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Kieran Kelly April 7, 2022

Effects of larval host plants and pyrrolizidine alkaloid containing plants on monarch butterfly (*Danaus plexippus*) mating behavior

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Abstract

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Plants and insects have co-existed for millions of years leading to intricate relationships. Danaine butterflies are a family of butterflies specialized on species of milkweed as their larval host plants and also sequester pyrrolizidine alkaloids from various plant sources during their adult life. Here we investigated how the monarch butterfly (*Danaus plexippus*) modulates its mating behavior based on pre- and post-eclosion interactions with both their milkweed host plants and plants containing pyrrolizidine alkaloid plants. We used a series of choice and no-choice mating trials to determine if monarch butterflies demonstrate assortative mating in regard to larval host plant. In choice trials, we found that male monarchs reared on a different species of milkweed host plant will outcompete male monarchs reared on the same milkweed host plant as the female monarch. Additionally, we exposed adult monarch butterflies, all reared on the same host plant, to plants containing pyrrolizidine alkaloids to determine how these immediate plant-insect interactions influenced mating behavior. We found that the addition of pyrrolizidine alkaloid containing plants did not alter monarch butterfly mating behavior. Overall, our data add to the growing list of studies demonstrating how distinct species of milkweed can influence and alter monarch butterfly behavior.

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INTRODUCTION

Plants and herbivorous insects have co-existed for millions of years, leading to intricate relationships. Countless species of insects have evolved to use certain species of plant as the host plant for their larval stages (Resh, 2009). During their adult life stages, the selection of plants for nutrients and various other chemicals can become much broader. These complex relationships are driven by the chemical compounds produced by the plant and the ways in which the insects consume and uptake these various chemicals (Jermy, 1984).

The subfamily of Danainae butterflies, or milkweed butterflies, have a very close relationship with their larval host plants. Female Danainae butterflies are specialized to lay their eggs on species of toxic milkweed. Milkweed species produce toxic, defensive compounds, called cardenolides, in their tissues. These cardenolides are produced by the milkweed as a chemical defense against predation. However, larvae within this family have evolved to incorporate these cardenolides into their own tissues (Malcolm, 1994). Cardenolides sequestered from the milkweed during the caterpillar life stage function as chemical defense against predators during the monarch's adult life (Agrawal et al., 2021). Moreover, consumption of high-cardenolide milkweeds reduces infection with a common and virulent protozoan parasites (Tan et al., 2018).

Additionally, during their adult life stage, male milkweed butterflies require nectar or plant material from pyrrolizidine alkaloid containing plants to produce the sexual pheromone required for their courtship behaviors (Edgar, 1982). Species within this subfamily of milkweed butterflies use dihydropyrrolizines in courtship behaviors where cuticular particles are released from hairpencils at the end of their abdomen onto an awaiting female. Studies have demonstrated the importance of plant-derived pheromones for the mating success of male butterflies (Honda et

al., 2018). For example, when the hairpencils of *Danaus gilippus berenice* were removed, males were capable of courting females but incapable of seducing them. Researchers were able to recover the male's mating competence by addition of a synthetic pheromone, demonstrating the importance of sex pheromones in butterfly courtship behaviors (Pliske and Eisner, 1969).

The monarch butterfly (*Danaus plexippus*) is a well-studied member of the milkweed butterfly family and is known for their transcontinental yearly migration across North America. In the fall, monarch butterflies begin their year migration to their overwintering sites in Mexico where they will hibernate for the winter (Malcolm, 1987). As spring begins, monarch butterflies awake from their hibernation, mate, and fly northwards following the growth of the milkweed (Reppert and de Roode, 2018). Like other members of the Danainae subfamily of butterflies, the monarch has a specialized relationship with its milkweed host plants (*Asclepias* spp). *Asclepias* species can vary drastically in their production and concentration of cardenolides, resulting in different chemical profiles in the adult monarch (Brower et al., 1967). In the United States, two species of milkweed available to monarch butterflies are *Asclepias curassavica* (tropical milkweed) and *Asclepias incarnata (*swamp milkweed). While the swamp milkweed is native to North America, the tropical milkweed is an invasive species that is becoming increasingly more abundant (Lemoine, 2015). Moreover, studies have shown that with an increasing rise in global temperatures, cardenolide concentrations within the tropical milkweed may become detrimental to the monarch (Faldyn et al., 2018). With its increase in range and possible deleterious effects, knowledge of how the tropical-invasive milkweed influences the subsequent mating behavior of the monarch would allow for more precise management of milkweed populations.

Furthermore, due to of their migratory abilities, monarch butterflies have adapted their mating behavior and lost the need for pheromones to initiate mating. Instead, male monarchs

participate in a mating behavior classified as forced copulation. To initiate an attempt at mating, male monarchs will grab females out of the air, wrestle them to the ground, and attempt to grasp the females' abdomens with their abdominal claspers (Hill et al., 1976). In this mating system, the direct courtship requirement for males to present females with their pheromones has been lost.

However, this does not mean that female monarch butterflies cannot sense the chemical profile of their potential mate. The hairpencilling behavior seen by other butterflies within this subfamily is not usually observed during the monarchs' forced matings, but these abdominal organs are often extruded during these encounters (Cannon, 2019). During struggles with males, females may be able to cryptically determine the chemical profile of the advancing male and may decide to resist their advances further. While monarchs no longer require pheromones to initiate mating, their associations with cardenolides and pyrrolizidine alkaloids to subsequent mating success have been largely understudied (Lawson et al., 2021)

Here we investigate the role of insect-plant interaction at two life stages of the monarch butterfly. First, we conducted multiple mating assays with monarchs raised on two different host plants, *Asclepias incarnata* and *Asclepias curassavica*. As the concentration of cardenolides varies between plant species, monarchs reared on one type of plant species have different chemical profiles and cardenolide concentrations than those raised on the other plant species. As monarch butterflies mate without the need for pheromones or chemical signals, we hypothesize that males will not assortatively mate with females and females will not preferentially avoid male attempts at mating. Second, we conduced additional mating assays with all monarchs reared on *Asclepias curassavica* and pyrrolizidine alkaloid plants added to the mating cages. Like their Danaine relatives, monarchs have been seen "scratching" at pyrrolizidine alkaloid containing

plants in nature, but there has been little research on the effect these alkaloid containing plants may have on monarch courtship and mating success (Lawson et al., 2021). We hypothesize that when given access to a pyrrolizidine alkaloid containing plants, monarch butterflies will not mate more frequently and achieve higher mating success rates.

METHODS:

Monarch Rearing:

Host Plant Choice Experiment

Monarchs used in this study were descendants of wild-caught, migratory monarchs from St. Marks, Florida and Atlanta, Georgia. We mated these wild-caught monarchs to rear the first batch of experimental monarchs. Offspring from these mated pairs were raised in a greenhouse at Emory University in Atlanta, GA under natural light and temperature conditions (range: 23.5-39.6°C). 216 monarchs were raised during February/March 2019 for the host plant mate choice assay.

Larvae raised were reared on one of two milkweed host plants, *Asclepias incarnata* or *Asclepias curassavica*. Caterpillars were housed individually on their host plant species in a clear-plastic tube (5-inch diameter x 22.5-inch height) with a netted covering fitted over the top. Newly emerged monarchs had their right forewings measured to the nearest 0.1mm using a ruler to size match males and females for the mating trials. As adults, butterflies were kept in 3.5x3.5-inch No.3 Glassine envelopes, segregated by sex, and fed on 10% honey water solution once a week before the trials. Adult monarch abdomens were taped and checked for infection by the parasite *Ophryocystis elektroscirrha* using methods outlined previously (De Roode et al., 2007). Only uninfected monarchs were used in these trials.

PA Influence Experiment

Monarchs used in this study were descendants of wild-caught monarchs from Florida, Georgia, and Puerto Rico. We mated these wild-caught monarchs to rear the first batch of experimental monarchs. Eastern North American and Puerto Rican monarchs were segregated

for all stages of the experiment. Offspring from these mated pairs were raised in a greenhouse at Emory University in Atlanta, GA under natural light and temperature conditions (range: 23.5-39.6°C). Sixty-eight monarchs were raised during August 2021 for the initial trials. Additionally, 112 monarchs were reared in October 2021 for subsequent trials.

Monarch larvae were all reared on one milkweed host plant, *Asclepias curassavica*. Caterpillars were housed individually or in pairs on the plant in a clear-plastic tube (5-inch diameter x 22.5-inch height) with a netted covering fitted over the top. After eclosion from the chrysalis, all adult monarchs were given an identification code and weighed. Newly emerged monarchs had their right forewings measured to the nearest 0.1mm using a ruler to size match males and females for the mating trials. Before the start of the trials, butterflies were kept in 3.5x3.5-inch No.3 Glassine envelopes, segregated by sex, and fed on 10% honey water solution once a week before the trials. Adult monarch abdomens were taped and checked for infection by the parasite *Ophryocystis elektroscirrha* using methods previously outlined (De Roode et al., 2007). Only uninfected monarchs were used in these trials.

Experimental design:

Overview

The overall goal of our studies was to test how larval and adult monarch-plant interactions influence monarch behavior and mating success. We conducted two experiments, one focused on the effect larval host plant on adult mating behavior and one focused on the effect of pyrrolizidine alkaloid containing plants. Both experiments involved mating trails where monarchs were placed in a 11-inch x 12-inch mesh popup insect cages (Carolina Biological Supply Company, Burlington, NC, USA). All trials occurred in a walk-in environmental

chamber (Environmental Specialties, Inc., Raleigh, NC, USA) set to a 14:10h light/dark cycle at 26˚C with 50% relative humidity.

Host Plant Mate Choice Experiment details

This experiment focused on how larval host plant influences the subsequent mating behavior of monarch butterflies. For the host plant mating trials, cages were divided into choice and no-choice trials (Figure 1). Choice cages contained one male raised on *Asclepias incarnata*, one male raised on *Asclepias curassavica*, and one female raised on either *Asclepias incarnata* or *Asclepias curassavica* (Figure 1a, 1b). In no-choice trials all monarchs were raised on either *Asclepias incarnata* or *Asclepias curassavica* (Figure 1c, 1d).

Prior to the start of the experiment, males in each cage were marked with a unique combination of 0.25-inch blue and yellow stickers placed on the ventral side of each wing. These markings were used for identifying which male was making an attempt or *in copula*. All mating trials lasted for approximately five days. Monarchs were provided with 10% honey water in a sponge in a petri dish that was refilled daily.

Additionally, a subset of cages from each trial were filmed continuously for the duration of the experiment. High-definition Owl AHD10-841-B security cameras were hung approximately 12-inches above each cage and provided a clear recording of the entire cage 24 hrs. a day. Cameras were equipped with infrared bulbs to film in complete darkness. Observers conducted spot-checks twice a day and recorded the identification of any mating pairs. Video analysis was conducted after all trials concluded.

PA Influence Experiment details

This experiment focused on how access to a pyrrolizidine alkaloid containing plant influences male monarch mating success. Mating trials consisted of two treatments: cages containing two clippings of PA plants and cages with no plants (Figure 2). The species of PA plant selected was the blue mistflower (see below for plant details). The clippings were placed in test tubes taped to the inside of the cage. Cages that did not receive clippings were also given taped test tubes.

This experiment was conducted using two different sex ratios across three trials. The first trial employed a 1:1 male to female sex ratio (Figure 2a, 2b). In the second and third trials, we used a 2:1 male to female sex ratio (Figure 2c, 2d). Previous work from our lab has shown a male dominated sex ratio in mating cages increases the interaction between males and females. All mating trials lasted for approximately five days. Monarchs were provided with 10% honey water in a sponge in a petri dish that was refilled daily.

Male and female monarchs in the 1:1 trial were given no identification markings before being placed in the cage. Marking the monarchs in this trial was not necessary as morphological traits can be used to differentiate males from females, and there were no other males in the cage. Prior to the 2:1 trials, male monarchs were each marked with a black Sharpie along the top most cell on the ventral side of their forewings. Males were identified by having their left or right ventral cell colored in. Females in these trials were left unmarked.

Additionally, a subset of cages from each trial were filmed continuously for the duration of the experiment. High-definition Owl AHD10-841-B security cameras were hung approximately 12-inches above each cage and provided a clear recording of the entire cage 24 hrs. a day. Cameras were equipped with infrared bulbs to film in complete darkness. Observers

conducted spot-checks twice a day and recorded the identification of any mating pairs. Video analysis was conducted after all trials concluded.

Selecting the PA plant

Conoclinium coelestinum, or the blue mistflower, was chosen as the plant species for this experiment as it has known pyrrolizidine alkaloids and natural history observations with the monarch (Herz et al., 1980; Lawson et al., 2021). Additionally, monarch butterflies have been seen in nature interacting and probing at the flowers and leaves of this species. Clippings of blue mistflower were taken from around the Emory University area and dried in a 65°C drying oven overnight. To confirm the clippings were of blue mistflower, we used the app Seek by iNaturalist. iNaturalist utilizes computer vision systems trained on users' photos and other databases, such as Catalogue of Life, uBio, and Wikimedia Commons, in order to provide automated taxon suggestions (INaturalist, 2020).

Quantification of mating behavior

For all trials described above, we quantified seven measures of mating performance. Monarch mating behavior was subdivided into two stages: attempt stage and copulatory stage. The attempt stage begins with the male monarch lunging on females to physically coerce them into mating. The pouncing behavior is easily identified from other random collisions that occur within the cage. Females during the attempt stage will apply various forms of resistance to deter the male's advances. An attempt is successful and ends when the pair achieve copulation. The attempt is classified as unsuccessful if either the male gives up or the female escapes the male's grasp. Only cages that were video-recorded were used to measure attempt time and success.

Observers watched the recordings and recorded which butterflies were involved in the attempt and the duration of each attempt.

During the copulation stage, multiple metrics of mating success were recorded. Copulation begins immediately after a successful attempt by a male. The pair has achieved copulation when the male latches onto the distal tip of the female's abdomen with his genital claspers (Brower et al., 2008). After the male and female are attached at the abdomen, they will orientate themselves into a stereotypical Lepidopteran mating posture. In this posture, the male and female will face opposite directions while the tips of their abdomens remained attached. Copulation ends the moment the mating pair splits up. To quantify the copulation stage, we used both twice-daily spot checks and video recordings. Mating cages were checked once in the morning and once before 7:00pm EST each day of the experiment. Spot checks were used to determine which butterflies were *in copula* and the total number of matings by each butterfly.

Statistical Analysis

For both experiments listed above, all analyses were conducted using JMP v15.0. We first computed the attempt success rate for each treatment by averaging each individual monarch's attempt success rate (number of matings/ total number of attempts) across each trial type. These rates were calculated using only cages that were recorded for the full length of the experiment. Next, we used daily observer spot checks to sum the total number of matings amassed by each trial over the course of the experiment. Finally, we quantified the percentage of males from each treatment who successfully mated.

To test for a difference between attempt success rates and the percentage of males that successfully mated, we conducted Fisher's exact test within the choice trials. Additionally, for

the analysis of the total matings amassed by each trial, we tested the totals observed against a random 50-50 mating success using Chi-squared tests with an $\alpha=0.05$.

The only analytical difference between the host plant mate choice and PA influence experiments was the determination of which male monarch achieved a successful copulation first in the host plant experiment. We tested to see if there was a difference in which male, either reared on the same or different host plant, was in the first successful mating with the female in the cage using a Chi-squared test with an $\alpha=0.05$ against a random 50-50 mate preference.

RESULTS

Host Plant Mate Choice Experiment Results

The host plant mate choice experiment included a total of 72 total mating trials. These trials consisted of 48 choice and 24 no-choice trials. The choice trials were divided into 24 cages containing a *curassavica* raised female and 24 with an *incarnata* raised female. The no-choice trials contained 12 cages with all *curassavica* raised monarchs and 12 cages with all *incarnata* raised monarchs. Within the choice trials, 50% (12/24) of the *curassavica* female cages and 50% (12/24) of the *incarnata* female cages were filmed continuously for the 5-day experiment. Within the no-choice trials, 50% (6/12) of the *curassavica* only monarchs and 50% (6/12) of the control cages were filmed continuously for the 5-day experiment.

For these trials, we first analyzed the first successful mating of each male and whether the female was reared on the same plant (Figure 3). In the no choice trials we observed a 100% success rate with males mating with a female of the same plant. This was expected, of course, because there was no other male-female combination in these cages. In the choice trials, 42.8% of the first matings observed occurred between monarchs reared on different host plants, whereas 57% of first matings occurred between monarchs reared on the same host plant. These proportions of first mating were tested against a random 50-50 mate preference for host plant using a Chi-squared test with an (alpha) = 0.05. We found that these observed proportions did not significantly deviate from random choice (Chi-squared test; n = 21, df = 1, $\chi^2 = 0.429$, P = 0.513).

Next, we used cage video recordings to quantify mating attempts from the 36 (12 choice *curassavica*, 12 choice *incarnata*, 6 no-choice *curassavica*, 6 no-choice *curassavica*) recorded cages. Over the course of the study, we recorded 88 attempts from these cages. We grouped the

attempts and matings from our choice trials into two categories: males from the same plant species and males from different plant species. Overall, males in the no-choice trials achieved an average attempt success rate of 42.9%. In the choice trials, males reared on the same host plant achieved an average attempt success rate of 55.6% and males reared on a different host achieved a success rate of 62.5% (Figure 4A). However, while on average males reared on different host plants seemed to achieve a higher attempt success rate than males reared on the same host plant, these differences were not statistically significant (Fisher's exact test within choice trials; n = 46, $P = 0.763$).

We further analyzed the total number of matings achieved by each type of mating combination over the course of the study (Figure 4B). In the no choice trials, males reared on the same plant achieved a total of 36 successful matings. In the choice trials, males reared on the different host plant achieved significantly more matings than males reared on the same type of host plant (Chi-squared test within choice trials; n = 74, df = 1, $\chi^2 = 5.41$, $P = 0.020$). While both treatments of males had similar attempt success rates, different host plant reared males outcompeted the same plant males by 20 total matings (same plant: n = 47, different plant: n = 27).

Lastly, we calculated the percentage of males that copulated with females. On occasion males did not copulate or attempt with a female over the course of the five days (Figure 4C). In the no choice trials, 60.4% (29/48) of males achieved a successful copulation with the female. On average, 62.5% (60/96) of males raised on the different host plants achieved a successful mating whereas 43.8% (42/96) of males raised on the same host plant successfully mated. However, these percent differences were not statistically different (Fisher's exact test; n = 96, $P = 0.678$).

PA Influence Experiment Results

The PA influence experiment included a total of 62 total mating trials. These trials consisted of 24 1:1 and 38 2:1-male to female trials. Within the 1:1 treatment, 50% (7/14) of the PA containing cages and 50% (5/10) of the control cages were filmed continuously for the 5-day experiment. Within the 2:1 treatment, 35% (7/20) of the PA containing cages and 33% (6/18) of the control cages were filmed continuously for the 5-day experiment.

For these sets of trials, we measured the same mating metrics as those in Figure 4. We first computed the average attempt success rate for males in cages with and without PA plants. Males in cages without PA containing plants achieved an attempt success rate of 25.4% and males in cages with PA plants achieved a success rate of 23.4% (Figure 5A). These attempt rates were not statistically different from each other (Fisher's exact test; n = 106, $P = 1.000$).

Next, we summed the total number of matings amassed by each trial type. In cages without PA plants, males amassed a total of 39 matings whereas males in cages with PA plants amassed a total of 28 total matings (Figure 5B). However, while males in cages without PA plants amassed 11 more overall matings, these totals were not statistically different (Chi-squared test; n = 67, df = 1, $\chi^2 = 1.81$, $P = 0.179$).

Finally, we calculated the percentage of males within each cage type who successfully mated over the course of the study. Across all the cages without PA plants, 45.5% (20/44) of males successfully mated. In all cages with PA plants, 36.4% (20/55) of males successfully mated (Figure 5C). However, while almost 10% more of the males in cages without PA plants achieved a successful mating, these proportions were not statistically different (Fisher's exact test; n = 99, $P = 0.413$).

DISCUSSION

Our results demonstrate the effects, or lack thereof, of how interactions with plants modulate monarch mating behavior. Overall, we found that when male monarchs are reared on different host plant species, males reared on a host plant that was not the same as the females, outcompeted males reared on the same species of host plant as the female (Figure 4B). This result is especially interesting as the male's host plant association to that of the females did not influence that males' attempt success ratio (Figure 4A). As the monarch mating system is male initiated, females have developed mechanisms for mate rejection. Previous research has investigated if female monarchs are capable of cryptic mate choice and tested to see if they can preferentially chose the paternity of their offspring (Mongue et al., 2015). This research found that females cannot choose the paternity of their offspring post copulation and so only have physical mechanisms of rejection to regulate their mating behavior. Future research should investigate if these host plant induced differences in the total observed matings are caused by an increase in the rejection of overall males by the female or a decrease in the interest of attempting to mate with a female of the same host plant by a male.

Unlike the influence of host plant on total mating success, we found no evidence that addition of pyrrolizidine alkaloid containing plants influenced monarch mating behavior. Within the study, males that were exposed to the plants did not perform better in their attempt success, total matings, or the proportion of males that successfully mated (Figure 5). However, while the plants did not increase mating success, they also did not significantly reduce mating success compared to the control cages. This demonstrates that addition of plant clippings to mating cages does not interfere, alter, or provide a barrier to natural monarch mating behavior. This

experimental design, along with cameras to record cages, can be used in subsequent studies investigating various other insects' interactions with species of specific plants.

Additionally, our research into the effects of pyrrolizidine alkaloid containing plants on monarch butterfly mating behavior follows up previous research into the influence of these plants in other milkweed butterflies. Recent studies into the other *Danaus* species located in the Americas (*Danaus erippus* and *Danaus gilippus*) found that, while *D. gilippus* requires high levels of pyrrolizidine alkaloids and *Danaus erippus* does not, their interaction with these plants did not differ (Ramos et al., 2020). However, it has been shown that pyrrolizidine alkaloids can be transferred between males and females during copulation as a nuptial gift in the male's spermatophore (Dussourd et al., 1989). Therefore, it may be that monarch butterflies do not utilize pyrrolizidine alkaloids to modify their mating behavior but to provide females with a "nuptial gift" to increase the success of fertilization. For that reason, future research should focus on the fitness of the offspring from males that did and did not interact with pyrrolizidine alkaloid containing plants.

When looking between studies, we noticed a decrease in both the attempt success rate and percentage of males that successfully mated. Males that were used in the host plant experiment, on average, were observed having attempt success rates more than 20% higher than males used during the pyrrolizidine alkaloid plant experiment. This difference in mating behavior may be explained by the time of year at which each experiment was conducted. The host plant mate choice experiment was conducted during the early spring (February/March) of 2019 and the pyrrolidine alkaloid plant influence experiment was carried out during the late fall and winter (August and October). As monarch butterflies are a migratory species, they mediate their reproductive development during their flight back to their overwinter grounds in Mexico.

This return flight occurs yearly during the late fall and winter (Urquhart and Urquhart, 1978). Both male and female monarch butterflies enter reproductive diapause during this time of year and experience a significant reduction in their physical reproductive tracts (Brower et al., 1977; Herman, 1973). Therefore, it could be possible that some of the monarchs reared in the late autumn for the pyrrolizidine alkaloid experiment were in reproductive diapause. Subsequent research investigating monarch butterfly mating behaviors should consider the time of year in which the study is conducted as this can greatly influence their behaviors. Alternatively, the sex ratios employed in the experiments could be responsible for the differences, as we have more recently found that a 2:1 ratio results in higher mating success.

Overall, we conducted one of the first tests investigating assortative mating based on the species of host plant the monarch butterfly was reared on. As climate change continues to increase global temperatures and alter the growth range for various milkweed species, understanding the mating dynamics between monarchs reared on these different species can inform conversationalists on the best ways to manage milkweed populations. As male monarchs reared on a different host plant than the female outcompeted males reared on the same host plant, it could benefit local monarch populations by sustaining populations of multiple species of milkweed over a monoculture. In addition, our experiment on pyrrolizidine alkaloid plants and monarch butterflies is the first to test for the immediate effects of interaction on monarch butterfly mating. Since there have been well documented accounts of interactions between monarchs and these alkaloid containing plants in nature, many have called for an increase into the investigation of this relationship (Lawson et al., 2021). While our study did not find an influence of interaction on mating success, pyrrolizidine alkaloid compounds may play various roles in monarch butterfly physiology such as defense against predation and resistance to

parasites (Majewska et al., 2019; De Roode et al., 2013). These studies continue to highlight the importance and interconnected relationship monarch butterflies have with plants.

Figures and Graphs

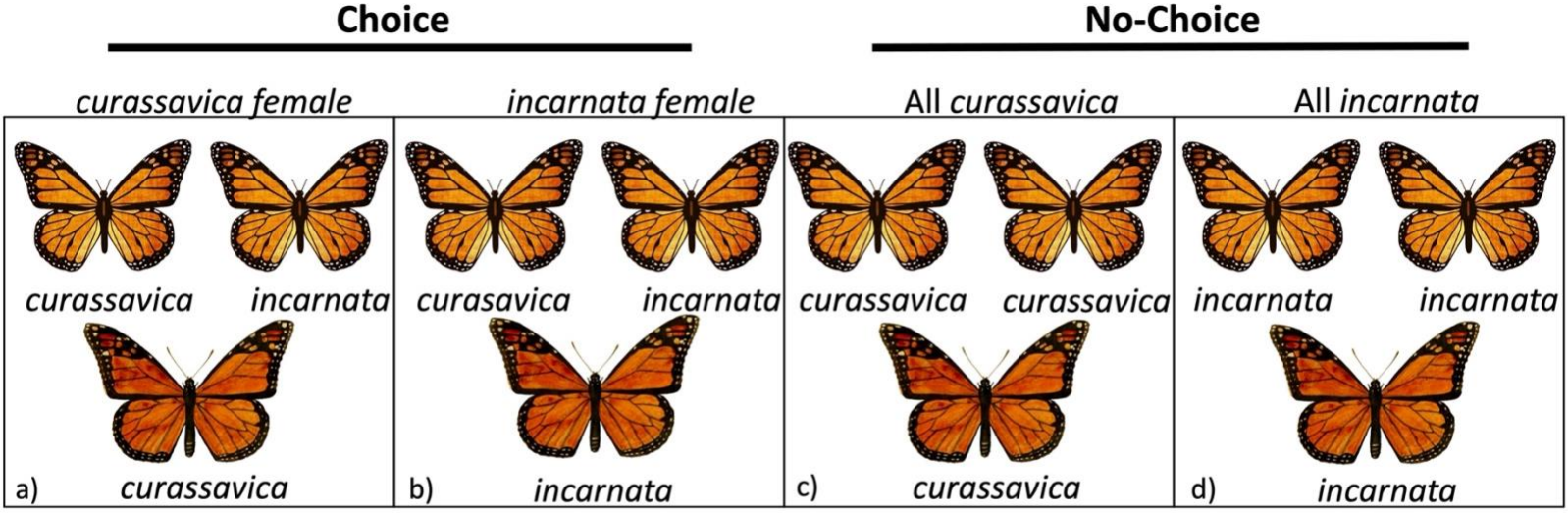

Figure 1: Host plant mate choice experimental design. See text for details. In trial schematic, males are on top, and females are on bottom.

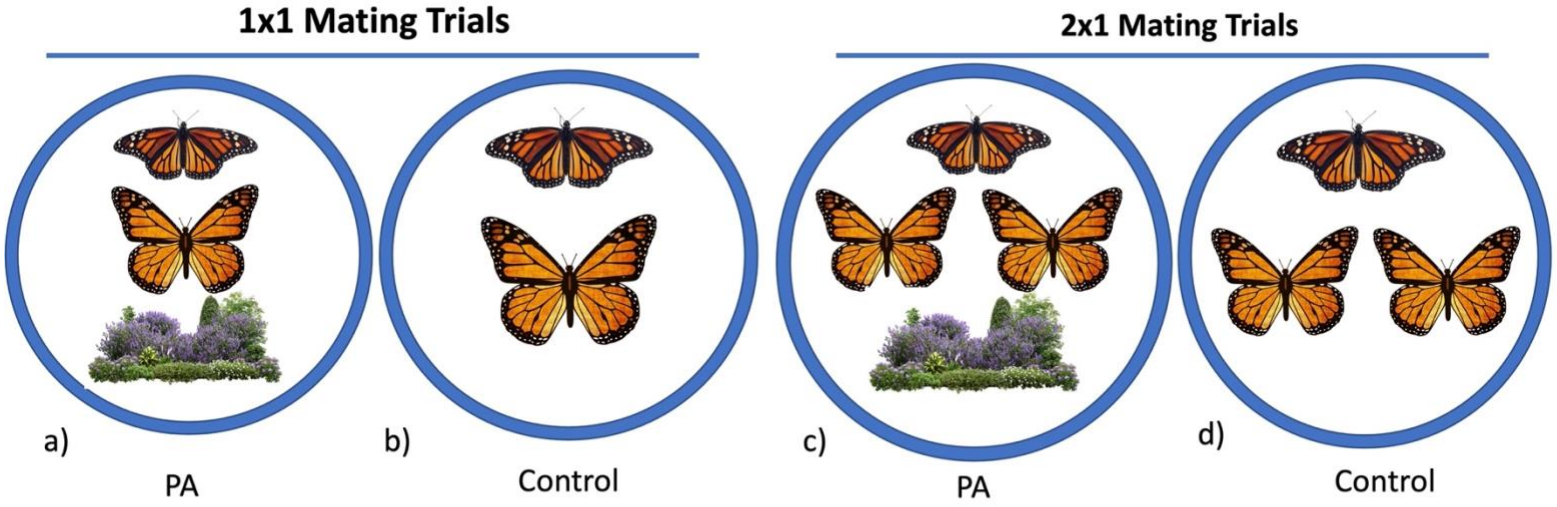

Figure 2: PA influence experimental design. See text for details. In trial schematics, females are on top, and males are on bottom.

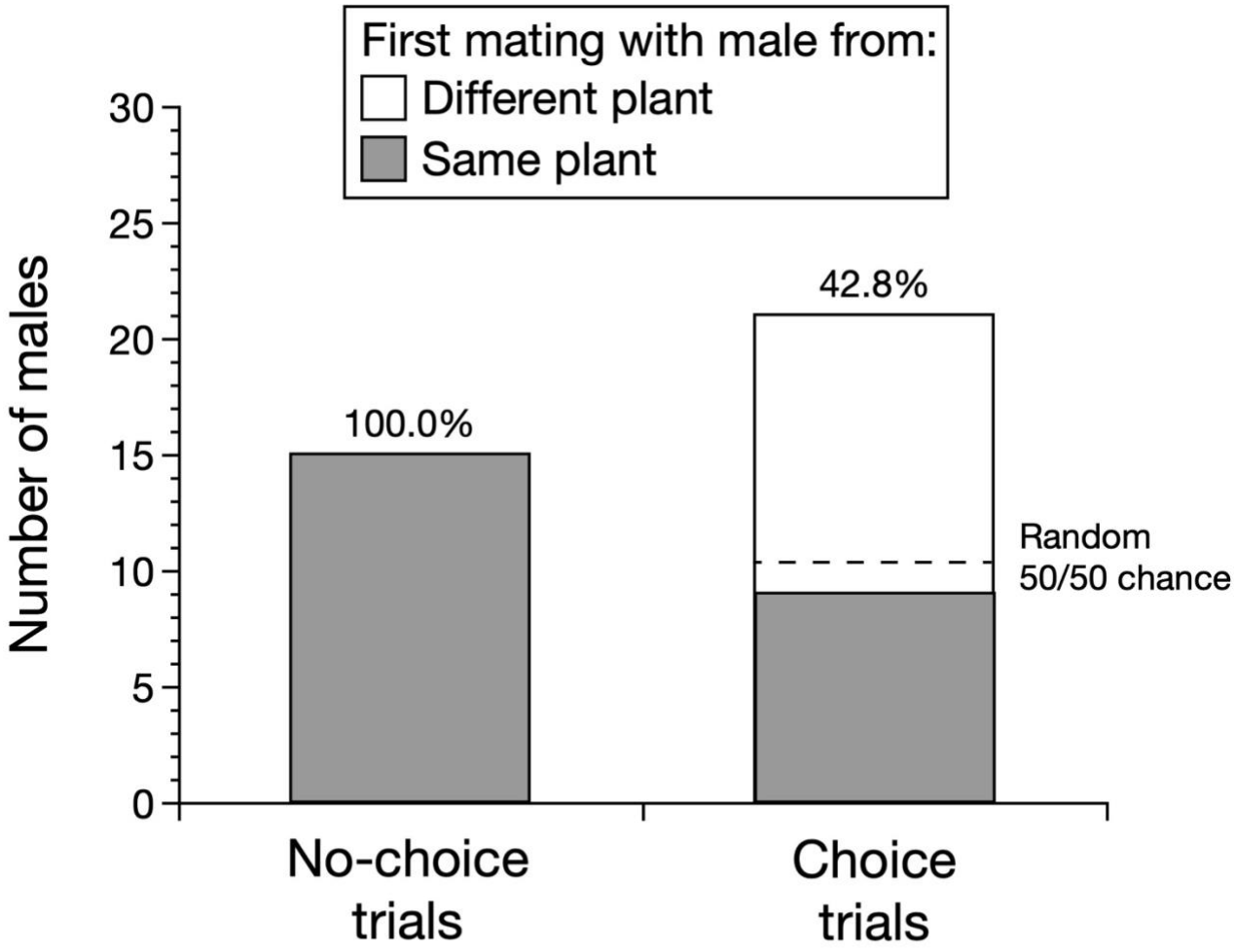

Figure 3: First mating from host plant mate choice experiment. No choice trials include both *curassavica* and *incarnata* only cages.

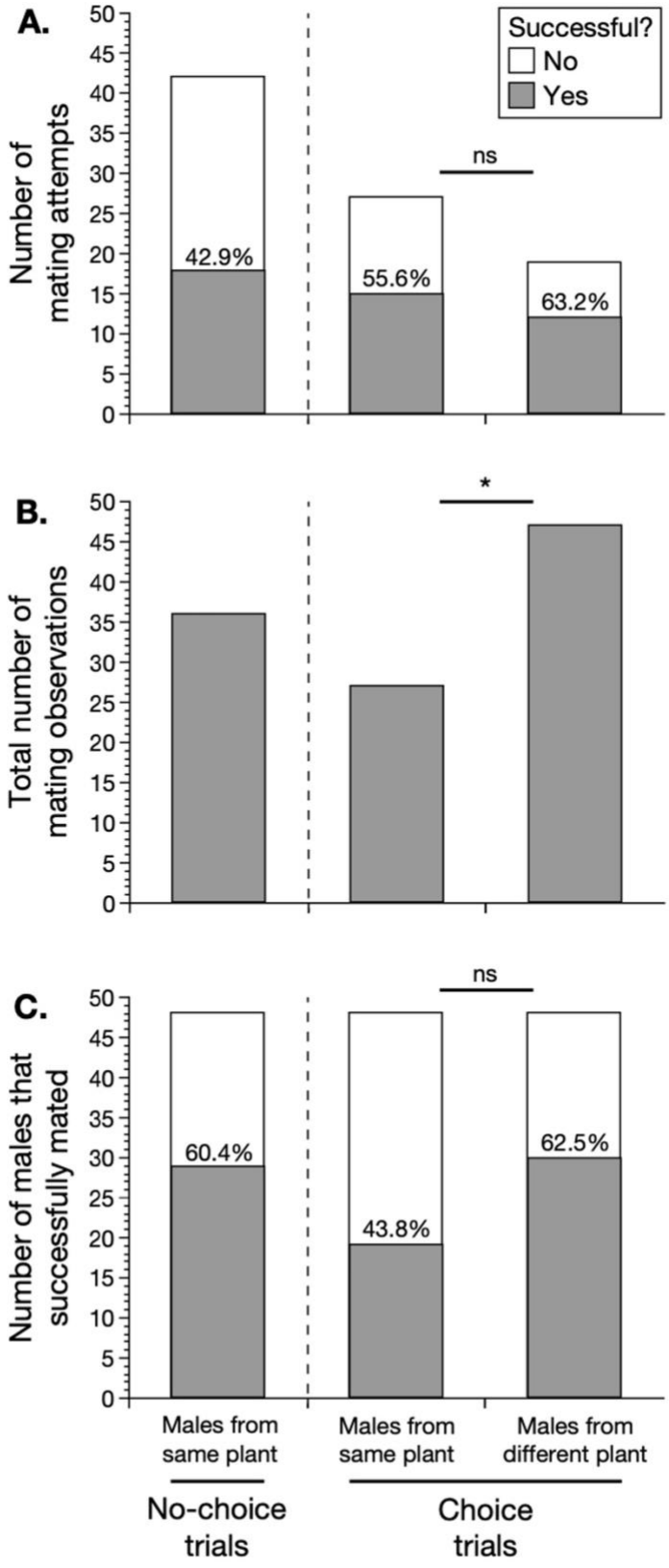

Figure 4: Mating outcomes from the host plant mate choice experiment. Each panel (A-C) uses the mating categories listed on the bottom.

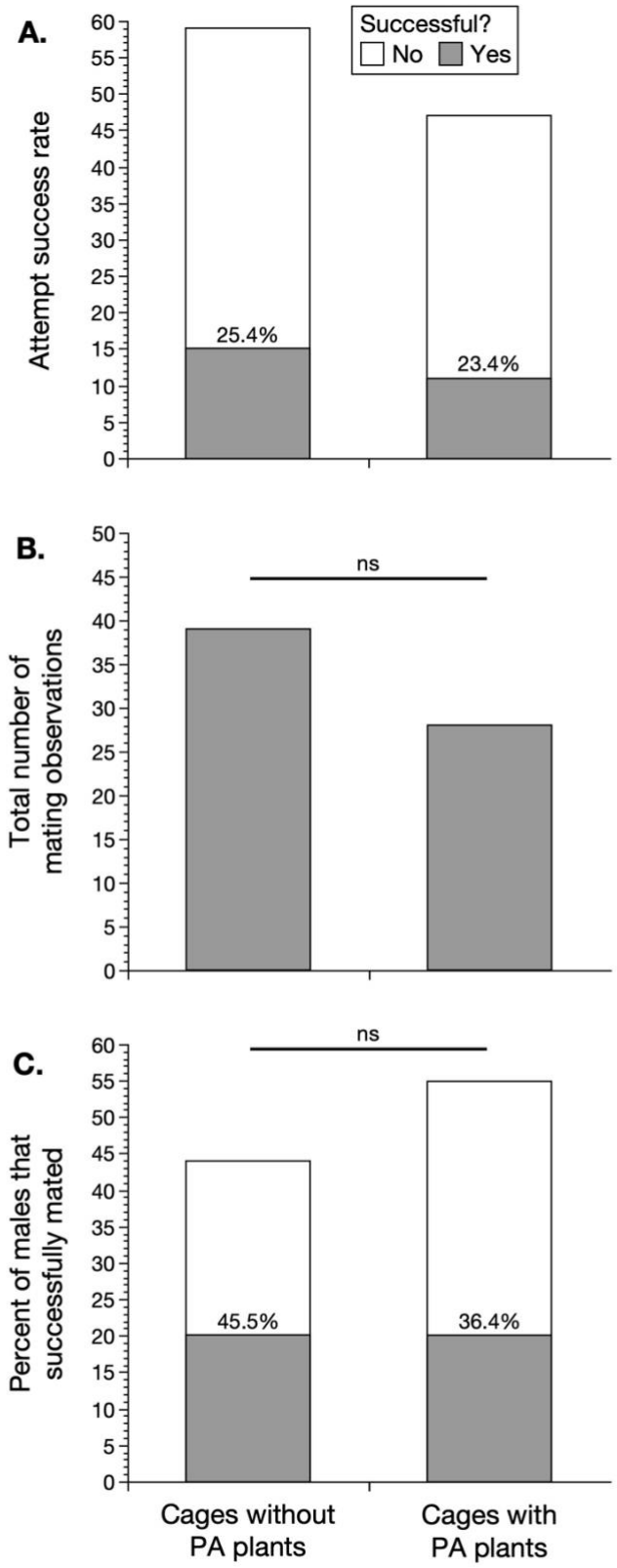

Figure 5: Mating outcomes from the PA influence experiment. Each panel (A-C) uses the trial categories listed on the bottom.

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