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From model to mimic: age-dependent unpalatability in monarch butterflies

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Abstract. Monarch butterflies (*Danaus plexippus*) are unpalatable to various vertebrate predators because their larvae sequester bitter and emetic cardiac glycosides (CGs) from milkweed plants (*Asclepias* spp.). Here we show that the concentration of the defensive CGs decrease as individual butterflies age, regardless of the CGs' initial amounts or specific chemical structures. Consequently, individual monarch butterflies can change from being unpalatable models to palatable mimics during their lifetime. Since monarchs breed continuously over the spring and summer in North America, freshly emerged adult butterflies may serve as noxious models for older individuals which become automimics as they age.
Key words. Cardiac glycoside loss; *Danaus plexippus*; aging; breakdown of chemical defense; three trophic level interactions; automimicry; Lepidoptera; *Asclepias*.

Monarch butterflies (*Danaus plexippus*) are considered unpalatable models of other palatable (Batesian) mimic species, as well as co-models (Müllerian) in systems involving two or more unpalatable species[1-2]. The larvae sequester cardiac glycosides (CGs) from numerous species of milkweed (*Asclepias* sp.) plants[3]. These bitter and emetic compounds reduce the susceptibility of adult butterflies to natural predation by birds[4] and mice[5]. In this study, we determine that the concentrations of CGs decrease as the butterflies age, regardless either of the initial amounts of CGs or their chemical structures. Monarchs therefore can progress from unpalatable models to palatable automimics as they age during their individual lifetimes.

Material and methods

Monarch butterflies were reared from eggs in the laboratory on *A. humistrata* leaves collected near Gainesville, Florida. *A. syriaca*-reared butterflies were gathered as late 5th instar larvae and chrysalids from wild populations in western Massachusetts and southeastern Vermont in August-September, 1989, and emerged in the laboratory. After excreting the meconium (i.e. excretory products accumulated during the pupal phase), day 1 adults were frozen late that evening. The rest of the butterflies that would age from 3 to 35 d were numbered with india ink and released into an outdoor 11 × 5 × 4.5 m high flight cage. The butterflies had continuous access to nectar from potted *Lantana* (Verbenaceae) and *Pentas* (Rubiaceae) plants.

Males and females were isolated from each other by a vertical net to prevent them from mating. We did this in order to simulate experimentally the natural autumn migrant and overwintering monarchs that do not mate until the approach of the vernal equinox[6]. This design also purposely excluded variance in CG concentrations that would result from multiple matings and the copulatory transfer of CGs[7-8].

The flight cage had a northern aspect on the edge of Paynes Prairie State Reserve in Gainesville, Florida, and was partially shaded by large oak trees. Ambient shade temperatures in the cage ranged from approximately 23–35 °C. The *A. humistrata* monarchs (n = 83) emerged and were aged during June and July 1989, while the *A. syriaca* monarchs (n = 86) did so during September and October 1989.

Individuals were harvested at random at 3, 5, 9, or 17 d after emergence. Some individuals were collected early if they had damaged their wings and a few were aged for up to 34 d. Once harvested, they were frozen and stored in liquid nitrogen until analyzed. Since the body regions of adult monarchs have different concentrations of CGs[9-10], abdomens, wings, and thoraces of individual butterflies were analyzed separately. Following lipid extraction[11], the lean material was spectroassayed to determine the concentration of CGs (equivalent to digitoxin[12]). Residues from the same sample extracts were then assayed by thin layer chromatography to assess whether qualitative changes in the array of different CGs occurred as the butterflies aged.

We used multiple regression analyses to determine which variables had significant effects on the CG concentration[13]. Days after emergence, initial wet weight, right fore-wing length, percentage lipid, lean weight, and final wet weight were the independent variables run against the concentration of CGs.

Results and discussion

The multiple regression analyses indicated that only days after emergence had a significant effect on the CG concentrations. Concentrations of CGs in the abdomens and wings decreased logarithmically with time,

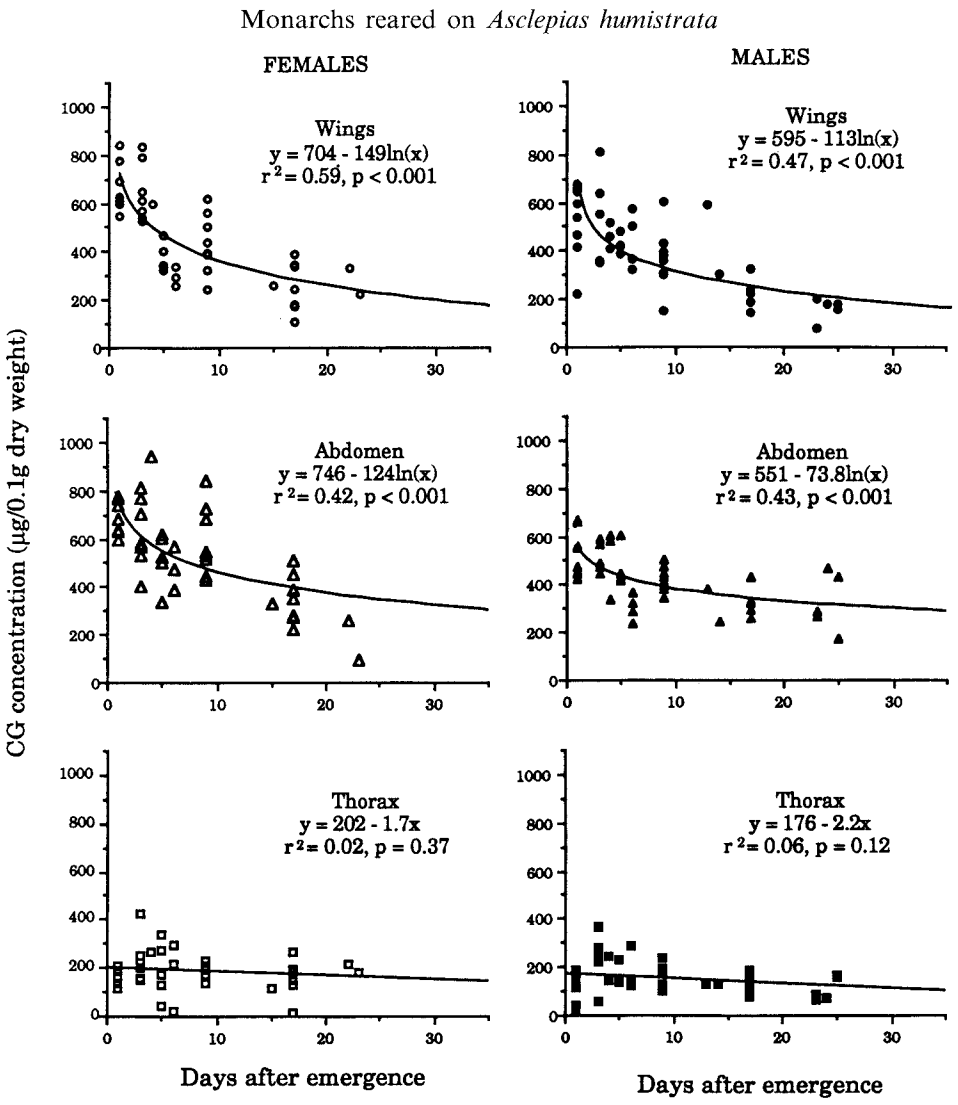

Figure 1. The effect of time on CG concentration in 3 body regions of monarch butterflies reared as larvae on *A. humistrata*. The concentration of CGs decreases with time in the abdomens and the wings of the butterflies, but not in the thoraces.

while CGs in the thoraces did not change (figs 1 and 2). Analysis of covariance indicated that CG loss rates were similar for both sexes and for abdomens and wings, irrespective of food plant. Concentrations in the wings decreased more than 60%; in abdomens, more than 30%.

Details of the statistical significances of slope and y-intercept differences are as follows:

In *A. humistrata*-reared monarchs 1) female abdomens and wings lost CGs at the same rate [$F(1, 78) = 0.95$, $p > 0.33$], but female abdomens had higher initial concentrations [$F(1, 79) = 6.01$, $p < 0.02$]; wings and abdomens of males had similar initial values and lost CGs at the same rate [$F(1, 88) = 0.96$, $p > 0.33$]. 2) Within body regions, abdomens of females and males had the same rate of CG loss [$F(1, 83) = 1.20$, $p > 0.27$], but female abdomens had higher initial concentrations [$F(1, 84) = 16.10$, $p < 0.001$]; wings of females and males lost CGs at the same rate [$F(1, 83) = 1.67$, $p > 0.19$] and had no differences in the initial concentrations.

In *A. syriaca*-reared monarchs 3) wings of both sexes lost CGs at a higher rate than abdomens [females, $F(1, 80) = 7.93$, $p < 0.005$; males, $F(1, 89) = 18.80$, $p < 0.001$]. 4) Abdomens and wings of females [$F(1, 84) = 0.19$, $p > 0.6$] and males [$F(1, 85) = 1.57$, $p > 0.21$] began with similar concentrations and had the same rates of loss. Between food plants, 5) the rate of loss of CGs from the wings was not different [females, $F(1, 80) = 0.51$, $p > 0.47$; males, $F(1, 88) = 0.54$, $p > 0.46$], and both had similar initial concentrations.

Thin layer chromatography of the butterflies reared on the two plant species confirmed the different arrays of CGs know to occur in *A. humistrata*[14] and *A. syriaca*[15]. For each plant, the abdomen, wings, thorax and meconium have the same arrays of CGs, and no qualitative

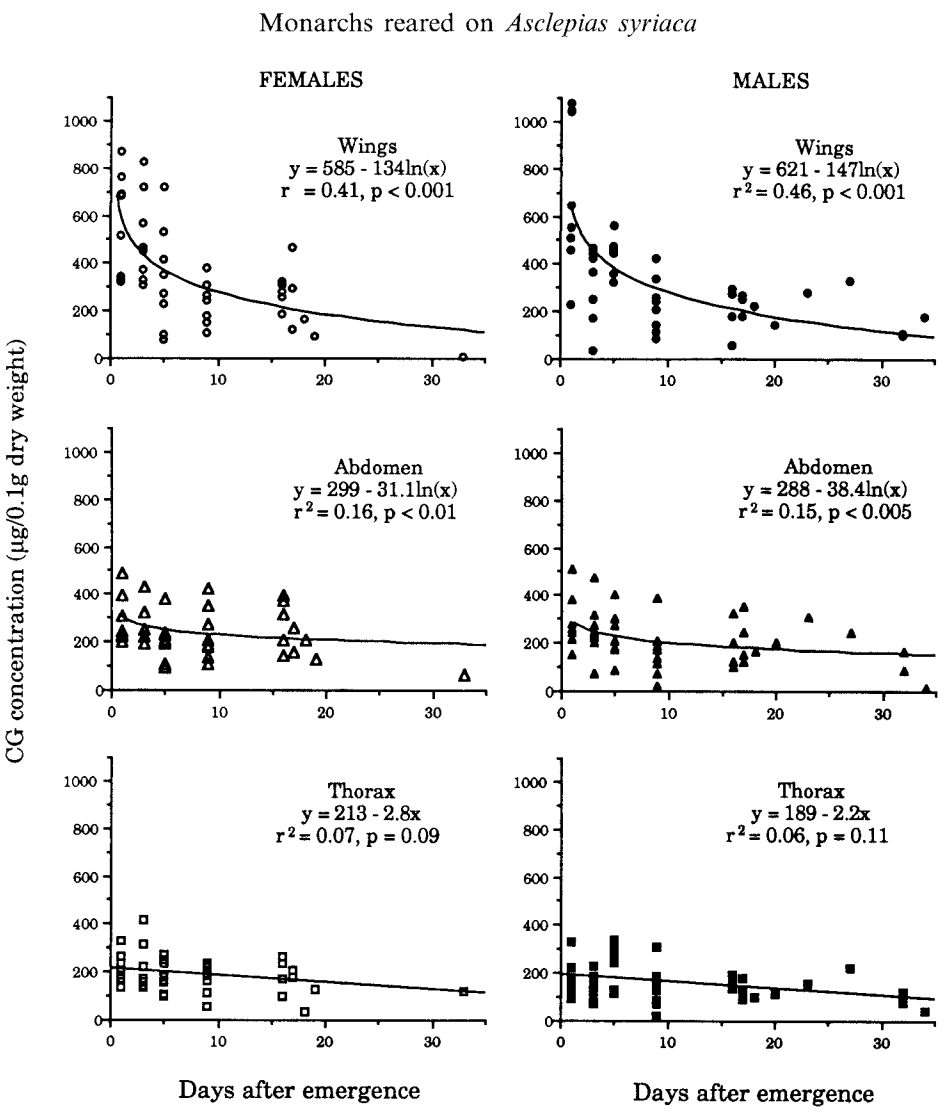

Figure 2. The effect of time on CG concentration in 3 body regions of monarch butterflies reared as larvae on *A. syriaca*. The overall patterns of rate of loss were similar in the butterflies reared on both plants.

changes of CGs occurred in any of the butterfly parts through time (fig. 3). Thus, even though the CGs are structurally different in the two milkweed plants, CGs decrease independently of molecular structure (calotropagenin-like CGs including humistratin occur in *A. humistrata* whereas polar epoxy CGs including syriobioside and aspecioside predominate in *A. syriaca*[16–17]). The mechanism of this age-related decline in CG content remains to be investigated. It may result from CG denaturation, physical loss of wings and abdomen scales, excretion, and/or an increased molecular binding of CGs to the exocuticle over time[10]. Binding could confound our quantitative estimates of CGs, but it is unlikely that unextractable CGs could be biologically active on the predators' gustatory or digestive systems. To predict the effect of the declining amounts of *A. syriaca*- and *A. humistrata*-derived CGs on the emetic strength of the butterflies as previously determined with blue jay predators[14], we plotted the total CG content found in the thorax and abdomen of individual butterflies against days after emergence. We excluded the wings because most predators tear off and reject them[18]. We based our predictions on two previously established facts: 1) the emetic dosages of monarchs that fed on *A. syriaca* are inversely related to the gross CG content of the butterflies (fig. 2 of Brower and Moffit[19]); and 2) at equal doses, *A. humistrata*-derived CGs are 5.7 times more emetic than *A. syriaca*-derived CGs[14].

Our results indicate that recently emerged monarchs reared on *A. syriaca* contain enough CG to cause emesis if eaten by a bird, but the older butterflies do not (fig. 4). Monarchs that fed on *A. humistrata* lost CGs at the same rate as *A. syriaca* butterflies. However, because *A. humistrata* monarchs have CGs of higher emetic potency, these butterflies would remain emetic for at least 30 days. This is the approximate life span of the spring

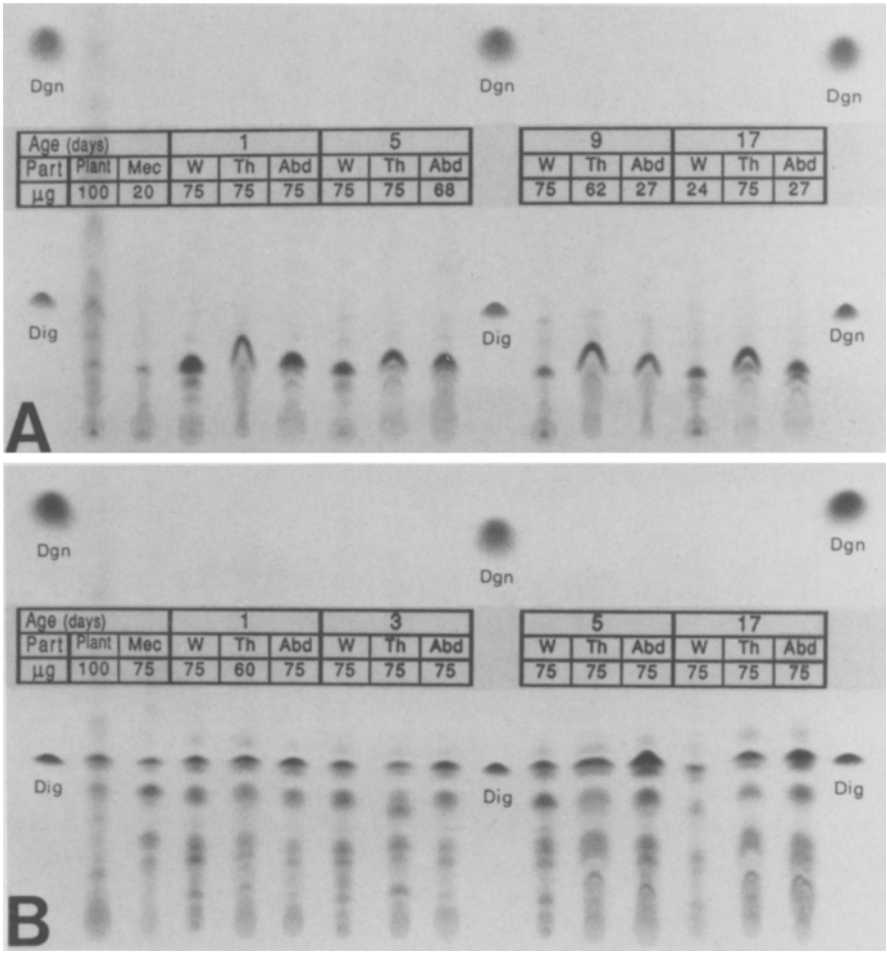

Figure 3. Thin layer chromatography of CGs found in **A** *A. syriaca* and **B** *A. humistrata* plants, the meconium (Mec) and the 3 body regions (wings, thorax, abdomen) of female butterflies aged for up to 17 days. The standards (std) are digitoxigenin (Dgn) and digitoxin (Dig)[15]. The estimated micrograms (µg) of CG spotted for each sample are indicated. The profiles show no evidence of differential loss of individual CGs in any of the body regions as the butterflies age. Male profiles were similar to those of the females.

and the summer generations[20] in which monarchs keep reproducing until they die[21].

Our data lead to two ecologically relevant conclusions. First, individual butterflies which as larvae sequestered large amounts of CGs will retain their potential emetic effect upon predators for a longer period of time than those that sequestered lesser amounts. Second, even though the monarchs may have the same initial concentrations of CGs, those that possess CGs of high emetic potency from certain milkweed species will be protected much longer than those with weakly emetic ones sequestered from other species[14]. There is in fact evidence that female monarchs prefer to oviposit on milkweed species that contain CGs of high emetic potency[22–23].

At the end of March, migrating monarchs from overwintering sites that reach the Gulf Coast states are at least 6 months old[24]. Here, they lay the majority of their eggs on the extremely toxic *A. humistrata* and *Asclepias viridis* milkweeds[25]. As new generations of adults emerge at the end of April they present the birds with maximal aposematism, i.e. their colors are bright and they have large initial amounts of CGs of high emetic potency[3]. By late May, these individual monarchs reach the northern parts of the United States and oviposit principally on *A. syriaca*. As this study shows, the butterflies of this generation will be emetic when they emerge, but their CG titers will dwindle below the emetic dosages. Nonetheless, since considerable overlapping of generations occurs over the summer[26], automimicry[27] is possible: the freshly emerged individual monarchs can serve as noxious models for the older individuals that have lost most of their CGs. Because of the loss of CGs, individual monarchs may progress from being models to mimics during their lifetime in these northern populations. Pyrrolizidine alkaloids, additional possible chemical defenses in monarch butterflies[28] appear to occur at concentrations too low to deter vertebrates[5].

We conclude that the CG-based chemical defense of adult monarch butterflies is a more dynamic three trophic-level interaction than previously envisioned[1,3].

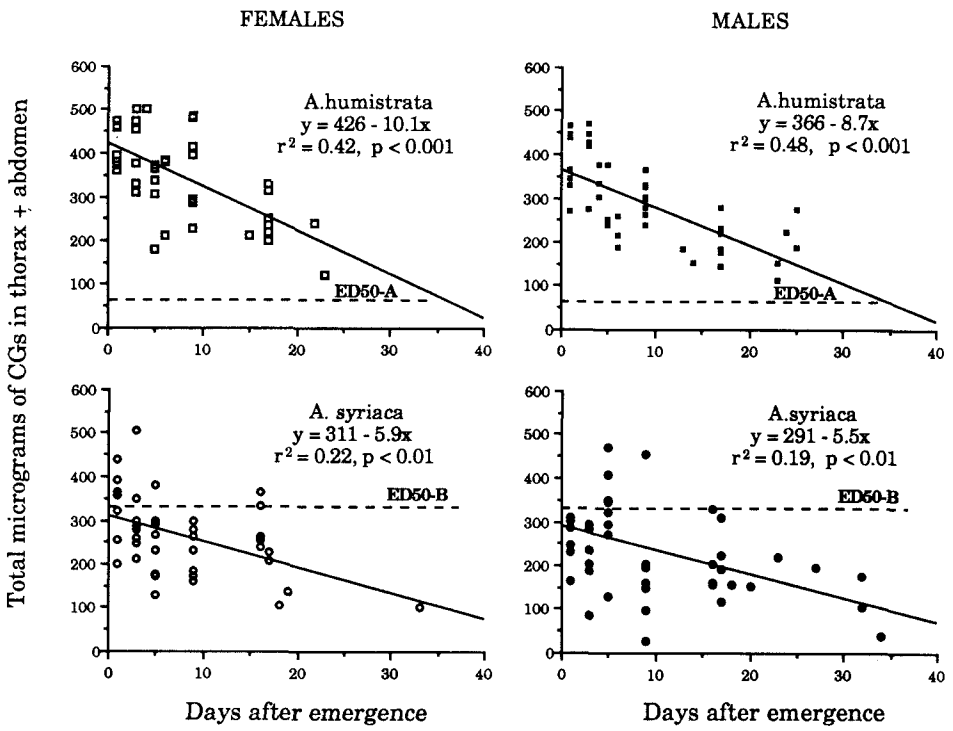

Figure 4. Individual monarchs become less emetic as they age because of the progressive loss of CGs. Dashed lines represent previously determined[14] emetic dosages (ED_{50}) to blue jays of adult monarchs reared on *A. syriaca* and *A. humistrata*, respectively. Data points above the dashed lines indicate individual butterflies that would be emetic to a blue jay. **ED50-A**: *A. humistrata* monarchs, which have CGs of higher emetic potency to blue jays, require only 57 μg/85 g bird to produce an emetic reaction in 50% of the birds. In contrast, 323 μg/85 g bird is the ED_{50} for *A. syriaca* monarchs (**ED50-B**). Because of this 5.7× higher emetic potency of the CGs in *A. humistrata*, monarchs reared on this plant remain emetic throughout their lives whereas those reared on *A. syriaca* rapidly become subemetic.

It involves not only the host plant species that the larvae of the butterflies have eaten and the diversity of predators to which they are exposed, but also the age structure of the butterfly populations.

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