium pumps found in heart muscle cells. In susceptible insects Cardenolides bind to the α subunits of Na⁺, K⁺ ATPase inhibiting the function ultimately affecting many important physiological process, including muscle contraction, neural function and ion transport. Without working pumps sodium levels in the cell rise, setting of a chain of events that ultimately disrupts the muscle contraction in the cardiac tissue. At high enough dose, can be lethal to insects. Feeding upon milkweed plants (Asclepiadaceae) as larvae, the butterflies sequester a series of cardenolides (found predominantly as cardiac glycosides, CGs) that render the adult unpalatable to predators. These toxins are degraded by inducible CYPs (P 450s) that are expressed in the midgut and also in the fat body. CGs are cardio-active steroids causing toxic effects in various vertebrates. Predatory birds avoid the butterflies primarily because they experience an obnoxious bitter taste and/or emesis after ingestion (Matthew *et al.*, 2012). While the *D. plexippus*-NaC/KC-ATPase is consistently more resistant than the non-adapted insect NaC/KC-ATPases of *E. core* and *S. gregaria*, 11 of the 16 cardenolides examined were more effective than ouabain against its NaC/KC-ATPase. Calactin and calotropin, which are preferentially sequestered by monarchs from *Asclepias* spp. (Cheung *et al.*, 1988; Groeneveld *et al.*, 1990)^[16, 35], were the most poisonous cardenolides, inhibiting *D. plexippus*-NaC/KC-ATPase up to 60 times more effectively than ouabain (Cheung *et al.*, 1988; Groeneveld *et al.*, 1990)^[16, 35]. Monarch caterpillars sequester large amounts of calactin and calotropin, as well as obtaining these substances enzymatically from other milkweed cardenolides like uscharidin (Seiber *et al.*, 1980)^[84]. Furthermore, monarchs appear to only sequester cardenolides

with a specific polarity range, and chemicals like calactin and calotropin may be easier to retain in bodily tissues than more lipophilic cardenolides (Roeske *et al.*, 1976)^[79]. While non-polar cardenolides are more easily absorbed via the gut, polar cardenolides appear to elicit emesis in vertebrates at lower doses, according to Duffey, (1977)^[27]. Cardenolide glycosides have been demonstrated to be more hazardous than equivalent genins in whole organism vertebrate experiments in the past (Hoch, 1961)^[44]. Sugar residues in cardiac glycosides have been shown to stabilise the NaC/KC-ATPase inhibitor complex by interactions of the sugar molecule's hydroxyl groups with proton-donating as well as proton-accepting groups on the NaC/KC-ATPase (Yoda, 1973)^[96], and sugars can prevent the enzyme from reactivating after cardiac glycoside inhibition (Yoda, 1973; Cornelius *et al.*, 2013)^[96, 20]. Furthermore, the sugar moiety is required for cardiac glycosides to be fixed to the heart muscle (Hoch, 1961)^[44]. Sugars' influence on any given NaC/KC-ATPase appears to be dependent on the number and chemical identity of the sugar molecules in general (Farr *et al.*, 2002)^[30]. Responses to sugar removal in the vertebrate NaC/KC-ATPase have been reported to diminish or increase inhibition strength (O'Brien *et al.*, 1993; Farr *et al.*, 2002; Paula *et al.*, 2005; Cornelius *et al.*, 2013)^[69, 30, 71, 20].

The peculiar properties of sugars found in cardiac glycosides suggest that they have an adaptive value for plants, but the processes of natural selection have yet to be discovered. Apart from absorption, these sugars may contribute to heart glycoside hydrolytic stability, which could be an anti-predator tactic (e.g., prevention of hydrolysis in the animal gut). Cardenolides from the Asclepiadoideae (milkweed) family, which feature cyclic bridges, are particularly resistant to acid hydrolysis (Seiber *et al.*, 1983)^[83] and should thus be given special attention in the future. Given the increased inhibition of NaC/KC-ATPase in glycosides compared to genins, we hypothesise that glycosylation of cardiac glycoside genins may result in metabolic activation rather than detoxification. Milkweed cardenolides is the trans-bent junction of rings A and B in the steroid, which is cis-bent in Digitalis and other medicinally significant cardenolides (Seiber *et al.*, 1983; Malcolm, 1991)^[83, 58]. Based on their toxicity (Brown and Thomas, 1984)^[13], these trans-bent 5 α -cardiac glycosides are thought to have a poor affinity for mammalian NaC/KC-ATPase and have a low biological efficacy (Paula *et al.*, 2005; Katz *et al.*, 2010)^[71, 49]. The fact that uzarigenin has a significantly lower water solubility than digitoxigenin highlights the importance of the A/B ring junction arrangement for basic physicochemical features, and hence might readily alter milkweed cardenolide absorption and sequestration in herbivorous insects.

Dog bane beetle (*Chrysochus* spp) which are resistant to ouabain, a toxic cardenolide found in milk weed host plant (Despres *et al.*, 2007)^[24]. Ouabain had a stronger inhibitory potency than ouabagenin on lamb-, shark-, and human NaC/KC-ATPase (Farr *et al.*, 2002; Paula *et al.*, 2005; Katz *et al.*, 2010; Cornelius *et al.*, 2013)^[30, 71, 49, 20], which was consistent with our data on insect NaC/KC-ATPase.

3.1.1 Cardenolides sequestration in large milk weed bug: *Onchopeltus fasciatus*

Chemical defence in the large milk weed bug *Onchopeltus fasciatus* derives from maternal but not from paternal. Newcombe *et al.*, (2013)^[66] investigated that weather transmission of Cardenolides from the host diet to the eggs in maternal,

paternal (or) biparental in the large milk weed bug *Oncopeltus fasciatus*. They reared individual bugs on either milkweed seeds [MW; *Asclepias syriaca* L. (Apocynaceae)] that contain Cardenolides, or on sunflower seeds [SF; *Helianthus annuus* L. (Asteraceae)] that do not contain cardenolides. They mated females and males so that all four maternal/paternal diet combinations were represented: MW/MW, MW/SF, SF/MW, and SF/SF. They conducted two-choice predation trials with green lace wing to assess whether maternal, paternal, or biparental transmission of cardenolides into the eggs of *O. fasciatus* increased protection against predation. They concluded that predation trials that a maternal diet of milkweed makes eggs more distasteful than a paternal diet of milkweed (Newcombe *et al.*, 2013) [66]

3.2 Pyrrolizidine alkaloids: (PAs)

Crotalaria (Leguminosae: Papilionoideae: Crotalariaeae) is a Pantropical weed genus that includes about 702 species (Polhill, 1982; le Roux *et al.*, 2013) [75, 53]. There are 31 native and 11 non-native species in the Neotropics, notably in Brazil (Flores, 2004) [32]. PAs are abundant in Crotalaria species (Roque-Albelo *et al.*, 2009) [80], with larger quantities in seeds than in leaves (Ferro *et al.*, 2006) [31]. Extrafloral nectaries (EFNs) are also present, which attract predatory ants and wasps (Ferro *et al.*, 2006; Guimarães *et al.*, 2006; Franco and Cogni, 2013; Pereira and Trigo, 2013) [31, 36, 33, 72]. PAs are toxic secondary metabolites found in wide variety of plant taxa, including *Senecio jacobae*, Eupatorium (Asteraceae), Crotalaria (Fabaceae), Parsonsia (Apocynaceae). Danaine butterfly, *Idea leuconoe*, specializing on *Parsonsia laevigata* (Apocynaceae). The larvae of this primitive Danaine acquire PAs and store them as N-oxides throughout all stages of their life. Males use volatile metabolites derived from one PA as a sex pheromone. Females use macrocyclic host PAs (*e.g.*, parsonsine) as specific oviposition cues (Nishida, 2002) [67]. The predatory neotropical orb-weaving spider *Nephila clavipes* (Nephilidae) weaves its web in forest clearings and corridors, which serve as insect flight lanes (Robinson and Mirick, 1971) [78]. PAs are responsible for *U. ornatix* and other arctiine moths' chemical defence (Trigo, 2011; Eisner and Meinwald, 1995; Conner and Jordan, 2009) [92, 28, 19], although a dose-dependent strategy has been less well described. As with other arctiines, the sequestration of unaltered PAs or the translation of plant PAs into insect PAs is varied (Hartmann *et al.*, 2004; Hartmann *et al.*, 2005; Hartmann *et al.*, 2004; Hartmann *et al.*, 2005) [39, 41, 40, 42].

3.3 Glucosinolates

The cabbage aphid is a damaging agricultural insect that feeds on plants in the Brassicales order, which includes crops like cabbages and mustards. They are found mainly in the family "Brassicaceae". The enzyme myrosinase, which is stored in specialised tissues. It converts the glucosinolates to the toxic isothiocyanates. Glucosinolates affects higher tropic levels, via reduced host (or) prey quality. Because specialist herbivores may sequester the glucosinolates for their own defence (Despres *et al.*, 2007) [24]. *Athalia rosea* (Turnip saw fly) sequesters ingested glucosinolates mainly in the hemolymph. *A. rosea* larvae easily bleed hemolymph containing the sequestered glucosinolate. A defensive response that offers them protection against the Ant (*Myrmica rubra*) (Hymenoptera: Formicidae) and the wasp (*Vespa vulgaris*) (Muller *et al.*, 2001) [63]. Harlequin bug, *Murgantia histrionica* (Heteroptera:

Pentatomidae) - also contains sequesters- bugs- rejected - two species of bird predators. Aphids are attacked by the predatory two-spotted lady beetle, *Adalia bipunctata*, sequestered glucosinolates and aphid myrosinase mix and volatile isothiocyanates and nitriles are released hydrolytically, conferring a toxic effect on the predator (Aliabadi *et al.*, 2002) [5]. Aphids *Brevicoryne brassicae* and *Lipaphis erysimi* special cases of sequestration of in that the concentration different types of glucosinolates 15- 20 times higher in their haemolymph (relative to the concentrations found in total leaf tissues) (Hopkins *et al.*, 2009) [45]. Although the mechanism governing the selective sequestration of specific glucosinolates is unknown, it is thought to include specific transporters such as the ABC transporters, which allow plant-derived molecules to traverse cellular membranes (Petschenka and Agrawal, 2016; Strauss *et al.*, 2013) [73, 89]. Specialist herbivores are more likely than generalist herbivores to sequester plant defence chemicals (Beran *et al.*, 2014; Bramer *et al.*, 2017; Kazana *et al.*, 2007; Müller *et al.*, 2001) [6, 10, 50, 63]. It's one of a number of adaptations made by Brassicales-feeding herbivores to help them colonise glucosinolate-rich host plants (Cao *et al.*, 2018; Cole, 1997; Louis *et al.*, 2012) [12, 17, 55]. These same glucosinolates, when activated by the insect, can function as defences against predators and parasitoids (Chaplin-Kramer *et al.*, 2011; Kos *et al.*, 2011) [15, 52]. The endogenous cabbage aphid myrosinase (BMY) allows the cabbage aphid to manufacture defensive ITCs from sequestered glucosinolates as long as the enzyme can take the sequestered glucosinolates as substrates (Jones *et al.*, 2002; Kazana *et al.*, 2007) [48, 50]. Because of their better interactions with enzyme binding sites, aliphatic glucosinolates may be favoured as substrates (Husebye *et al.*, 2005) [49].

3.4 Cyanogenic glycosides

Six spot Burnet moth *Zygaena filipendulae* are toxic, when they are injured, they released cyanide, highly poisonous chemical. Cyanide compound are found at some levels at each stage of the life cycle from egg to adult. The larvae sequester and store some cyanogenic compound from their food plants – Birds foot trefoil – *Lotus corniculatus*. Cyanogenic glucosides are one of plants' most common defensive compounds (Jensen *et al.*, 2011; Simon *et al.*, 2010) [47, 89]. These glucosides are degraded to a reactive hydroxynitrile, which releases lethal hydrogen cyanide when plant tissue is disrupted (Agerbirk and Olsen, 2012) [4]. Herbivorous arthropods duplicated an old horizontal transfer of a gene originally involved in Sulphur amino acid production in bacteria to detoxify plant-produced cyanide (Wybouw *et al.*, 2014) [95]. The occurrence of CNGs in animals appears to be restricted to a single phylum out of the currently known 31 Arthropod species, in contrast to the taxonomically extensive distribution of CNGs within the plant kingdom (Nahrstedt, 1996) [64].

3.4.1 *Euptopia hegesia* (Papilionoidea)

The Cyclopentenyl glycine derived (CNGs) from its host plant *Turnera ulmifolia* were hypothesized and sequestered by *Euptopia hegesia* (Heliconiinae: Papilionoidea). The sequestering was proposed because of significantly higher CNG levels in larvae reared on cyanogenic plants compared to siblings reared on a cyanogenic plants (Zagrobelyny *et al.*, 2004) [97].

3.4.2 Cyanogenic glucosides in *Zygaenidae*

The poisonous amino acid -cyano-L-alanine has been discovered in 21 *Zygaenidae* species that synthesise and store the cyanoglucosides linamarin and lotaustralin. -cyanoalanine is also reported for the first time from other cyanogenic lepidoptera, such as the Acraeinae and Heliconiinae of the nymphalid family, as well as other Nymphalidae species that were previously unknown to be cyanogenic. For the first time, cyanogenesis is demonstrated in Heterogynidae, with the existence of -cyano-alanine and the cyanoglucosides linamarin and lotaustralin (Witthohn and Naumann, 2014) ^[94]. *Zygaena* species have long been known to be resistant to HCN, and they may, for example, survive for an hour in a concentrated HCN atmosphere and then swiftly recover when exposed to clean air (Noriega, 2014) ^[68].

4. Conclusions

Among the enormous number of secondary metabolites with known toxic properties only few classes of chemicals have been selected as sequesterable elements by insects, ultimately selected by natural enemies through a food chain. The density of sequestered plant secondary metabolites may progress as follows: a) Produced by a plant, b) Ingested by insects without deterrent, c) Absorbed through the gut without elimination, d) Stored in the particular tissues without disturbing the sensitive tissues, e) Retained and Redistributed to the adults without major loss. Picked up by a bird and taste rejected (or) delivered to the stomach and then vomited (or) and causing other pharmacological affects. Male insects uses sequestered compound for production of sex pheromone. Female insects used for oviposition cues. Gaining unpalatability for an insect may not be a simple process, in that the process deals with consecutive selective factors, each of which may involve physiological and ecological costs.

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6. References

- Aardema ML, Zhen Y, Andolfatto P. The evolution of cardenolide-resistant forms of Na⁺,K⁺-ATPase in Danainae butterflies. *Molecular Biology*, 2012, 21(2). <https://doi.org/10.1111/j.1365-294X.2011.05379.x>
- Abe F, Yamauchi T, Honda K, Omura H, Hayashi N. Phenanthroindolizine alkaloid by *Asclepiadacea* feeding Danoide butterfly *Ideopsis similis*. *phytochemistry*. 2000;56:697-701.
- Adler LS, Schmitt J, Bowers MD. Genetic variation in defensive chemistry in *Plantago lanceolata* (Plantaginaceae) and its effect on the specialist herbivore *Junonia coenia* (Nymphalidae). *Oecologia*. 1995;101:75-85.
- Agerbirk N, Olsen CE. Glucosinolate structures in evolution. *Phytochemistry*. 2012;77:16-45.
- Aliabadi A, Renwick JAA, Whitman DW. Sequestration of glucosinolates by harlequinbug *Murgantia histrionica*. *Journal of chemical ecology*. 2002;28:9.
- Beran F, Pauchet Y, Kunert G, Reichelt M, Wielsch N, Vogel H *et al.* *Phyllotreta striolata* flea beetles use host plant defense compounds to create their own glucosinolate-myrosinase system. *Proc Natl Acad Sci USA*. 2014;111(20):7349-7354.
- Björkman C, Larsson S. Pine sawfly defence and variation in host plant resin acids: a trade-off with growth. *Ecol Entomol*. 1991;16:283-289.
- Bowers MD. Plant allelochemistry and mimicry. In: Barbosa P, Letourneau D, editors. *Novel aspects of plant-insect interactions*, New York: John Wiley, 1988, 273-311p.
- Bowers MD. Unpalatability as a defense strategy of *Euphydryas phaeton* (Nymphalidae). *Evolution*. 1980;34:586-600.
- Bramer C, Friedrich F, Dobler S. Defence by plant toxins in milkweed bugs (Heteroptera: Lygaeinae) through the evolution of a sophisticated storage compartment. *Syst Entomol*. 2017;42(1):15-30.
- Brower LP, Moffitt CM. Palatability dynamics of cardenolides in the monarch butterfly. *Nature*. 1974;249:280-283.
- Brower LP, Seiber JN, Nelson CJ, Lynch SP, Tuskes PM. Plant determined variation in the cardenolide content, thin-layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus plexippus* reared on the milkweed, *Asclepias eriocarpa* in California. *J Chem Ecol*. 1982;8:579-633.
- Brown L, Thomas R. Comparison of the inotropic effects of some 5 alpha-cardenolides on guinea pig left atria. *Arzneimittelforschung*. 1984;34:572-574.
- Cao HH, Zhang ZF, Wang XF, Liu TX. Nutrition versus defense: why *Myzus persicae* (green peach aphid) prefers and performs better on young leaves of cabbage. *PLoS One*. 2018;13(4):e0196219
- Chaplin-Kramer R, Kliebenstein DJ, Chiem A, Morrill E, Mills NJ, Kremen C. Chemically mediated tritrophic interactions: opposing effects of glucosinolates on a specialist herbivore and its predators. *J Appl Ecol*. 2011;48(4):880-887
- Cheung HTA, Nelson CJ, Watson TR. New glucoside conjugates and other cardenolide glycosides from the monarch butterfly reared on *Asclepias fruticosa* L. *J. Chem. Soc. Perkin Trans*. 1988;1:1851-1857. doi: 10.1039/P19880001851
- Cole RA. The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated *brassica* species. *Entomol Exp Appl*. 1997;85(2):121-133.
- Conner WE, Iyengar VK. Male pheromones and courtship. In: Allison JD, Cardé RT, editors. *Pheromone communication in moths: Evolution, behavior and application*. California: University of California Press; In press, 2015.
- Conner WE, Jordan AT. From armaments to ornaments: the relationship between chemical defense and sex in tiger moths. In: Conner WE, editor. *Tiger moths and woolly bears. Behavior, ecology, and evolution of the Arctiidae*. New York: Oxford University Press, 2009, 155-152p.
- Cornelius F, Kanai R, Toyoshima C. A structural view on the functional importance of the sugar moiety and steroid hydroxyls of cardiotonic steroids in binding to Na, K-ATPase. *J. Biol. Chem*. 2013;288:6602-6616. doi: 10.1074/jbc.M112.442137
- Da Costa MA. Phylogeny of *Utetheisa* s. str. (Lepidoptera: Noctuidae: Arctinae) with comments on the

- evolution of colour, hind wing scales and origin of New World species. *Invert Syst.* 2010;24:113-130.
22. Dar SA, Wani AB, Wani MY, Hussain S, Majid MS. Plant-Insect Interactions-Cyanogenic Glucosides. *IJIR*, 2016, 2(11).
 23. de Roode JC, Pederson AB, Hunter MD, Altizer. Host plant species affects virulence in monarch butterfly parasites. *J Anim Ecol.* 2008;77:120-126.
 24. Despres L, David JP, Gallet C. The evolutionary ecology of insects resistance to plant chemical. *Trend in ecology and evolution.* 2007;22(6):299-307.
 25. Dimarco RD, Fordyce JA. Not all toxic butterflies are toxic: high intra- and interspecific variation in sequestration in subtropical swallowtails. *Ecosphere.* 2017;8(12):e02025.
 26. Duffey SS, Scudder GGE. Cardiac glycosides in north American Asclepiadaceae, a basis for unpalatability in brightly coloured Hemiptera and Coleoptera. *J Insect Physiol.* 1972;18:63-78.
 27. Duffey SS. "Arthropod allomones: chemical effronteries and antagonists," in *Proceedings of the XV International Congress Entomology, Washingt, DC, 1977, 19-27.*
 28. Eisner T, Meinwald J. The chemistry of sexual selection. *Proc Natl Acad Sci USA.* 1995;92:50-55. PMID: 7816847
 29. Eisner T, Rossini C, González A, Iyengar VK, Seigler MVS, Smedley SR. Paternal investment in egg defense. In: Hilker M, Meiners T, editors. *Chemoecology of insect eggs and egg deposition.* Berlin: Blackwell Verlag, 2002, 91-116p.
 30. Farr CD, Burd C, Tabet MR, Wang X, Welsh WJ, Ball WJ. Three-dimensional quantitative structure-activity relationship study of the inhibition of NaC, KC-ATPase by cardiotonic steroids using comparative molecular field analysis. *Biochemistry.* 2002;41:1137-1148. doi: 10.1021/bi011511g
 31. Ferro VG, Guimarães PR, Trigo JR. Why do larvae of *Utetheisa ornatrix* penetrate and feed in pods of *Crotalaria* species? Larval performance vs. chemical and physical constraints. *Entomol Exp Appl.* 2006;121:23-29.
 32. Flores AS. Taxonomia, números cromossômicos e química de espécies de *Crotalaria* L. (Leguminosae-Papilionoideae) no Brasil. Dr. Sc. Thesis, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil, 2004. Available: <http://www.bibliotecadigital.unicamp.br/document/?code=vtls000343516>.
 33. Franco MS, Cogni R. Common-garden experiments reveal geographical variation in the interaction among *Crotalaria pallida* (Leguminosae: Papilionoideae), *Utetheisa ornatrix* L. (Lepidoptera: Arctiidae), and extrafloral nectary visiting ants. *Neotrop Entomol.* 2013; 42:223-229. doi: 10.1007/s13744-013-0114-8 PMID: 23949803
 34. Gowler CD, Leon KE, Hunter MD, de Roode JC. Secondary defense chemicals in milkweed reduce parasite infection in monarchs butterflies, *Danaus plexippus*. *J Chem Ecol.* 2015;41:520-523.
 35. Groeneveld HW, Steijl H, Berg B, Elings JC. Rapid, quantitative HPLC analysis of *Asclepias fruticosa* L. and *Danaus plexippus* L. cardenolides. *J. Chem. Ecol.* 1990;16:3373-3382. doi: 10.1007/BF00982104
 36. Guimarães PR, Raimundo LG, Bottcher C, Silva RR, Trigo JR. Extra floral nectaries as a deterrent mechanism against seed predators in the chemically protected weed *Crotalaria pallida* (Leguminosae). *Austral Ecol.* 2006;31:776-782.
 37. Hagg JF, Zagrobelny M, Jørgensen K, Heiko V, Møller BL, Søren B. Chemical Defense Balanced by Sequestration and De Novo Biosynthesis in a Lepidopteran Specialist. *Plos One.* 2014;9(10):e108745. doi: 10.1371:0108745.
 38. Hartman T, Ober D. Defense by pyrrolizidine alkaloids: Developed by plants and recruited by insects. In: Schaller A, editor. *Induced plant resistance to herbivory*, Springer Science + Business Media BV, 2008, 213-231p.
 39. Hartmann T, Theuring C, Beuerle T, Bernays EA. Phenological fate of plant-acquired pyrrolizidine alkaloids in the polyphagous arctiid *Estigmene acrea*. *Chemoecology.* 2004;14:207-216.
 40. Hartmann T, Theuring C, Beuerle T, Ernst L, Singer MS, Bernays EA. Acquired and partially de novo synthesized pyrrolizidine alkaloids in two polyphagous arctiids and the alkaloid profiles of their larval food-plants. *J Chem Ecol.* 2004;30:229-254. PMID: 15112722
 41. Hartmann T, Theuring C, Beuerle T, Klewer N, Schulz S, Singer MS *et al.* Specific recognition, detoxification and metabolism of pyrrolizidine alkaloids by the polyphagous arctiid *Estigmene acrea*. *Insect Biochem Mol Biol.* 2005;35:391-411. PMID: 15804574
 42. Hartmann T, Theuring C, Beuerle T, Bernays EA, Singer MS. Acquisition, transformation and maintenance of plant pyrrolizidine alkaloids by the polyphagous arctiid *Grammia geneura*. *Insect Biochem Mol Biol.* 2005; 35:1083-1099. PMID: 16102415
 43. Hay-Roe MM, Nation J. Spectrum of cyanide toxicity and allocation in *Heliconius erato* and *Passiflora* host plants. *J Chem Ecol.* 2007;33:319-329. PMID: 17200887
 44. Hoch J. A Survey of Cardiac Glycosides and Genins. Charleston, SC: University of South Carolina Press, 1961.
 45. Hopkins RJ, Van Dam NM, Van Loon JJ. Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annu. Rev. Entomol.* 2009;54:57-83.
 46. Husebye H, Arzt S, Burmeister WP, Härtel FV, Brandt A, Rossiter JT *et al.* Crystal structure at 1.1Å resolution of an insect myrosinase from *Brevicoryne brassicae* shows its close relationship to β-glucosidases. *Insect Biochem Mol Biol.* 2005;35(12):1311-1320.
 47. Jensen NB, Zagrobelny M, Hjærnø K, Olsen CE, Bak S. Convergent evolution in biosynthesis of cyanogenic defence compounds in plants and insects. *Nature Communications.* 2011;2:273.
 48. Jones AME, Winge P, Bones AM, Cole R, Rossiter JT. Characterization and evolution of a myrosinase from the cabbage aphid *Brevicoryne brassicae*. *Insect Biochem Mol Biol.* 2002;32(3):275-284.
 49. Katz A, Lifshitz Y, Bab-Dinitz E, Kapri-Pardes E, Goldshleger R, Tal DM *et al.* Selectivity of Digitalis glycosides for isoforms of human Na, K ATPase. *J. Biol. Chem.* 2010;285:19582-19592. doi: 10.1074/jbc.M110.119248
 50. Kazana E, Pope TW, Tibbles L, Bridges M, Pickett JA, Bones AM *et al.* The cabbage aphid: a walking mustard oil bomb. *Proc Royal Soc B: Biol Sci.* 2007;274(1623):2271-2277.
 51. Kebeish R, Aboelmy M, Peterhansel YC. Simultaneous

- overexpression of cyanidase and formate dehydrogenase in *Arabidopsis thaliana* chloroplasts enhanced cyanide metabolism and cyanide tolerance. *Environmental and Experimental Botany*, 2015, 110.
52. Kos M, Kabouw P, Noordam R, Hendriks K, Vet LEM, Van Loon JJA *et al.* Prey-mediated effects of glucosinolates on aphid predators. *Ecol Entomol.* 2011;36(3):377-388.
 53. le Roux MM, Boatwright JS, van Wyk BE. A global infrageneric classification system for the genus *Crotalaria* (Leguminosae) based on molecular and morphological evidence. *Taxon.* 2013;62:957-971.
 54. Lechtenberg M. Cyanogenesis in higher plants and animals. eLS McIntosh, JA. Christopher, CF and Arnold, FH. 2014. Expanding P450 catalytic reaction space through evolution and engineering. *Curr Opin Chem Biol.* 2011;0:126-134.
 55. Louis J, Singh V, Shah J. *Arabidopsis thaliana*-Aphid Interaction. *The Arabidopsis Book.* The American Society of Plant Biologists, Rockville, 2012.
 56. Macel M. Attract and deter: a dual role for pyrrolizidine alkaloids in plant-insect interactions. *Phytochem Rev.* 2011;10:75-82. PMID: 21475391
 57. Malcolm SB, Cockrell BJ, Brower LP. Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca* L. *J Chem Ecol.* 1989;15:819-853.
 58. Malcolm SB. "Cardenolide-mediated interactions between plants and herbivores," in *Herbivores: their Interactions with Secondary Plant Metabolites, the Chemical Participants*, 2nd Edn, Vol. I, eds G. A. Rosenthal and M. R. Berenbaum (San Diego, CA: Academic Press), 1991, 251-296. doi: 10.1016/B978-0-12-597183-6.50012-7.
 59. Martin RA, Lynch SP, Brower LP, Malcolm SB, van Hook T. Cardenolide content, emetic potency, and thin-layer chromatography profiles of monarch butterflies, *Danaus plexippus*, and their larval host-plant milkweed, *Asclepias humistrata*, in Florida. *Chemoecology.* 1992;3:1-13.
 60. Martins CHZ, Cunha BP, Solferini VN, Trigo JR. Feeding on Host Plants with Different Concentrations and Structures of Pyrrolizidine Alkaloids Impacts the Chemical-Defense Effectiveness of a Specialist Herbivore. *PLoS One.* 2015;10(10):e0141480. doi:10.1371/journal.pone.0141480
 61. Mason PA, Bowers MD. Localization of defensive chemicals in two congeneric butterflies (Euphydryas, Nymphalidae). *J Chem Ecol.* 2017;43:480-486.
 62. Matthew L, Aardona Zhen K, Andofatto P. The evolution of cardenolides resistant forms of Na⁺/K⁺-ATPase in *Danainae* butterflies. *Molecular ecology.* 2012;21:340-349.
 63. Müller C, Agerbirk N, Olsen CE, Boevé JL, Schaffner U, Brakefeld PM. Sequestration of host plant glucosinolates in the defensive hemolymph of the sawfly *Athalia rosae*. *J Chem Ecol.* 2001;27(12):2505-2516.
 64. Nahrstedt A, Mueller E. β -Glucosidase (Linamarase) of the Larvae of the Moth *Zygaena trifolii* and Its Inhibition by Some Alkaline Earth Metal Ions. *ACS Symposium Series.* 2009;533(9):132-144.
 65. Nelson C. A model for cardenolide and cardenolide glycoside storage by the monarch butterfly. In: Zalucki MP (ed) Malcolm SB. *Biology and Conservation of the Monarch Butterfly*, Los Angeles, 1993, 83-90p.
 66. New Combe D, Blount JD, Mitchell C, Moore JA. Chemical egg defence in the large milk weed bug, *Oneopeltus fasciatus*, derived from maternal but not paternal diet. *Entomologia Experimentalis applicata*, 2013, 5.
 67. Nishida R. Sequestration of defensive substances from plant by Lepidoptera. *Annu. Rev. Entomol.* 2002;47:57-92.
 68. Noriega FG. Juvenile Hormone Biosynthesis in Insects: What Is New, What Do We Know, and What Questions Remain? *International Scholarly Research. Notices.* 2014;2014:16.
 69. O'Brien WJ, Wallick ET, Lingrel JB. Amino acid residues of the Na, K-ATPase involved in ouabain sensitivity do not bind the sugar moiety of cardiac glycosides. *J. Biol. Chem.* 1993;268:7707-7712.
 70. Opitz SEW, Müller C. Plant chemistry and insect sequestration. *Chemoecology.* 2009;19:117-154.
 71. Paula S, Tabet MR, Ball WJ. Interactions between cardiac glycosides and sodium/potassium-ATPase: three-dimensional structure activity relationship models for ligand binding to the E2-Pi form of the enzyme versus activity inhibition. *Biochemistry.* 2005;44:498-510. doi: 10.1021/bi048680w
 72. Pereira MF, Trigo JR. Ants have a negative rather than a positive effect on extrafloral nectaried *Crotalaria pallida* performance. *Acta Oecol.* 2013;51:49-53.
 73. Petchanka G, Agarwal AA. How herbivores coopt plant defenses: natural selection, specialization and sequestration. *Current opinion in insect science.* 2016;14:17-24.
 74. Petschenka G, Fei CS, Araya JJ, Schröder S, Timmermann BN, Agrawal AA. Relative selectivity of plant cardenolides for Na⁺/K⁺-ATPases from the monarch butterfly and non-resistant insects. *Front Plant Sci.* 2018;9:1424.
 75. Polhill RM. *Crotalaria* in Africa and Madagascar. Rotterdam: AA Balkema, 1982.
 76. Rasmann S, Johnson MD, Agrawal AA. Induced responses to herbivory and jasmonate in three milkweed species. *J Chem Ecol.* 2009; 35:1326-1334.
 77. Robert CAM, Zhang X, Machado RAR, Schirmer S, Lori M, Mateo P *et al.* Sequestration and activation of plant toxins protect the western corn rootworm from enemies at multiple trophic levels, 2017. NCBI: doi: 10.7554/eLife.29307
 78. Robinson MH, Mirick H. The predatory behavior of the golden-web spider *Nephila clavipes* (Araneae: Araneidae). *Psyche.* 1971;78:123-139.
 79. Roeske CN, Seiber JN, Brower LP, Moffitt CM. Milkweed cardenolides and their comparative processing by monarch butterflies (*Danaus plexippus* L.). *Recent Adv Phytochem.* 1976;10:93-167.
 80. Roque-Albelo L, Garrett SE, Conner WE. Darwin's moth: *Utetheisa* in the Galápagos Islands. In: Conner WE, editor. *Tiger moths and woolly bears. Behavior, ecology, and evolution of the Arctiidae.* New York: Oxford University Press, 2009, 207-222p.
 81. Ruxton GD, Sherratt TN, Speed MP. Avoiding attack: the evolutionary ecology of crypsis warning signals and mimicry. Oxford: Oxford University Press, 2004.
 82. Scudder GGE, Duffey SS. Cardiac glycosides in the Lygaeinae (Hemiptera: Lygaeidae). *Can J Zool.*

- 2011;50:35-42.
83. Seiber JN, Lee SM, Benson JM. Cardiac glycosides (cardenolides) in species of *Asclepias* (Asclepiadaceae). In RF Keeler & AT Tu (Eds.), *Handbook of Nat Toxins*, Vol. I: plant and fungal toxins. Amsterdam, the Netherlands, 1983, 43-83p.
84. Seiber JN, Tuskes PM, Brower LP, Nelson CJ. Pharmacodynamics of some individual milkweed cardenolides fed to larvae of the monarch butterfly (*Danaus plexippus* L.). *J. Chem. Ecol.* 1980;6:321–339. doi: 10.1007/BF01402911
85. Simon J, Gleadow RM, Woodrow IE. Allocation of nitrogen to chemical defence and plant functional traits is constrained by soil N. *Tree Physiology.* 2010;30:1111-1117.
86. Smilanich AM, Dyer LA, Chambers JQ, Bowers MD. Immunological cost of chemical defence and the evolution of herbivore diet breadth. *Ecol Lett.* 2009;12:612-621.
87. Sourakov A. You are what you eat: native versus exotic *Crotalaria* species (Fabaceae) as host plants of the Ornate Bella Moth, *Utetheisa ornatrix* (Lepidoptera: Erebidae: Arctiinae). *J Nat Hist.* 2015;49:2397-2415.
88. Sternberg ED, Levèvre T, Li J, Fernandez de Castillejo CL, Li H, Hunter MD *et al.* Food plant-derived disease tolerance and resistance in a natural butterfly-plant-parasite interactions. *Evolution.* 2012;66(11):3367-3376.
89. Strauss AS, Peters S, Boland W, Burse A. ABC transporter functions as a pacemaker for sequestration of plant glucosides in leaf beetles. *eLife.* 2013;2:e01096–e01096.
90. Szewczyk K, Zidorn C. Ethnobotany, phytochemistry, and bioactivity of the genus *Turnera* (Passifloraceae) with a focus on damiana-*Turnera diffusa*. *J Ethnopharmacol.* 2014;28:152(3):424-43.
91. Termonia A, Hsiao TH, Pasteels JM, Milinkovitch MC. Feeding specialization and host-derived chemical defense in Chrysomeline leaf beetles did not lead to an evolutionary dead end. *PNAS.* 2001;98:3909-3914.
92. Trigo JR. Effects of pyrrolizidine alkaloids through different trophic levels. *Phytochem Rev.* 2011;10:83-98.
93. Trigo JR. The chemistry of antipredator defense by secondary compounds in Neotropical Lepidoptera: facts, perspectives and caveats. *J Braz Chem Soc.* 2000;11:551-561.
94. Witthohn C, Naumann M. Die Verbreitung des β -Cyan-L-alanin bei cyanogenen Lepidopteren. The Distribution of β -Cyano-L-alanine in Cyanogenic Lepidoptera. *Zeitschrift für Naturforschung C.* 2014;39(7-8):837-840.
95. Wybouw N, Dermauw W, Leeuwen TV. A gene horizontally transferred from bacteria protects arthropods from host plant cyanide poisoning. 2014;3:e02365.
96. Yoda A. Structure-activity relationships of cardiotonic steroids for the inhibition of sodium- and potassium-dependent adenosine triphosphatase. *Mol. Pharmacol.* 1973;9:766-773.
97. Zagrobelny M, Rasmussen AV, Nauman CM, Moller LB. Cyanogenic glucosides and plant –insect interaction. *Phytochemistry*, 2003, 65-293-306.
98. Zalucki MP, Brower LP, Alonso MA. Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. *Ecolog Entomol.* 2001;26:212–224.
99. Zvereva EL, Kozlov MV. The costs and effectiveness of chemical defenses in herbivorous insects: a meta-analysis. *Ecol Monogr.* 2016;86:107-124.
100. Zvereva EL, Zverev V, Kruglova OY, Kozlov MV. Strategies of chemical anti-predator defences in leaf beetles: is sequestration of plant toxins less costly than de novo synthesis? *Oecologia.* 2017;183:93-106