Monarch butterflies gather pyrrolizidine alkaloids from dead and injured plants: a call for citizen science contributions

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Monarch butterflies (*Danaus plexippus*) are peculiar in having special relationships with two kinds of plants: one with milkweeds (species of *Asclepias*) on which the caterpillars develop and from which they usually obtain cardiac glycosides (CGs; cardenolides), the second with various unrelated plants which provide them with 1,2-dehydropyrrolizidine ester alkaloids (PAs), another large and diverse group of 'toxic' compounds. PAs are gathered by the adult butterflies only, mainly from *dry* or *injured* plant matter, usually independent from feeding behavior and for purposes not related to nutrition, a syndrome that has been coined as "PA-pharmacophagy" (Boppré 1984). Research on monarchs and plants has focused on larval hostplants, CG-based defense, and adult nectar sources—the relationship between monarchs and PA-containing plants, however, has been largely neglected, as Lawson *et al.* (2021) have documented.

Pyrrolizidine alkaloids play important roles in the lives of many organisms (e.g., Boppré 2011). Like CGs, they are natural chemicals (plant secondary metabolites) that are produced by plants of several unrelated families for protection from antagonists (see Boppré & Monzón 2022). Various insects store PAs for their defense; many male clearwing and milkweed butterflies (Ithomiini and Danaini but not *D. plexippus*) as well as tiger and wasp moths (Arctiini) also use odors (pheromones) derived from PAs in their courtship behavior. For humans and livestock PAs pose serious health risks (JECFA 2020).

PA-pharmacophagous Lepidoptera obtain PAs mostly from withering or dead plants, and sometimes from injured ones. In living plants, PAs are concealed within the cell walls and inaccessible to butterflies until a plant becomes dry or injured and cells break open. Also, PAs often occur only in particular parts of the plant (such as roots or seeds) and amounts vary inter- and intraspecifically, even intra-individually, and over time. Since PAs do not provide nourishment, butterflies do not 'feed' on PA-plants in the literal sense but instead 'take up' PAs. Loosely speaking, for adult PA-insects, PA-plants do not represent 'grocery shops' but rather 'pharmacies'. Plants that provide food for larvae and adults can be called primary hostplants, while those that provide PAs we call secondary hostplants.

At first glance, gathering nectar or PAs seems to reflect the same behavior – both kinds of compounds are ingested *via* the proboscis. However, it is two distinct behaviors: different sensory cues are used to locate sources, the ingested substances are treated very differently by the metabolic

Three examples of monarchs (*Danaus plexippus*) with expanded proboscides gathering pyrrolizidine alkaloids from withered inflorescences and withered leaves, respectively, of boneset (*Eupatorium serotinum*) in a garden in Maryland. Photos by Nancy Lawson.

system, and the motivation is different. While monarchs digest nectar and must consume it steadily throughout their lives, they store PAs. Once they have accumulated sufficient, they will not continue searching; this implies that gathering PAs is not a regularly occurring but a temporary, additional activity. Also, it is costly not only because sources of PAs must be actively sought but also because the PAs in dry plant matter have to be dissolved by regurgitating gut content and/or saliva that is sucked up again, a time-consuming and expensive process. At a wound, sometimes, butterflies scratch with their tarsal claws to damage further cells and gain access to more PAs. The amount of alkaloids that can be taken from plants varies greatly; this results in individuals with unique defensive characters. A monarch can live without PAs—but its success in obtaining these chemicals can extend its lifetime and/or increase its biological fitness.

Surprisingly, only a few field records of monarchs and PA-plants are available in the literature, although as long ago as 1955 the celebrated American naturalist William Beebe found *D. plexippus* at withered heliotrope (*Heliotropium indicum*) in Trinidad. However, he had no knowledge of PAs and could not explain the phenomenon. Since then, counterintuitive sightings of monarchs sucking at dry plant matter likely were made occasionally but did not seem to be considered newsworthy. After PAs were recognized as target chemicals for some insects in the mid-1970s, two studies documented that PAs are indeed stored by monarchs. PA-pharmacophagy is without doubt a basic character of monarch butterflies, even though it has been largely ignored (see Lawson *et al*. 2021 for details and references). Perhaps monarchs are less eager to take up PAs than their relatives, but gathering PAs must play an important role in their biology; otherwise they would never do it.

Currently, many questions about monarch PA-pharmacophagy remain, including first and foremost: Which plants serve as natural sources of PAs for monarchs? Do male and female monarchs gather PAs in equal manner or extent, or is there a sex-bias? Do monarchs engage in PA-pharmacophagy obligately or facultatively? Is PA-gathering in monarchs a plastic behavior that only occurs in certain circumstances, and if so, which ones?

The floral nectar of PA-plants usually is devoid of PAs, but some flowers do have these alkaloids in their nectar; they can be thought of as 'supermarkets' where butterflies get food and drugs at the same time. For most nectar foragers, however, PAs are deterrents. Are flowers that contain PAs in their nectar visited preferably or even exclusively by monarchs that primarily seek PAs and not food? Which other insects visit them? Are monarchs found at withering inflorescences, too? Candidate plants for PAs in nectar include some species of *Eupatorium, Conocilium coelestinum*, and *Gymnocoronis spilanthoides*.

Scientific natural history studies are needed to fill the knowledge gaps on PA-pharmacophagy in monarchs, an intrinsic behavior of these iconic butterflies. Sightings of monarchs with expanded proboscides at dead plant parts are of particular relevance. Much of the initial information required will probably come from observations that most likely can be made only by chance, not intentionally or by design.

WE INVITE butterfly lovers, insect enthusiasts, gardeners, bird watchers, and other naturalists, from as many regions of the United States (plus Latin America, Australia, the Pacific and Atlantic islands, and wherever else the monarch is found), to volunteer for our citizen science project "Monarch Rx" (CitSci 2021, Mertz 2021) and contribute observations of monarchs at dry or injured plants on as many occasions and from as many habitats as possible. For sure, members of the Lepidopterists' Society are the most appropriate audience to call for assistance, given their time spent in the field and capacity for conducting systematic studies by baiting with PA-plants. The primary goal is to create a list of plants that are attractive to monarchs due to their PA content. Then the health of visiting butterflies, the frequency of visits, and many further details can be studied quantitatively through PA-baiting.

Keep eyes open for monarchs with uncoiled proboscides at dry or injured plant leaves, stems or exposed roots. Identify the attractive plant, record parts visited (leaves, roots) and their condition (withered, injured), the sex of visiting monarchs, and the time they spent sucking. Was wetting with solvent or scratching observed? Plant determination is sometimes a problem because of ambiguous vernacular names and continuing name changes; however, exact plant ID is crucial because even closely related species may differ significantly in their PAs. Date and place, weather conditions, estimated abundance of monarchs flying in the vicinity at the time of observation, and any remarkable experiences should also be put on record. If possible, record how many days a particular plant remained attractive to monarchs. Fortuitous field observations can be substantiated by testing the attractive plant in other locations.

Information on incidence and level of infection with *Ophryocystis elektroscirrha* ('*Oe*') parasites is much wanted; if time permits, check monarchs found at PA-sources for spores of *Oe* (see Monarch Health 2022). PAs might not only provide protection against predators but also harm *Oe* and regulate infection by them: if female monarchs deposit PAs in their egg shells (like queen butterflies do), first instar larvae will take them up, and PAs might act as medicine against sporozoites of *Oe* in the caterpillars' guts. This hypothesis seems worth refining. Perhaps infected monarchs are more eager to gather PAs than healthy individuals?

A most valuable additional approach is to conduct PA-baiting: take a homemade or commercial butterfly trap traditionally used for monitoring fruit feeding butterflies

Danaus plexippus nigrippus at PA-baits in Panguana, Peru. Upper left: female at a slit twig of *Prestonia* sp. (photo by Phillip Klein); lower left: female and two day-active arctiine moths, *Corematura chrysogastra,* at bag with dry *Heliotropium indicum* (photo by Michael Boppré); right: male at bag with dry *Heliotropium indicum* (photo by Dirk Sichelschmidt).

(e.g., Shuey 1997, DeVries *et al*. 2016) and use uprooted PA-plants as bait. When monarchs are around, baiting with PA-plants is useful for checking if the butterflies are looking for PAs. Of course, baiting records should include the same information listed above for field observations. Trapped specimens can be marked, released and continue to be observed. Note that PA-baits become less attractive over time, PA-content within a given plant may vary and in some cases is highest in the roots, and that humidity is needed for generating volatile PA-derivatives as attractive odors (for practical tips see Boppré & Monzón 2022). Note that a PA-bait may compete with PA-sources occurring naturally in the habitat. The intraspecific as well as intra-individual variation in PA-content of many PA-plants suggests the use of different plants in parallel. Negative records (such as: leaves are not attractive but roots are) can provide valuable information, too. However, in general, documenting non-attraction is challenging, while demonstrating attraction is straightforward.

Studies of other PA-pharmacophagous insects including other species of milkweed butterflies have revealed that they do not have relationships with particular PA-plants but only with the alkaloids as such. Strictly speaking we are not dealing with an insect-plant relationship in the common sense but rather with an insect-chemical relationship: insects have an association with a whole variety of plants in their environment that contain the target chemicals; they can also be lured with some milligrams of pure PAs offered in a dish. Hundreds of PAs are known from hundreds of plant species and many more will be found. A given plant contains a bouquet of several PAs. As such, PAs are not volatile and cannot be detected by Lepidoptera over a distance, but attractive odors are formed when PAs degrade by hydrolysis in a humid environment. Of the many variables influencing attractiveness of PA-plants, currently only some basics are understood (see Boppré & Monzón 2022).

In any case, for the USA we can plausibly assume that many Echiteae (Apocynaceae), Senecioneae, Eupatorieae (Asteraceae, "Compositae"), Heliotropiaceae, Boraginaceae (Boraginales) and Crotalarieae (Fabaceae) including species of the genera *Amsinckia* (fiddlenecks), *Artemisia* (mugwort), *Chromolaena* (Siam weed), *Crotalaria* (rattlebox), *Delairea* (Cape ivy), *Erechtites* (burnweed), *Eupatorium* (thoroughwort), *Eutrochium* (Joe Pye weed), *Gymnocoronis* (Senegal tea), *Hackelia* (stickseed), *Heliotropium* (heliotrope), *Onosmodium* (false gromwell), *Senecio* (ragwort, groundsel), *Symphytum* (comfrey), and *Tournefortia* (soldierbush) are potential sources of PAs for monarchs. Since monarchs use all kinds of habitats with many plant communities in different assemblages, it is important to survey as many habitats as possible.

In many PA-plants, PAs are concentrated mainly in the roots, which are usually inaccessible to butterflies and therefore do not represent frequently used PA-sources in nature. Nevertheless, they can be good bait with which to determine the 'appetite' of monarchs for PAs. Species with highest PA levels in their roots and widespread occurrence include *Symphytum officinale* (common comfrey; a garden herb) and *Erechtites hieraciifolius* (American burnweed, fireweed; a pioneer therophyte).

A peculiar PA-containing plant is Senegal tea (*Gymnocoronis spilanthoides*, see next page), an invasive aquatic species introduced to the USA (USDA 2017). Not only its flowers (Minolli 2019) but also withering roots are very attractive to monarchs (Krauska 2009). It is commercially available as an ornamental for aquaria, it can be cultivated and propagated easily, and its drying roots can be used as bait for monitoring monarchs' relationship to PAs. However, this plant must be handled with great care to avoid release into the natural environment.

In a recent issue of the News, Badon (2021) published striking photographs of "*Euploea* butterflies nectaring *en*

masse". In fact, they are not "nectaring" but rather gathering PAs from drying infructescences of *Heliotropium* plants. We are convinced that similar pictures can be taken with monarchs on PA-plants if one is in the right place at the right time.

Twenty seven monarch butterflies gathering PAs at withering roots of Senegal tea (*Gymnocoronis spilanthoides*) in Missouri. Photo by Tom Krauska.

We are eagerly anticipating the participation of many readers willing to look out for monarchs at dry or injured plants, conduct baiting with PA-plants, or record past sightings on "Monarch Rx" (CitSci 2021), ideally accompanied by photographs. The website provides various resources on background topics and instructions on what to record and report. Comments and questions are most welcome. In due course, we will compile and analyze the data from citizen scientists for a future report that will acknowledge all contributors. At this moment the approach for studying monarchs and PAs is clear and vague at the same time—we are only at the very beginning of elucidating this poorly known and complex facet of monarch biology. With a great number of records, knowledge will grow, research tasks can be formulated more specifically, and eventually it will also be possible to assess whether PA-plants need to be taken into account in conservation efforts for the monarch.

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