Supplementary Information: Bayesian life-stage model

Asclepias syriaca degree-day development submodel

In the monarch colony maintained at Iowa State University, eggs take four days at 27.8°C (82°F) and five days at 21.1°C (70°F) to hatch (K. Bidne, personal communication). This equates to 106.2 mean degree days (Table S1), which is consistent with Oberhauser (2004). For larval stage durations, we used data from larvae raised on A. syriaca in the laboratory (N. Krishnan, unpublished data) and published developmental rates (Krishnan et al. 2021a; Table S1). Development time for North American monarchs reared on Asclepias syriaca (Table S1) is substantially longer than developmental time for Australian monarchs reared on Gomphocarpus fruticosus (Zalucki 1982). Development time may not be the same in the laboratory and field. In the laboratory, larvae are fed ad libitum, but larvae in nature may not always have high-quality food available, especially when moving between plants (Fisher et al. 2020b), which can slow development. On the other hand, daily temperature fluctuations can accelerate development (Zalucki 1982), and basking behavior may decrease development time by up to 30% (Rawlins and Lederhouse 1981). We used the laboratory-based degree-day estimates for development from egg through 5th instar and decreased them by 15%, the halfway point between 0% and 30% reported by Rawlins and Lederhouse (1981) (Table S1; Table S2). Using this new A. syriaca model for development time, we revised average cumulative survival probability from 0.014 (Grant et al. 2020) to 0.036 (Figure 3). New estimates for individual sites range from 0.006 to 0.13 (Figure S1). Future analyses of larval survival in North America, where most monarchs develop on common milkweed, should employ this updated model. Code for the updated Bayesian survival model and for the current version of the agent-based model are accessible at www.github.com/tgrant7.
Table S1. Degree days from different monarch development experiments. Australia data is from Zalucki (1982). Niranjana Krishnan (NK) and Keith Bidne (KB) data (personal communication data combined with data reported in Krishnan et al. 2021a) are from North American monarchs raised on common milkweed (*Asclepias syriaca*) in the laboratory under constant temperature (21°C). The final row is the NK and KB data reduced by 15%.

|                | Egg | First | Second | Third | Fourth | Fifth | Total |
|----------------|-----|-------|--------|-------|--------|-------|-------|
| Australia      | 45  | 32.3  | 27.8   | 24.5  | 35.7   | 66.6  | 231.9 |
| NK, KB data    | 106.2 | 54.7 | 47.0 | 44.1 | 63.8 | 106.7 | 422.5 |
| NK, KB data less 15% | 90.2  | 46.5  | 39.9  | 37.5  | 54.2  | 90.7  | 359.1 |

Table S2. Development time in days at 21°C for the degree days in Table 1. Niranjana Krishnan (NK) and Keith Bidne (KB) data (personal communication data combined with data reported in Krishnan et al. 2021a) are from North American monarchs raised on common milkweed (*Asclepias syriaca*) in the laboratory under constant temperature (21°C). The final row is the NK and KB data reduced by 15%.

|                | Egg | First | Second | Third | Fourth | Fifth | Total |
|----------------|-----|-------|--------|-------|--------|-------|-------|
| Australia      | 2.1 | 1.5   | 1.3    | 1.2   | 1.7    | 3.2   | 11.0  |
| NK and KB data | 5.0 | 2.8   | 2.0    | 2.5   | 3.0    | 5.4   | 20.7  |
| NK, KB data less 15% | 4.3  | 2.4   | 1.7    | 2.1   | 2.5    | 4.6   | 17.6  |
**Figure S1:** Monarch larval survival estimates from a reanalysis of the individual field sites from Grant et al. (2020). Point estimates are the median of the posterior distribution and error bars are the 0.025 and 0.975 quantiles of the posterior distribution. Site names are comprised of a state or province (IA-Iowa or ON-Ontario) and a habitat type (ROW-right of ways, AGR-agriculture field border, and NAT-natural area), and a sequential number. Sites are grouped by state or province and habitat type.

![Graph showing survival probability for different sites and groups.](image)

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Supplementary Information: Simulating adult monarch production

Figure S2. Ecoregions across Iowa’s 99 counties (based on Griffith et al. 1994).
Figure S3. The Des Moines Lobe ecoregion in Iowa with 17 landcover types. Major rivers and streams (fourth, fifth, and sixth order) and counties are also shown (GM – Genetically Modified; Dev – Development; ROW - Right-of-ways). (Tyler Grant, unpublished data; based on methods described in Grant et al. 2018, 2021).
**Figure S4.** A small area of southern Webster County in the Des Moines Lobe of Iowa showing landcover types used in this analysis (GM – Genetically Modified; ROW – Right of Ways; DEV-Development). (Tyler Grant, unpublished data; based on methods described in Grant et al. 2018, 2021).
Figure S5. Eggs laid by landcover type for Scenario 1, 2, and 3. The five roadside categories were combined. Landcover types with no eggs (water, high intensity development), or with so few eggs that they were not visible on the graph (forests, other agriculture) were not included. Inset bar graph shows total eggs laid per scenario. Scenario 1 is the current milkweed density on the landscape; Scenarios 2 and 3 reflect maximum and moderate habitat establishment, respectively (IMCC 2018).
Figure S6. Density plots (smoothed histograms) of eggs laid per monarch agent for the 200,000 monarch agents in Scenario 1 (solid line), Scenario 2 (long dashes), and Scenario 3 (short dashes). Scenario 1 is the current milkweed density on the landscape; Scenarios 2 and 3 reflect maximum and moderate habitat establishment, respectively (IMCC 2018).
Figure S7. Generalized additive model regression fit to egg density by shape index. The 63,375 plotted points are represented using kernel density where darker blue represents a higher density of points. $R^2 = 0.0122$, $p < 0.001$. The 95% confidence interval is shown as a gray band.
Figure S8. Generalized additive model regression fit to egg density by fractal index. The 63,375 plotted points are represented using kernel density where darker blue represents a higher density of points. $R^2 = 0.0164$, $p < 0.001$. The 95% confidence interval is shown as a gray band.
**Figure S9.** Generalized additive model regression fit to egg density by perimeter length. The 63,375 plotted points are represented using kernel density where darker blue represents a higher density of points. $R^2 = 0.0164$, $p < 0.001$. The 95% confidence interval is shown as a gray band.

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Supplementary Information: Monarch movement ecology and habitat utilization

Figure S10. Radio-tagged monarchs showing A) attachment site on ventral abdomen and antenna extending behind; and B) tagged female feeding on coneflower nectar.

Because methods for tracking insect movement are limited at the landscape scale (Osborne et al. 2002, Asplen 2018, Kral-O’Brien and Harmon 2021), movement is often inferred with indirect methods such as trapping, molecular markers, and mass mark-recapture (Urquhart 1987, Reynolds et al. 1997, Showers 1997, Southwood and Henderson 2000, Ide 2002, Osborne et al. 2002, Kim and Sappington 2013, Nagoshi et al. 2015). However, directly quantifying movement characteristics of individual butterflies, including natural flight step lengths and flight directionality, can provide finer-scale data with which to interpret movement patterns. Within 50 m of an observer, movement patterns have been characterized for over 25 butterfly species with visual observations in tandem with Geospatial Positioning System units (Schultz and Crone 2001, Schultz et al. 2012, 2017, Kallioniemi et al. 2014, Fernández et al. 2016). Our research group has pushed the limit of current technology to directly track large, flying insects at a landscape scale (~64-ha). We used a combination of handheld and automated (Fisher et al. 2021) VHF radio telemetry techniques to estimate the locations of 108 radio tagged monarch butterflies in several field scenarios, including within resource-rich habitat (Figure S10) (Fisher et al. 2020), within resource-devoid habitat (Fisher and Bradbury 2021), and across a mosaic of habitat including prairie, grass-dominated fields, crop, roadsides, and habitat edges (Fisher and
Bradbury 2022). To date, our efforts provide the most robust dataset of directly tracked movement of individual breeding-season monarch butterflies to assess utilization of agricultural landscapes.

**Figure S11.** Outdoor tethered flight experiments to track orientation of monarch butterflies relative to an artificial cluster of potted flowering forbs and milkweed placed at different distances upwind of the flight mill platform. Data were analyzed from a total of 93 monarchs of mixed sexes. Flight mill design allowed pivoting around the central post, and tether attachment also allowed the butterfly to swivel around the tether pin. Overhead video camera footage was analyzed for butterfly orientation and wind direction at 5-sec intervals over a 10-min flight trial.
Attaching insects to devices that allow flight within a constrained space, known as tethered flight, has proven an effective means to study flight behaviors in controlled settings (Naranjo, 2019). One such device, the flight simulator, allows an insect to pivot atop a central pillar (Dreyer et al. 2021) and has been used extensively in studies of monarch butterfly migratory flight and orientation (Mouritsen and Frost 2002, Reppert et al. 2004, Wilcox et al. 2021). Flight mills are another type of system, which allow a tethered insect to fly in a circular path around a central axis (e.g., Jones et al. 2016, Yu et al. 2019), and occasionally have been used to characterize monarch migratory flight performance (Bradley and Altizer 2005, Talla et al. 2020). However, few experiments have examined tethered flight of non-migratory monarchs (Garlick 2007, Franzke et al. 2020). Most tethered flight studies, whether of migratory or non-migratory monarchs, have been conducted either indoors or outdoors with minimal sensory information available. To probe monarch perceptual range, we needed animals to have a full array of sensory cues available. Therefore, we incorporated flight mills into field settings, asking how monarch orientation varied at different distances from a cluster of potted milkweed and nectar plants (Mullins 2021). We designed low resistance flights mills, based on those of Yu et al. (2019) and Garlick (2007) in which monarchs were attached to one end of an arm that rotated in the horizontal plane around a central axis (see Figure S11). We attached monarchs via a 3D-printed tether, which was affixed to the butterfly’s dorsal thorax with superglue and connected to the arm through an eyelet on a swivel-pin. This enabled 360° rotation in the horizontal plane around both the central axis of the flight mill and the point of attachment. Using video recorded from directly above the mill, we assessed individual orientation in relation not only to resource location, but also wind direction, using a small piece of tissue paper mounted to the top of the flight mill as a wind pennant. Wild-caught females and males were held together in a laboratory cage without food for 48 h before testing. Preliminary experiments indicated food restriction for this time period increased feeding behavior, and thus presumably motivation to forage, without preventing untethered flight to floral resources 3 m away. Flight-mill tests were conducted with males and females in an open field of mowed grass on days of winds < 16 km/h and temperatures between 20-30°C. Male and female behavior on the flight mills did not differ, so data from the sexes were combined for analyses.
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Supplementary Information: Evaluating risks of pesticide use to milkweed and monarchs.

Experimental designs of insecticide toxicity studies conducted on monarch butterflies.

Oberhauser et al. (2006): Experiment 1. Common milkweed leaves were collected from sites sprayed with permethrin barrier treatments; individual leaves were provided to individual monarch larvae until leaves dried out or were consumed; untreated leaves were provided thereafter and in the control group. Experiment 2. Tropical milkweed plants were sprayed with diluted permethrin formulations (0.1 and 0.5%); control leaves were unsprayed or sprayed with diluent. Larvae were provided new treated or control leaves daily until pupation. Experiment 3. Mated females were either introduced into cages with unsprayed plants (control cages), unsprayed, diluent-sprayed, and permethrin-sprayed plants (choice cages), or permethrin-sprayed plants (permethrin cages) and allowed to oviposit for two days.

Oberhauser et al. (2009): Common milkweed leaves, monarch larvae, monarch larvae on milkweed leaves/plants, and adult monarchs were placed in separate cages at different distances upwind and downwind of a resmethrin application spray path. Following application, adult monarchs were monitored in the lab for three days and larvae were either provided untreated or resmethrin-treated leaves until pupation.

Krischik et al. (2015): Formulated imidacloprid was applied in the soil of potted tropical milkweed plants three weeks prior to start of experiment. Early instar larvae and adults were exposed for over two weeks to plants containing 6000 and 10,000 ng imidacloprid/g flower (leaf and nectar concentrations not provided). Another set of adults were force-fed 15 and 30 ng/mL imidacloprid in sugar syrup every two days for 29 days.

Pecenka and Lundgren (2016): First instars were exposed to swamp milkweed leaves containing a range of clothianidin concentrations for 36 h. A concentration lethal to 50% of the test population (LC$_{50}$) corresponded to 0.2 ng/cm$^2$ or 9.8 ng/g leaf. However, this paper was excluded as other acute and chronic larval studies with clothianidin (see Krishnan et al. 2020, Bargar et al. 2020, Olaya-Arenas et al. 2020, Krishnan et al. 2021b, and Knight et al. 2021) suggest the toxicity was overestimated by 100 to 1000-fold (see Krishnan et al. 2021b for details).

James (2019): Monarch adults had 22-day exposure to a formulated imidacloprid foliar product that was applied on cotton wool. As monarchs likely had both topical and oral exposure
to imidacloprid from the cotton wool, the results of this study were not used to estimate adult risk to nectar containing seed-treatment insecticide residues.

Krishnan et al. (2020): Different instars of monarch larvae reared on tropical milkweed leaf tissue received topical (single application) and dietary exposure (one or two-days) to multiple concentrations of six individual insecticides: beta-cyfluthrin, chlorantraniliprole, chlorpyrifos, imidacloprid, thiamethoxam, and clothianidin. The observation period was until pupation for fifth instars or 96 hours for other instars. Exposure was estimated from representative foliar insecticide labels and the spray drift model AgDRIFT (USEPA 2011a).

Bargar et al. (2020): A granular clothianidin product was applied to the soil of pots containing swamp milkweed. Larvae were exposed to clothianidin concentrations ranging from 11 to 2200 ng/g leaf from neonate to pupation.

Olaya-Arenas et al. (2020a): Monarch larvae received chronic dietary exposure to common milkweed leaf discs containing mean or maximum detectable field concentrations of six individual pesticides: three fungicides (azoxystrobin, pyraclostrobin, and trifloxystrobin), two herbicides (atrazine and s-metolachlor), and one insecticide (clothianidin).

Olaya-Arenas et al. (2020b): Caged, mated monarch females were provided a choice of four common milkweed plants for seven days: unsprayed, diluent-sprayed, or sprayed with mean or maximum detectable field concentrations of six pesticides. The pesticide mixture consisted of three fungicides (azoxystrobin, pyraclostrobin, and trifloxystrobin), two herbicides (atrazine and s-metolachlor), and one insecticide (clothianidin). In a separate experiment, monarch larvae were given a choice of untreated/diluent treated or pesticide-treated leaf discs for 24 hours. Pesticide treatments consisted of the six individual compounds at mean and maximum detectable field concentrations.

Giordano et al. (2020): Tropical milkweed plants were placed 25 and 50 m downwind of a deltamethrin Ultra Low Volume spray path. Control milkweed was placed 100 m upwind. Twelve hours later, larvae were provided leaves from these plants until pupation.

Krishnan et al. (2021b): Larvae had chronic dietary exposure to tropical milkweed leaves containing five individual insecticides: chlorantraniliprole, chlorpyrifos, imidaclorpid, thiamethoxam, and clothianidin. Eggs, pupae, and adults received topical exposure to the same insecticides, in addition to beta-cyfluthrin. Adults received dietary exposure to the three
neonicotinoids. Mortality from foliar insecticides was estimated from AgDRIFT (USEPA 2011a) and representative foliar products.

Mullins et al. (2021): Mated females were released in cages containing imidacloprid-treated or diluent-treated tropical milkweed plants and allowed to oviposit for three days. Imidacloprid treatment of milkweed mimicked either seed treatment (400,000 to 1,800,000 ng/g leaf) or foliar (35,000 to 62,000 ng/g leaf) route of exposure.

Krueger et al. (2021): Monarch larvae reared on an artificial diet were topically exposed to multiple concentrations of bifenthrin and beta-cyfluthrin. The observation period was 72 hours. Exposure was estimated from representative foliar insecticide labels and the spray drift model AgDRIFT (USEPA, 2011a).

Wilcox et al. (2021a): Technical grade clothianidin was applied to the soil of pots containing swamp milkweed. Larvae were chronically exposed to a clothianidin leaf concentration of 1.2 ng/g or less. Female adults that emerged from the clothianidin treatment were allowed to mate and oviposit on untreated swamp milkweed plants for seven days.

Knight et al. (2021): Larvae were chronically exposed to common milkweed transplanted into plots containing either clothianidin-treated or untreated maize seeds. Oviposition from visiting females and survival of larvae from eggs laid were monitored.

Attributes to consider when evaluating a terrestrial insect toxicology study [a synopsis of USEPA (2011b)].

**Sound conceptual design:** Rationale for life stages, application routes, exposure durations, number of doses/concentrations, and endpoints based on realistic scenarios of pesticide applications and physiochemical properties of compounds(s) being tested.

**Statistical design considerations:** Randomization of treatments, replications (optimum of 30 insects at each chemical dose/concentration) and rationale for statistical analyses. Whenever possible, dose- or concentration-response curves should be generated for different effects. This increases the reliability of the generated data and allows estimation of effects at various doses/concentrations.

**Use of a well-established colony:** Ideally, insects should be used from colonies that are healthy and free from chemical exposures, parasites/infections, genetic abnormalities, stress, and lengthy periods of inbreeding. First-generation insects should be avoided as they could have had
prior exposure to stressors. Insects used in testing should optimally be derived from at least two or three generations to account for intragenerational susceptibilities. Insects should be reared under optimal and consistent temperature, humidity, and light settings. Ad libitum food should be provided.

**Appropriate controls:** A true negative control consists of all components of a treatment solution/suspension minus the pesticide active ingredient being tested; the ratio of non-active ingredient components should match that of the treatment group. Ideally, mortality in the negative control group should not differ from that of the untreated group.

**Acceptable and accountable control mortality:** If standardized guidelines for an insect bioassay are unavailable, a specified and reasonable control mortality should be established prior to start of the bioassay. Control mortality should be based on historical colony performance and background mortality rates. All bioassays yielding control mortality rates (or other endpoints of interest) equal to or lower than the acceptable mortality should be included in statistical analyses, while all exceeding the acceptable control mortality should be excluded. Treatment mortality should be normalized to account for control mortality (e.g., use of Abbott’s formula [Abbott 1925]).

**Relevant dose/concentration units:** The dose or concentration unit(s) should reflect the route of exposure to the insect. For example, if the insect is exposed to an insecticide within a diet, the insecticide concentration in the diet (e.g., ng/g leaf, ng/cm² leaf tissue or ng/cm³ artificial diet) should be reported and used in statistical analyses and generation of dose-response curves; the concentration of the insecticide solution that was incorporated in the diet should not be used. Avoid use of ppm (parts per million) and ppb (parts per billion) as they do not provide unambiguous information on the metrics (weight, surface area, volume, etc.) or matrices (solution, diet, insect, etc.) being analyzed.

**Verified concentrations in exposure matrices:** Analytical verification of the pesticide concentrations should be carried out to confirm insect exposure concentrations.

**Comparison with environmental exposure concentrations:** In isolation, toxicity data are of limited utility. Efforts should be made to place the toxicity data in context of environmental exposure concentrations.
Detailed methods and analyses: Method and analyses sections of studies should be of sufficient detail to allow other scientists to replicate the research. As needed, supplementary file options can be used if journals have strict word/page limits.

Raw data and metadata: Raw data and associated metadata should be publicly available via university, journal, or other online digital repositories and websites.

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Supplementary Information: Butterfly Species of Conservation Concern in the North Central USA

The agricultural North Central region of the USA is critical for implementing conservation practices for at-risk flower-visiting insects (Raven and Wagner 2021), including native bees (Koh et al. 2016) and lepidopterans of conservation concern (Swengel et al. 2011). Among Lepidoptera, the Dakota skipper (*Hesperia dacotae*) is designated as threatened (USFWS 2018) under the Endangered Species Act (ESA), while the Mitchell’s satyr (*Neonympha mitchellii mitchellii*), Karner blue (*Lycaeides melissa samuelis*), and Poweshiek skipperling (*Oarisma poweshiek*) are designated as endangered (USFWS 1997, 2003, 2019). The regal fritillary (*Speyeria idalia*) is being considered for listing under the ESA (USFWS 2015). The monarch butterfly (*Danaus plexippus*) was designated a candidate species for listing under the ESA in 2020 (USFWS 2020). Several factors contribute to the declines and/or threaten the recovery of these species, including habitat loss, climate change, increased susceptibility to diseases, and pesticide exposure; however, habitat loss is typically identified as a leading cause of decline (USFWS 1997, 2002, 2003, 2014, 2018, 2019, 2020).

These at-risk butterfly species have all experienced habitat loss due to extensive agricultural development. Habitat restoration within or adjacent to cultivated areas is needed (USFWS 1997, 2003, 2014, 2018, 2019, 2020), but could increase potential pesticide exposure to the butterflies and/or their host plants. Characterizing the spatial-temporal benefits and risks of restoring habitat in agroecosystems requires an understanding of species-specific life history, host plant requirements, movement ecology, habitat utilization, and pesticide risks. Based on information gained through our research, our findings for the monarch butterfly are not directly transferrable to other at-risk butterfly species in the North Central states.

Several ecological and behavioral factors contribute to population responses of butterfly species to habitat loss and degradation (Kotiaho et al. 2005, Thomas 2016, Habel et al. 2019). In general, these factors include whether adults oviposit on one or several plant species, which depends on larvae being monophagous, oligophagous, or polyphagous; adult mobility; and the number of generations per year (voltinism) (Thomas 2016). Adult mobility is especially important in determining spatial population structure and responses to habitat changes in landscapes (Thomas and Kunin 1999) as movement and navigation abilities directly influence population dynamics, persistence, and distribution (Hanski 1998, Barton et al. 2009, Stevens et
Movement between habitats with different resource attributes requires an individual to emigrate from its current location, transit across the landscape, and find and enter the new habitat (Bowler and Benton 2005, Zalucki and Lammers 2010, Kral-O’Brien and Harmon 2021). Closed populations result when a species exhibits low mobility or when individuals are reluctant to cross habitat edges (Thomas 2016). In contrast, open or “patchy” populations (Harrison 1991, Thomas and Kunin 1999) are associated with vagile (mobile) species with high emigration and immigration rates. During their lifetime, adult butterflies seek habitats with nectar resources and/or larval host plants. High dispersal capacity allows rapid transit across landscapes, resulting in eventual colonization or utilization of resources within the dispersal range of individuals (Harrison 1991, Hanski and Gaggiotti 2004). Metapopulations of sedentary species with closed populations are more vulnerable to habitat loss because of their low capacity to colonize unoccupied or new habitats in a fragmented landscape (Wiens 1997, Thomas 2016).

A range of factors determines the spatial population structure of the butterfly species at risk in the North Central USA (Table S3 below). Dakota skipper, Poweshiek skipperling, and Mitchell’s satyr larvae are oligophagous, while Karner blue, monarch, and regal fritillary larvae are monophagous. The Dakota skipper (McCabe 1981, USFWS 2002), Poweshiek skipperling (COSEWIC 2014), Mitchell’s satyr (Szymanski et al. 2004), and regal fritillary (Wagner et al. 1997, Selby 2007) are univoltine, while the Karner blue is bivoltine (USFWS 2003). The monarch is multivoltine (USFWS 2020).

Compared to other lepidopterans of conservation concern in the North Central USA, our research suggests monarchs are more vagile and less affected by habitat edges and zero or low-density habitat (see Monarch movement ecology and habitat utilization in the main body of the overview). In our studies (Fisher et al 2020a, Fisher and Bradbury 2021, 2022), monarchs emigrated from high and intermediate-density habitats within an hour of experimental release, and individuals were observed crossing multiple habitat boundaries with flight steps as long as 1,900 m. Although regal fritillaries are vagile in some respects (Swengel 1996) and can traverse at least 1,500 m of matrix between prairies (Selby 2007), they avoid leaving prairie habitat (Ries and Debinski 2001). Similarly, Poweshiek skipperlings move up to 1,600 m during their lifespan; however, adults avoid crossing dense woodlands, crop fields, or other landcover not dominated by native grasses (Burke et al. 2011, COSEWIC 2014). Although long-distance movements are
possible for regal fritillaries and Poweshiek skipperlings, they are infrequent because edge and matrix crossings are rare. Other species, like the Karner blue, Dakota skipper, and Mitchell’s satyr are relatively sedentary species that traverse lifetime maximum distances of 190 m (USFWS 2003), 300 m (USFWS 2002), and 345 m (Szymanski et al. 2004), respectively; and almost never cross matrix habitat.

In conclusion, North Central butterfly species of conservation concern, except the monarch, have sedentary flight characteristics and closed population structures. The monarch is highly vagile during the breeding season with an open population structure. Thus, the spatial habitat configurations that facilitate functional connectivity for monarchs across a landscape are likely not appropriate for other species with closed population structures. Consistent with an open population structure, the monarch is resilient to insecticide exposure across the landscape (see Evaluating risks of pesticide use to milkweed and monarchs in the main body of the overview). However, for the other, more sedentary, species of conservation concern it is likely that insecticide exposure could result in population sinks. The extent to which sinks could depress landscape populations requires metapopulation analyses.

While the findings we report for monarchs cannot be assumed for the other butterfly species of conservation concern, the basic approach we employed (see Figure 2 in the main body of the overview) could be adapted for other species. Given their movement ecology, a new agent-based model would be needed to address female movement and egg laying at smaller scales. This advancement would entail creating new GIS models and movement algorithms. Adapting an agent-based model for bees (e.g., see Becher et al. 2014, 2018; Everaars et al. 2018) to butterflies that move at similar scales could also be an efficient approach for moving forward. Currently, the monarch is the only USA butterfly species of conservation concern for which there is sufficient insecticide toxicity data to undertake a landscape-scale risk assessment. Given constraints to undertake research with ESA-listed species, as well as the lack of reliable rearing methods, it is unlikely that insecticide toxicity testing with the other butterfly species of conservation concern will be undertaken. Alternative methods to generate toxicity estimates, including Species Sensitivity Distribution (SSD) models, could provide a means to extrapolate toxicity data from easier-to-rear lepidopteran pest species. For example, we developed lepidopteran SSD models for topical exposures to pyrethroids and organophosphates (Hall et al. 2021b), the only insecticide classes with adequate toxicity data. This exploratory analysis
showed that monarch and other lepidopteran larvae are similarly sensitive. Generation of dose-response data for other insecticide classes and routes of exposure using easier-to-rear Lepidoptera species could support development of additional SSDs to estimate toxicity for species of conservation concern.
### Table S3. Attributes of at-risk butterfly species in the North Central USA (ESA- Endangered Species Act; T – threatened; E-endangered; CS-candidate species; LIR- Listing in review).

| Common Name       | Latin Name               | Status under ESA | Migratory | Generations per Year | Larval Host Plant Specificity                                                                 | Population Structure | Reference                                           |
|-------------------|--------------------------|------------------|-----------|----------------------|-----------------------------------------------------------------------------------------------|----------------------|-----------------------------------------------------|
| Dakota skipper    | *Hesperia dacotae*       | T                | No        | Univoltine           | Oligophagous: Short, fine-stemmed bunch grasses, such as little bluestem *(Schizachyrium scoparium)*, and *Dichanthelium* spp. | Closed               | (McCabe 1981, USFWS 2002, 2018)                   |
| Karner blue       | *Lycaeides melissa samuelis* | E                | No        | Bivoltine            | Monophagous: Wild lupine *(Lupinus perennis)*                                                 | Closed               | (USFWS 2003)                                      |
| Mitchell’s satyr  | *Neonympha mitchelli mitchelli* | E                | No        | Univoltine           | Oligophagous: Sedges *(Thelypteris palustris* and *Carex* spp.)                                | Closed               | (USFWS 1997, Szymanski et al. 2004)                |
| Poweshiek skipperling | *Oarisma poweshiek*     | E                | No        | Univoltine           | Oligophagous: Native grasses and sedges (e.g., big bluestem *[Andropogon gerardii]*, little bluestem, prairie drop seed *[Sporobolus heterolepis]*, muhly grass *[Muhlenbergia richardsonis]*, and *Carex* spp.) | Closed               | (COSEWIC 2014, USFWS 2019)                      |
| Regal fritillary | *Speyeria idalia*        | LIR              | No        | Univoltine           | Monophagous: Violets *(Viola* spp.)                                                            | Closed               | (Selby 2007, USFWS 2015)                         |
| Monarch butterfly | *Danaus plexippus*       | CS               | Yes       | Multivoltine         | Monophagous: Milkweed *(Asclepias* spp.)                                                       | Open                 | (Brower et al. 2012, USFWS 2020)                 |
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