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**Supplementary information**

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**Changes in climate drive recent monarch butterfly dynamics**

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## Supplementary Information for

### Changes in climate drive recent monarch butterfly dynamics

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**Covariates that were not included in annual-cycle models.** We performed an extensive search of the literature on monarch butterflies to identify environmental factors that could explain temporal variation in the size of the eastern migratory population. After careful consideration, we excluded several factors from our models because evidence from previous studies suggested that the effects on the monarch population were minimal (i.e., autumn temperatures, infection with protozoan parasites) or because available data were insufficient (i.e., neonicotinoid [insecticide] use, parasitism by tachinid flies). Below, we describe each of these seasonal factors and outline the reasons why the factor was not included in our analyses.

*Neonicotinoid use*—Neonicotinoids are insecticides that are used on agricultural crops throughout the world<sup>86</sup>. Both the extent of croplands treated and the amount of chemicals applied have increased since 2000, especially in the Midwestern U.S.<sup>56</sup>. Growing evidence suggests that neonicotinoids can negatively affect bees and other non-target organisms, including butterflies<sup>56,87</sup>. The extent to which insecticide use affects monarchs on the summer breeding grounds, however, is less clear. Neonicotinoids are most commonly applied as a seed coat, limiting exposure risk to larval butterflies that ingest milkweed adjacent to treated crops, where chemicals are found in low concentrations in soil or dust<sup>88</sup>. We attempted to account for neonicotinoid use on the summer breeding grounds but were unable to include this factor in our models because estimates of use, for neonicotinoids and all other insecticides (via application of foliar spray, soil drench, and seed coating), were not publicly available for the Midwestern U.S. and southern Ontario after 2014. However, given that milkweed abundance in agricultural areas declined prior to 2004, and limited evidence of lethal or sub-lethal concentrations of neonicotinoids in milkweed adjacent to crops<sup>88</sup>, there is little evidence to support the assertion that neonicotinoid use was a primary driver of dynamics in the eastern monarch population during our study period. Further research is needed to understand the potential impacts of neonicotinoids, as well as other pesticides that vary in their frequency of use across U.S. agricultural fields<sup>89</sup>, on monarch abundances.

*Parasitism by tachinid flies*—Biotic factors, including effects of predators and parasites, have also been suggested as possible drivers of monarch declines<sup>90</sup>. Monarchs are host to many parasitoids, the most common of which is *Lespesia archippivora*, a tachinid fly that attacks monarch larvae and results in the death of late instars or pupae<sup>91</sup>. Parasitism rates vary over space and time but are generally <25% in the Midwestern U.S.<sup>91,92</sup>. We used data from the Monarch Larva Monitoring Project ([www.mlmp.org](http://www.mlmp.org)) to estimate annual rates of parasitism on the summer breeding grounds (proportion of individuals collected as 5<sup>th</sup> instars or pupae that were parasitized). Ultimately, we did not include this variable in our models because (1) the amount of available data was limited (no data were collected prior to 2000 and annual rate estimates between 2000–2007 were based on observations from  $\leq 2$  sites); and (2) annual parasitism rates were positively correlated with summer temperatures ( $\text{diffGDD}_{c,k,t}$ ,  $r = 0.58$  between 2004–2018), which is a stronger predictor of monarch population sizes.

*Autumn temperatures*—Unfavorable weather conditions in autumn in the central and southern U.S. could reduce survival rates or delay arrival of monarchs migrating south to overwintering grounds. However, we did not include autumn temperatures in our model because recent analyses have found little evidence that autumn temperatures within the migratory corridor contributed to variation in early-winter population size<sup>11,18</sup>.

*Disease*—We examined whether annual rates of disease among migrating individuals influenced early-winter population sizes. Monarchs can be infected by the protozoan parasite *Ophryocystis elektroscirrha* when larvae ingest spores deposited on eggs or milkweed surfaces<sup>93</sup>. Infections can result in lower rates of survival or mating success as well as reduced flight speed and endurance, characteristics that affect the ability of monarchs to successfully migrate to overwintering grounds<sup>94,95</sup>. We used data from Project Monarch Health ([www.monarchparasites.org](http://www.monarchparasites.org)) to calculate the proportion of adults in late summer that were infected prior to commencing autumn migration. We calculated annual infection rates, by city (scale based on data availability), in the summer breeding range based on samples collected on or after 19 July (week 21), and then averaged across locations to calculate the annual proportion of adult monarchs infected (range for 2006–2018 = 0.03–0.32). Notably, infection rates were positively correlated with summer population size ( $r = 0.49$ ), and we were thus unable to include this variable in models. Previous studies have found similar associations between infection rates and monarch larval densities<sup>93,96</sup>, making it difficult to determine which factor may be driving observed dynamics. Given these patterns, as well as a positive correlation between infection rates at the end of summer and population size the following December ( $r = 0.42$ ), it is unlikely that *O. elektroscirrha* infection was the primary driver of dynamics in the eastern monarch population. This is consistent with other research that did not find a negative impact of disease on overwintering population size<sup>18</sup>.

**Differentiating factors associated with trends from those associated with annual variations in monarch population size.** Differentiating trends from annual fluctuations in the eastern migratory population of monarch butterflies is inherently difficult. Like many insect species, monarch abundance varies greatly from one year to the next, which in conjunction with the relatively short-time series of our analyses (1994–2003, 2004–2018), can obscure long-term population trends. Modeling long-term trends in the monarch population is also challenging given their unique, multi-generational migratory cycle, which results in two disparate indices of population size (counts of adult butterflies from surveys conducted throughout the summer breeding range and measures of area occupied from the overwintering colonies). Trends or fluctuations in one seasonal index may not correlate with temporal changes in the other due to sampling biases, seasonal environmental stressors, or some combination of these factors. Finally, trends and fluctuations are difficult to differentiate given that the summer breeding population is dispersed throughout an immense geographic area. Integrating counts of adult monarchs throughout the summer breeding range allowed us to make reliable inferences about the population as a whole. Using these counts as indices of population size, however, complicates efforts to model trends (see below).

To assess the extent to which factors in our model explained trends versus annual variation in overwintering population size, we evaluated patterns in residuals from the winter component of the 2004–2018 model (see Methods and Results). We were unable to perform a similar evaluation of residuals from the 1994–2003 model given that we had only 10 observations of the

total area occupied during that period (i.e., we did not have estimates of the area occupied in each supercolony).

To further differentiate factors that were associated with trends from those that were associated with fluctuations in population size, we attempted to construct modified versions of the annual-cycle models, wherein we removed temporal trends from covariates and estimated the extent to which detrended covariates explained annual variation in monarch population size (i.e., by adding population-level trends to both the summer and winter submodels)<sup>97,98</sup>. We ultimately decided not to pursue this approach, however, given that the detrended models depended on strong assumptions that we could not independently verify. Specifically, a trend in the summer model assumed uniform declines in abundance across the summer breeding range, rather than a range contraction, for example. Similarly, a detrended 2004–2018 model assumed uniform declines in the area occupied at each supercolony over time, but no decline in the probability of monarch presence.

**Evaluating spatiotemporal variation in glyphosate use and its role in monarch population dynamics, 2004–2018.** While there is a clear correlation between glyphosate use and monarch population size during the period when glyphosate-resistant crops were introduced and widely adopted throughout the Midwest (Fig. 1, left of dashed line), the extent to which glyphosate use has driven recent population dynamics is less clear. Because there was more spatial than temporal variation in glyphosate use (~74% of total variation in the proportion of crops sprayed was attributable to differences in county means whereas only ~26% of total variation was attributable to differences over time), we extracted annual, county-level estimates of peak summer monarch counts and used a linear mixed-effects model to assess whether temporal trends in monarch counts over the 15-year period varied with glyphosate use. Among counties that used glyphosate on corn or soy crops (i.e.,  $\geq 1\%$  of crops sprayed), we found no evidence that county-level trends (mean =  $-0.21$  adult monarchs/yr) differed among counties depending on mean glyphosate use (interaction term =  $-0.04$ , 95% CI =  $-0.14, 0.05$ ).

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**Supplementary Table 1 | Attributes of overwintering monarch colonies and newly-designated supercolonies in Mexico.** We used mapped locations and expert opinion to delineate supercolonies by combining colonies in close proximity to one another that were unlikely to function as independent units. ‘Inside reserve’ and ‘Outside reserve’ refer to the Monarch Butterfly Biosphere Reserve (MBBR) located on the border of the states of Michoacán and México. Each supercolony was surveyed for monarch butterflies each year, from 1994–2018.

Location	Sanctuary	Colony ( $n = 19$ )	Supercolony ( $n = 13$ )
Inside reserve	Cerro Pelón	E. El Capulín	Cerro Pelón W
		C.I. San Juan Xoconusco	Cerro Pelón W
		E. Nicolás Romero	Cerro Pelón W
		E. Mesas Altas de Xoconusco	Cerro Pelón E
		C.I. San Pablo Malacatepec	Cerro Pelón E
	Sierra Campanario	E. La Mesa	E. La Mesa
		E. El Rosario	E. El Rosario
	Cerro Altamirano	E. Contepec	E. Contepec
	Chivati-Huacal	C. I. Carpinteros	C. I. Carpinteros
	Sierra Chincua	Propiedad Federal	Sierra Chincua
Propiedad Estatal		Sierra Chincua	
E. Cerro Prieto		Sierra Chincua	
E. El Calabozo Fracción		Sierra Chincua	
Lomas de Aparicio	E. Crescencio Morales	E. Crescencio Morales	
Outside reserve	Cerro del Amparo	E. San Francisco Oxtotilpan	E. San Francisco Oxtotilpan
	Palomas	E. San Antonio Albarranes	E. San Antonio Albarranes
	Piedra Herrada	E. San Mateo Almomoloa	E. San Mateo Almomoloa
	Los Azufres	P. P. San Andrés	P. P. San Andrés
	Mil Cumbres	E. Río de Parras	E. Río de Parras

**Supplementary Table 2 | Sources of monarch count data on the summer breeding grounds, 1994–2018.** We used monarch count data from surveys conducted between 14 June–15 August by the North American Butterfly Association (NABA) and four state-specific butterfly monitoring networks (BMNs) to model monarch population dynamics in 1994–2003 and 2004–2018. We excluded NABA surveys in Canada from the 1994–2003 analyses. See Extended Data Fig. 1 for locations of surveys.

Monitoring program	Years available	Mean no. locations per year (range)		Mean no. surveys per year at each location (range)	
		1994–2003	2004–2018	1994–2003	2004–2018
NABA	1994–2018	58 (41–80)	92 (80–99)	1.0 (1–1)	1.0 (1–2)
State BMNs					
Illinois	1994–2018	49 (24–97)	100 (72–129)	4.4 (1–9)	4.5 (1–9)
Ohio	1995–2017	21 (1–39)	43 (27–52)	6.7 (1–9)	6.6 (1–9)
Iowa	2006–2018	NA	16 (1–60)	NA	4.8 (1–9)
Michigan	2011–2018	NA	44 (4–77)	NA	4.3 (1–9)

**Supplementary Table 3 | Parameter estimates from the full annual-cycle model describing monarch population dynamics between 2004–2018.** We present mean, standard deviation (SD), and 95% credible intervals (95% CI) based on 7,500 samples from the posterior distributions of parameters in the 2004–2018 annual-cycle model. Parameters in the summer submodel describe variation in the number of monarchs observed during surveys conducted on the summer breeding grounds. Parameters in the winter submodel describe variation in the area occupied by monarchs in supercolonies on the overwintering grounds in December (conditional on presence). Bold text indicates that the associated 95% CI excluded zero. Notations correspond with those used in the text. PCP = precipitation.

Parameter	Notation	Mean	SD	95% CI
<i>Summer submodel</i>				
Intercept	$\alpha_0$	<b>2.46</b>	<b>0.10</b>	<b>2.25, 2.65</b>
Week (linear)	$\text{week}_k$	<b>0.68</b>	<b>0.07</b>	<b>0.54, 0.81</b>
Week (quadratic)	$\text{week}_k^2$	<b>-0.24</b>	<b>0.07</b>	<b>-0.38, -0.11</b>
Late winter population size	$\text{Feb}_t$	<b>0.14</b>	<b>0.02</b>	<b>0.11, 0.17</b>
Spring GDD (linear)	$\text{spGDD}_t$	<b>0.32</b>	<b>0.03</b>	<b>0.27, 0.37</b>
Spring GDD (quadratic)	$\text{spGDD}_t^2$	<b>-0.27</b>	<b>0.03</b>	<b>-0.33, -0.22</b>
Spring PCP (linear)	$\text{spPCP}_t$	<b>-0.26</b>	<b>0.02</b>	<b>-0.30, -0.22</b>
Spring PCP (quadratic)	$\text{spPCP}_t^2$	<b>-0.33</b>	<b>0.02</b>	<b>-0.36, -0.30</b>
Summer GDD, average	$\text{avgGDD}_c$	-0.05	0.09	-0.22, 0.13
Summer GDD, difference (linear)	$\text{diffGDD}_{c,k,t}$	<b>0.34</b>	<b>0.02</b>	<b>0.31, 0.38</b>
Summer GDD, difference (quadratic)	$\text{diffGDD}_{c,k,t}^2$	<b>-0.05</b>	<b>0.01</b>	<b>-0.08, -0.03</b>
Summer GDD, average * difference	$\text{avgGDD}_c * \text{diffGDD}_{c,k,t}$	<b>-0.14</b>	<b>0.02</b>	<b>-0.18, -0.11</b>
Summer PCP, average	$\text{avgPCP}_c$	0.02	0.08	-0.14, 0.19
Summer PCP, difference (linear)	$\text{diffPCP}_{c,t}$	<b>0.17</b>	<b>0.01</b>	<b>0.15, 0.20</b>
Summer PCP, difference (quadratic)	$\text{diffPCP}_{c,t}^2$	<b>-0.02</b>	<b>0.01</b>	<b>-0.03, 0.00</b>
Summer PCP, average * difference	$\text{avgPCP}_c * \text{diffPCP}_{c,t}$	<b>0.04</b>	<b>0.02</b>	<b>0.01, 0.07</b>
Glyphosate use	$\text{gly}_{c,t}$	<b>-0.11</b>	<b>0.03</b>	<b>-0.16, -0.06</b>
Crop cover	$\text{crop}_c$	0.11	0.07	-0.03, 0.24
Glyphosate use * Crop cover	$\text{gly}_{c,t} * \text{crop}_c$	-0.02	0.02	-0.06, 0.02
IA BMN (indicator)	$\text{IA}_i$	0.17	0.21	-0.24, 0.58
IL BMN (indicator)	$\text{IL}_i$	0.22	0.15	-0.09, 0.51
MI BMN (indicator)	$\text{MI}_i$	<b>-1.07</b>	<b>0.15</b>	<b>-1.36, -0.79</b>
OH BMN (indicator)	$\text{OH}_i$	<b>-0.92</b>	<b>0.16</b>	<b>-1.24, -0.62</b>
Unforested area	$\text{open}_i$	<b>0.13</b>	<b>0.04</b>	<b>0.04, 0.21</b>
<i>Winter submodel</i>				
Intercept	$\gamma_0$	<b>-2.04</b>	<b>0.58</b>	<b>-3.22, -0.95</b>
Summer population size	$\text{summer}_t$	<b>0.43</b>	<b>0.09</b>	<b>0.25, 0.62</b>
Nectar availability	$\text{nectar}_t$	-0.01	0.07	-0.15, 0.13
Reserve (indicator)	$\text{reserve}_s$	0.62	0.74	-0.79, 2.13
Dense forest cover	$\text{forest}_{s,t}$	0.08	0.38	-0.66, 0.86



**Supplementary Table 4 | Relative importance of seasonal factors in driving monarch population dynamics between 1994–2018.** Results of hierarchical partitioning analyses assessing the percent of explained variance in summer monarch counts (*Summer submodel*) that can be attributed to late-winter population size ( $Feb_t$ ), spring weather ( $spGDD_t$ ,  $spGDD_t^2$ ,  $spPCP_t$ ,  $spPCP_t^2$ ), summer weather ( $diffGDD_{c,k,t}$ ,  $diffGDD_{c,k,t}^2$ ,  $avgGDD_c \cdot diffGDD_{c,k,t}$ ,  $diffPCP_{c,t}$ ,  $diffPCP_{c,t}^2$ ,  $avgPCP_c \cdot diffPCP_{c,t}$ ), and summer land-use ( $gly_{c,t}$ ,  $crop_c \cdot gly_{c,t}$ ), and the percent of explained variation in the area occupied by monarchs in early winter (*Winter submodel*) that can be attributed to peak summer population size ( $summer_t$ ), autumn nectar availability ( $nectar_t$ ), and forest cover at the overwintering sites ( $forest_{j,t}$ ). Estimates of late-winter population size were not available for 1994–2003. We did not assess the relative importance of factors in the winter submodel for 1994–2003 because measures of the area occupied in early winter were aggregated among supercolonies, resulting in only a single measure of population size each year.

Seasonal factors	Percent of explained variance attributable to seasonal factors (%)	
	1994–2003	2004–2018
<i>Summer submodel</i>		
Late-winter population size	NA	4.6
Spring weather	92.1	58.4
Summer weather	6.1	28.8
Summer land-use	1.8	8.2
<i>Winter submodel</i>		
Peak summer population size	NA	91.9
Autumn nectar	NA	0.0
Early-winter forest cover	NA	8.1

**Supplementary Table 5 | Parameter estimates from “reduced” annual-cycle models describing monarch population dynamics in 1994–2003 and 2004–2018.** We present mean and standard deviation (SD) based on 7,500 samples from the posterior distributions of parameters in reduced annual-cycle models for 1994–2003 and 2004–2018. To ensure valid comparisons between the two time periods, both models excluded count data from Canada, used an aggregate measure of overwintering population size in December, and excluded effects of late-winter population size ( $Feb_t$ ) and nectar availability ( $nectar_t$ ) because those data were unavailable for the earlier time period. Parameters in the summer submodel describe variation in the number of monarchs observed during surveys conducted on the summer breeding grounds. Parameters in the winter submodel describe variation in the total area occupied by monarchs on the overwintering grounds in December. Bold text indicates that the associated 95% credible interval excluded zero. State monitoring programs in Iowa (IA) and Michigan (MI) began after 2003. Notations correspond with those used in the text. PCP = precipitation. We provide estimates from the reduced annual-cycle model during 2004–2018 to verify that estimates were consistent with those from the full annual-cycle model during the same period (see Extended Data Table 3).

Parameter	Notation	1994–2003		2004–2018	
		Mean	SD	Mean	SD
<i>Summer submodel</i>					
Intercept	$\alpha_0$	<b>1.80</b>	<b>0.12</b>	<b>2.17</b>	<b>0.11</b>
Week (linear)	$week_k$	<b>0.98</b>	<b>0.07</b>	<b>0.68</b>	<b>0.06</b>
Week (quad)	$week_k^2$	-0.24	0.13	<b>-0.25</b>	<b>0.07</b>
Spring GDD (linear)	$spGDD_t$	0.22	0.12	<b>0.33</b>	<b>0.02</b>
Spring GDD (quad)	$spGDD_t^2$	<b>-0.42</b>	<b>0.07</b>	<b>-0.27</b>	<b>0.03</b>
Spring precipitation (linear)	$spPCP_t$	<b>0.33</b>	<b>0.06</b>	<b>-0.24</b>	<b>0.02</b>
Spring precipitation (quad)	$spPCP_t^2$	<b>0.26</b>	<b>0.09</b>	<b>-0.31</b>	<b>0.02</b>
Summer GDD, average	$avgGDD_c$	<b>-0.56</b>	<b>0.14</b>	-0.05	0.09
Summer GDD, difference (linear)	$diffGDD_{c,k,t}$	-0.03	0.04	<b>0.31</b>	<b>0.02</b>
Summer GDD, difference (quadratic)	$diffGDD_{c,k,t}^2$	0.01	0.02	<b>-0.05</b>	<b>0.01</b>
Summer GDD, average * difference	$avgGDD_c * diffGDD_{c,k,t}$	0.00	0.03	<b>-0.13</b>	<b>0.02</b>
Summer PCP, average	$avgPCP_c$	0.02	0.10	0.03	0.09
Summer PCP, difference (linear)	$diffPCP_{c,t}$	0.01	0.03	<b>0.19</b>	<b>0.01</b>
Summer PCP, difference (quadratic)	$diffPCP_{c,t}^2$	0.03	0.02	<b>-0.02</b>	<b>0.01</b>
Summer PCP, average * difference	$avgPCP_c * diffPCP_{c,t}$	-0.01	0.03	0.02	0.02
Glyphosate use	$gly_{c,t}$	-0.03	0.05	<b>-0.10</b>	<b>0.03</b>
Crop cover	$crop_c$	<b>0.28</b>	<b>0.11</b>	0.11	0.07
Glyphosate use * Crop cover	$gly_{c,t} * crop_c$	<b>-0.07</b>	<b>0.03</b>	<b>-0.04</b>	<b>0.02</b>
IA BMN (indicator)	$IA_i$	NA	NA	0.15	0.21
IL BMN (indicator)	$IL_i$	0.24	0.21	0.22	0.16
MI BMN (indicator)	$MI_i$	NA	NA	<b>-1.10</b>	<b>0.16</b>
OH BMN (indicator)	$OH_i$	<b>-0.67</b>	<b>0.22</b>	<b>-0.89</b>	<b>0.17</b>
Unforested area	$open_i$	<b>0.25</b>	<b>0.08</b>	<b>0.14</b>	<b>0.05</b>
<i>Winter submodel</i>					
Intercept	$\gamma_0$	<b>2.28</b>	<b>0.27</b>	<b>1.18</b>	<b>0.20</b>
Summer population size	$summer_t$	-0.05	0.29	0.37	0.21
Dense forest cover	$forest_t$	0.07	0.25	0.20	0.20