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Butterfly abundance declines over 20 years of systematic monitoring in Ohio, USA

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## 20 **Abstract**

21           Severe insect declines make headlines, but are rarely based on systematic monitoring  
22 outside of Europe. We estimate the rate of change in total butterfly abundance and the population  
23 trends for 81 species using 21 years of systematic monitoring in Ohio, USA. Total abundance is  
24 declining at 2% per year, resulting in a cumulative 33% reduction in butterfly abundance. Three  
25 times as many species have negative population trends compared to positive trends. The rate of  
26 total decline and the proportion of species in decline mirror those documented in long-term  
27 European monitoring. Multiple environmental changes such as climate change, habitat  
28 degradation, and agricultural practices may contribute to these declines in Ohio and shift the  
29 makeup of the butterfly community by benefiting some species over others. Our analysis of life-  
30 history traits associated with population trends shows an impact of climate change, as species  
31 with northern distributions and fewer annual generations declined more rapidly. However, even  
32 common and invasive species associated with human-dominated landscapes are declining,  
33 suggesting widespread environmental causes for these trends. Declines in common species,  
34 although they may not be close to extinction, will have an outsized impact on the ecosystem  
35 services provided by insects. These results from the most extensive, systematic insect monitoring  
36 programs in North America demonstrate an ongoing defaunation in butterflies that on an annual  
37 scale might be imperceptible, but cumulatively has reduced butterfly numbers by a third over 20  
38 years.

39

## 40 **Introduction**

41           Defaunation, or the drastic loss of animal species and declines in abundance, threatens to  
42 destabilize ecosystem functioning globally (1). In comparison to studies of vertebrate

43 populations, monitoring of changes in insect diversity is more difficult and far less prevalent  
44 (2,3). Despite this, a global analysis of long-term population trends across 452 species estimated  
45 that insect abundance had declined 45% over 40 years (1). Recently, more extreme declines in  
46 insect biomass have been observed upon resampling after 2-4 decades (4,5). Losses of total  
47 biomass or total abundance across all species may be more consequential than local declines in  
48 species diversity, as common insect species contribute the most to ecosystem functions, such as  
49 pollination (6). However, our knowledge of insect declines is skewed towards European  
50 monitoring programs, including in global analyses (1). In this study, we analyze long-term,  
51 region-wide trends in abundance across a diversity of species for an entire insect group in North  
52 America to examine the scope of insect defaunation.

53         The best source of data to assess insect defaunation comes from large-scale, systematic  
54 monitoring programs of multiple species (3). Through these efforts, trained volunteers or citizen  
55 scientists have contributed much of the evidence for biotic responses to anthropogenic climate  
56 warming through changes in insect phenology and range distributions (7,8). Unlike citizen  
57 science reporting of opportunistic observations or species checklists, many insect monitoring  
58 programs use a systematic protocol developed specifically for volunteers to track butterfly  
59 abundances through time, both within and between seasons, and over large spatial scales (9).  
60 Pollard-based monitoring programs, modeled after the first nationwide Butterfly Monitoring  
61 Scheme launched in the United Kingdom in 1977 (UKBMS), use weekly standardized counts on  
62 fixed transects (10). Their widespread adoption enables regional comparisons of insect responses  
63 to environmental change or defaunation (11,12). We compare our analysis with exemplary long-  
64 term monitoring schemes from Europe to test if the rate of insect declines generalizes across  
65 continents.

66           The best source of abundance data for assessment of chronic insect decline, and the most  
67 prominent source of data in (1), is within the butterflies. Due to the relative ease and popularity  
68 of monitoring butterflies, environmental assessments use them as an indicator taxa for the  
69 general trajectory of biodiversity, assuming that they experience comparable pressures from  
70 land-use change, climate change, and habitat degradation as other insect taxa (13–15). Intensive  
71 long-term monitoring of individual butterfly species has provided rigorous, quantitative  
72 estimates of declines. Most prominently, the Eastern North American Monarch has declined by  
73 over 85% (16) and the Western North American Monarch by over 95% (17) over the past two  
74 decades. Severe declines have also been observed in some of the rarest butterflies (18,19). These  
75 data from individual species of conservation concern may not represent a broader trend across  
76 butterflies, which is what we aim to document in this study.

77           Volunteers, organized and trained by The Ohio Lepidopterists, have assembled the most  
78 extensive dataset of systematic butterfly counts that stands alone in North America in terms of  
79 the spatial extent and sampling frequency of Pollard walks (9). Three other monitoring programs  
80 in the United States have documented long-term, multi-species population trends. In  
81 Massachusetts, based on species lists from field trips, climate-driven community shifts explain  
82 how the relative likelihood of species observations change over 18 years (20). Art Shapiro and  
83 colleagues have made biweekly presence/absence observations on 11 fixed transects along an  
84 elevational gradient in California over 40 years to document species richness changes in  
85 response to climate and land-use, increasing abundance at a high elevation site, and impacts of  
86 agricultural practices on abundance at low elevation sites (21,22). Several teams have monitored  
87 declines in specialist butterflies restricted to native prairie patches in the Midwestern states with  
88 transect or timed survey methods over 26 years (23,24). The number of Pollard-based monitoring

89 programs has increased sharply since 2010 in the United States (9), which could potentially track  
90 to test how widespread and consistent butterfly trends are.

91 Here, we used 21 years of weekly butterfly surveys across 104 sites to assess abundance  
92 trends for butterflies in Ohio. We estimate population trends for 81 species and test for their  
93 association with life-history traits and phylogenetic relatedness. We review findings from  
94 European butterfly monitoring schemes for quantitative comparison with the rate of abundance  
95 changes in Ohio. This analysis provides evidence of widespread insect defaunation and species'  
96 declines from the most extensive, systematic monitoring program in North America.

97

## 98 **Materials and methods**

### 99 *Study sites*

100 We studied butterfly population trends across the state of Ohio in the Midwestern USA.  
101 Over its 116,100 km<sup>2</sup> land area, Ohio has a mosaic of habitat types due to its partially glaciated  
102 history and its place at the confluence of Midwestern prairies, the Appalachian Mountains, and  
103 the boreal forest (25). Only remnants of wetland and prairie habitat remain in the state due to  
104 human modification of the landscape. Some rare butterflies have declined due to forest  
105 succession following suppression of disturbances (26). Agriculture and pastures (50%), forest  
106 (30%), and urban development (10%) are the predominant land-use/land cover classes (27).

107 Monitoring sites have a Northeast to Southwest gradient in their mean annual  
108 temperatures (mean 18.8°C, range from 14.0°C to 23.6°C) from interpolated daily temperatures  
109 from Daymet over 1996-2016 (Thornton et al. 1997). Mean annual temperatures at these sites  
110 grew at a linear trend of 0.3°C per decade and growing season length has increased by 60 degree-  
111 days (base 5°C) per decade from 1980-2016. Monitoring sites span the state but are concentrated

112 near cities (Fig 1). On average, within a radius of 2 kilometers, monitoring sites have 24%  
113 cropland and pasture, 34% forest, and 30% urban land-use based on the National Land Cover  
114 Dataset (29). Although not considered in this study, impervious surfaces from urban  
115 development influence temperature-dependent butterfly phenology in Ohio through the urban  
116 heat island effect, which may not be captured in these gridded temperature interpolations (30).

117  
118 **Fig 1: Transect locations monitored by volunteers with the Ohio Lepidopterists.** Of the 147  
119 sites, this analysis used the 104 sites monitored for three or more years.

120  
121 *Monitoring surveys*

122 Trained volunteers contributed 24,405 butterfly surveys from 1996 to 2016 as part of the  
123 Ohio Lepidopterists Long-term Monitoring of Butterflies program. Volunteers survey on fixed  
124 paths at approximately weekly intervals during the entire growing season from April through  
125 October (median 23 of 30 weeks surveyed per year per site) and count every species within an  
126 approximate 5-meter buffer around the observer (10). Surveys are constrained to times of good  
127 weather to increase the detectability of butterflies and last a mean 85 minutes in duration. The  
128 annual number of monitored sites ranged from 13 in 1996 to a maximum of 80 in 2012. We  
129 limited our analysis of abundance trends to the 104 sites with three or more years of monitoring  
130 data and 10 or more surveys per year at each site (Fig 1). We included observations of all sites  
131 with at least 5 surveys per year in phenology models that we used to interpolate missing counts  
132 before estimating abundance (31).

133 All species (102) with population indices estimated by phenology models contributed to  
134 the total abundance analysis. We limited species-specific analysis to 81 with sufficient

135 population indices for estimating trends (present at five or more sites and for 10 or more years).  
136 Species naming conventions in the monitoring program follow those used in Opler and Krizek  
137 (1984) and Iftner et al. (1992) except for combining all observations of *Celastrina ladon* (Spring  
138 Azure) and *Celastrina neglecta* (Summer Azure) as an unresolved species complex.

#### 139 *Population indices*

140 We estimated population indices for each site x year x species by adapting methods  
141 established for the UKBMS that account for missing surveys and butterfly phenology over the  
142 season (31,33). We used generalized additive models for each species to estimate variation in  
143 counts in order to interpolate missing surveys with model predictions (31,34). To account for  
144 seasonal, spatial, and interannual variation in species phenology, we extended the regional  
145 generalized additive model approach (12, Supplement 1) by including spatially-explicit site  
146 locations and converted calendar dates of observations to degree-days (35), which can improve  
147 butterfly phenology predictions. We calculated the population index by integrating over the  
148 weekly counts and missing survey interpolations using the trapezoid method (31).

#### 149 *Controlling for confounding factors*

150 We accounted for differences in sampling across sites and years so that our modeled  
151 trends would capture changes in abundance rather than changes in detection probability (36).  
152 True abundance is confounded with detection probability when using counts from Pollard walks  
153 (37). Butterfly monitoring protocols that account for detection probability like distance sampling  
154 are commonly used for single-species studies (38), but untenable for scaling up to a statewide  
155 program. Most analyses of Pollard walks assume no systematic change in detectability (but see  
156 (39)) because counts correlate closely with true abundance estimates from distance sampling  
157 (40,41). We used two covariates to account for variation in sampling and its influence on

158 population indices (20,36,42). We tracked the number of species reported in each survey, or list-  
159 length, which is a synthetic measure of factors influencing detectability such as weather  
160 conditions, site quality, and observer effort (20,43,44). We treated the total duration of surveys in  
161 minutes as an offset in the models of population trends, which converts the population indices to  
162 a rate of butterflies observed per minute. Because we interpolated missing surveys for the  
163 population indices, we projected what the total duration would be if all 30 weeks had been  
164 surveyed at the mean duration reported for that site x year.

165         Sampling across the state is nonrandom because participants choose transect locations, a  
166 common practice in volunteer-based monitoring programs. Since sites generally cluster near  
167 human population centers with a greater proportion of developed land-use and a lesser  
168 proportion of agriculture, we assumed that population trends at the 104 sites across the state  
169 sufficiently capture the broader statewide trends (36). Comparisons between the UKBMS  
170 volunteer-placed transects and a broader survey with stratified, random sampling show  
171 congruence between species trends estimated from each monitoring strategy (45).

## 172 *Population Trends*

173         We used generalized linear mixed models to estimate temporal trends in relative  
174 abundance for 81 species from their population indices (46). We modeled population indices at  
175 each site and year as an over-dispersed Poisson random variable with covariates on the log-link  
176 scale.

$$\log(\text{PopulationIndex}) = \beta_0 + \beta_1 \times \text{year} + \beta_2 \times \text{listlength} + \log(\text{duration}) + \text{siteID} \\ + \text{yearID} + \text{siteyearID}$$

177         We included the year and mean list length for each population index as covariates, which  
178 were centered to aid in model fitting and interpretation (47). We used the coefficient for year ( $\beta_2$ )



179 as the annual trend in population indices as our main result. We controlled for changes in  
180 sampling by using the total duration of surveys as a model offset, converting the dependent  
181 variable to a rate of butterflies counted per minute. Random effects of individual sites and years  
182 account for spatial and temporal variation in population counts deviating from the statewide  
183 trend. We accounted for over-dispersion in the Poisson-distributed counts with the random effect  
184 *siteyearID* for each unique observation (48). We modeled trends in total abundance using the  
185 same modeling approach, but summed across 102 species' population indices for each site x year  
186 observation. We interpreted trends as an annual rate by taking the geometric mean rate of change  
187 between the predicted abundance between two points in time after setting the list-length  
188 covariate to its mean and excluding the random effects (46). For comparisons with other  
189 monitoring programs, we used a *p*-value threshold of 0.05 to classify trends as positive, negative,  
190 or stable.

191 Our approach is similar to that used by the UKBMS and other European monitoring  
192 programs which use generalized linear models in TRIM software (49). One key difference is that  
193 our site and annual fluctuations from the temporal trend were derived from random effects rather  
194 than fixed effects, which reduces spurious detection of trends (42). Another key difference is that  
195 TRIM does not allow for continuous covariates, which we used to account for sampling variation  
196 instead of assuming no confounding pattern in sampling effort. To validate that our modeling  
197 choices did not unreasonably influence the results, we used three alternative approaches: (1) a  
198 Poisson-based generalized linear model (equation 1 without the random effect *siteyearID*); (2) a  
199 nonlinear generalized additive mixed model with a smoothing spline replacing the linear  
200 temporal trend (42); and (3) a TRIM model with over-dispersion and serial temporal correlation  
201 but no sampling covariates or offsets (49). We compared similarity in the total abundance trends,

202 the correlation of species' trends between model alternatives, and the classification of species'  
203 trends.

#### 204 *Comparison with other studies*

205 We compare our findings to three European long-term, regional butterfly monitoring  
206 programs with systematic Pollard walks that publish regular updates on total abundance and  
207 species' trends (39,50,51). Although all programs analyzed counts with Poisson regression, we  
208 had to standardize them differently depending on the data available and their modeling  
209 approaches. The UKBMS reports total abundance indicators as the geometric mean of species  
210 trends from two groups: specialist and countryside species (50). We used the reported smoothed  
211 annual index values for these indicators because the first year of monitoring is an outlier that  
212 exaggerates declines (UK Biodiversity Indicators 2018, <http://jncc.defra.gov.uk/page-4236>). We  
213 used the Dutch Butterfly Monitoring Scheme's reported cumulative annual trend in total  
214 butterflies counted across all transects after correction for missing surveys (51). For the Catalan  
215 Butterfly Monitoring Scheme, we extracted annual population indices from the 2015-2016  
216 annual report (52) with WebPlotDigitizer 4.1 (53) and performed a Poisson regression over time  
217 with annual random effects to obtain a comparable abundance trend. We converted total  
218 abundance trends into annual percent rates for comparison. We tallied the increases and  
219 decreases in species' trends for each region reported by the monitoring program, without  
220 accounting for differences in their statistical approaches.

#### 221 *Species' traits*

222 To explore potential mechanisms that might explain species-level variation in abundance  
223 trends, we modeled the estimates of species' temporal trends ( $\beta_1$ ) as a response to life history  
224 traits (20,30). Of the 81 species, 14 were classified as migratory species and 4 (*Colias*

225 *eurytheme*, *Lycaena phlaeus*, *Pieris rapae*, and *Thymelicus lineola*) were considered naturalized  
226 species that were introduced to the state 90 or more years ago (25,32). We analyzed traits models  
227 both across all species and after excluding migratory species, which would have population  
228 trends driven by factors outside of Ohio. We collected traits that relate to insect responses to  
229 climate change and habitat change, as these are two primary drivers of butterfly community  
230 changes (7,20,21).

231 We tested if butterflies with traits making them more adaptive to a warming climate have  
232 more positive population trends. Voltinism, or the number of generations per year, increases in  
233 warmer years and warmer regions within many species in Ohio (54), compared with obligate  
234 univoltine species that do not adjust their lifecycle based on changing growing season length. We  
235 assigned voltinism observed in Ohio (1-4 generations per year) to an integer scale based on  
236 visualization of phenology models and (25). We compared species with different range  
237 distributions, assuming that species distributed in warmer, Southern regions would be more  
238 likely to increase in Ohio as the climate warms. We assigned species' ranges as Southern, core,  
239 or Northern by range maps and county records (25,32). The life stage in which species  
240 overwinter contributes to its ability to respond to warming with shifts in phenology (20,55).

241 We would expect more generalist species, in host plant requirements and habitat  
242 preferences, to have more positive population trends in a landscape heavily modified by human  
243 use (21,50). For host plant requirements, we gathered two traits from the literature that describe  
244 host plant category (forb, graminoid, or woody) and whether the butterfly's host plant  
245 requirements span multiple plant families or are limited to one plant family or genus (25). Mean  
246 wing size from (32) was used as a surrogate of dispersal ability between habitats, which is  
247 expected to increase ability to access resources in a fragmented landscape. Three of the authors

248 assigned species as wetland-dependent or human-disturbance tolerant species, which we  
249 aggregated into two binary variables to test if specialist or generalist habitat preferences correlate  
250 with abundance trends.

251 We used univariate linear models for each life history trait both for all 81 species and  
252 with the 14 migratory species excluded. To account for the phylogenetic relatedness and the non-  
253 independence across species, we compared phylogenetic generalized least squares models that  
254 estimated branch length transformations with Pagel's lambda by maximum likelihood (56). The  
255 phylogenetic models excluded three species without gene sequences available.

#### 256 *Phylogenetic tree*

257 We obtained coding sequences for the most widely used DNA barcoding locus, the  
258 mitochondrial cytochrome c oxidase subunit I gene COI-5P, from GenBank (57). For species not  
259 found in GenBank, we obtained coding sequences from The Barcode of Life Data System (58).  
260 When possible, we obtained sequences from multiple sampling locations in North America.

261 Owing to the relatively small size of our multiple-species alignment—i.e. a single  
262 mtDNA locus, 651 base pairs in length—we decided to take both a constrained and  
263 unconstrained maximum likelihood approach to estimate the genealogical relationships of our  
264 samples. Some of the species from our analysis, though not all, were recently used in a more  
265 comprehensive phylogenetic analysis of butterflies (59), thus prompting us to constrain the  
266 phylogenetic backbone of our tree using family-level relationships. We report details of our  
267 workflow in Supplement 1.

#### 268 *Statistical analysis*

269 We used R 3.5.2 for analysis (60) and share the data and our code on Dryad. We fit  
270 generalized additive models with the *mgcv* package (34), generalized linear mixed models with

271 the *lme4* package (Bates et al. 2015), and phylogenetic generalized least squares models with the  
272 *ape* and *caper* packages (62,63). Confidence intervals for the temporal trends were estimated  
273 with bootstrapped model fits with the *merTools* package (64). We estimated the goodness of fit  
274 with  $R^2$  developed for generalized linear mixed models that give marginal and conditional  $R^2$   
275 values for the fixed effects and the fixed + random effects, respectively (65,66).

276

## 277 **Results**

278 The statewide relative abundance across all species declined at an annual rate of 2.0% ( $\beta_1$   
279 = -0.020, std. err. 0.005,  $p < 0.001$ ), accumulating a 33% decline over 1996-2016 (Table 1, Fig  
280 2). Among population trends, more than three times as many species are declining than  
281 increasing in abundance at our threshold of  $p < 0.05$  (32 versus 9, respectively) (Table 2, Fig B-  
282 D in S1 Appendix). Positive and negative species trends are distributed across the phylogenetic  
283 tree (Fig A in S1 Appendix).

284

285 **Table 1: Generalized linear mixed model of total abundance across all species.** The natural  
286 logarithm of the total survey duration across the monitoring season was an offset in the model.  
287 The model's marginal  $R^2$  was 0.20 for its fixed effects and its conditional  $R^2$  was 0.61 when  
288 including variation in sites, years, over-dispersion with random effects parameters.

Fixed effects	<i>B</i>	std.error	z statistic	p.value
Intercept	1.33	0.0506	26.4	<0.001
Year (numeric)	-0.0203	0.00496	-4.11	<0.001
List-length	0.104	0.00587	17.7	<0.001
		#		
Random effects	std. dev.	groups		
Site x year	0.278	1005		
Site	0.417	104		
Year (factor)	0.121	21		

289

290 **Table 2: Species' abundance trends over time.** Trends are the coefficient of year in our regression models with the accompanying  
 291 standard error and *p*-value for the coefficient (equation 1). Included are the number of population indices calculated for each species  
 292 for use in abundance model (Site x year) and total number of butterflies recorded for all years. Bold font indicates trends classified as  
 293 increasing or decreasing (*p* < 0.05).

Common	Species	Sample size				GLMM temporal trend		
	Latin	Total # counted	Sites	Years	Site/year	Trend coef.	Std. error	P
Aphrodite Fritillary	<i>Speyeria aphrodite</i>	477	9	16	131	<b>-0.233</b>	<b>0.060</b>	<b>&lt;0.001</b>
Baltimore	<i>Euphydryas phaeton</i>	818	7	17	83	<b>-0.224</b>	<b>0.071</b>	<b>0.002</b>
American Copper	<i>Lycaena phlaeas</i>	10,255	31	21	359	<b>-0.193</b>	<b>0.024</b>	<b>&lt;0.001</b>
Hoary Edge Skipper	<i>Achalarus lyciades</i>	291	7	19	88	<b>-0.178</b>	<b>0.061</b>	<b>0.003</b>
Milbert's Tortoise Shell	<i>Nymphalis milberti</i>	140	8	16	101	<b>-0.174</b>	<b>0.065</b>	<b>0.008</b>
European Skipper	<i>Thymelicus lineola</i>	46,549	57	21	609	<b>-0.173</b>	<b>0.021</b>	<b>&lt;0.001</b>
Southern Cloudywing	<i>Thorybes bathyllus</i>	667	15	20	194	<b>-0.129</b>	<b>0.037</b>	<b>&lt;0.001</b>
Falcate Orangetip	<i>Anthocharis midea</i>	756	8	18	103	<b>-0.123</b>	<b>0.040</b>	<b>0.002</b>
Dreamy Duskywing	<i>Erynnis icelus</i>	879	18	21	260	<b>-0.120</b>	<b>0.024</b>	<b>&lt;0.001</b>
Swarthy Skipper	<i>Nastra lherminier</i>	448	7	17	78	<b>-0.114</b>	<b>0.041</b>	<b>0.006</b>
Tawny Emperor	<i>Asterocampa clyton</i>	937	27	19	308	<b>-0.114</b>	<b>0.036</b>	<b>0.002</b>
Leonard's Skipper	<i>Hesperia leonardus</i>	1,348	11	20	144	<b>-0.110</b>	<b>0.025</b>	<b>&lt;0.001</b>
White M Hairstreak	<i>Parrhasius m-album</i>	95	7	15	110	-0.105	0.081	0.195
Northern Cloudywing	<i>Thorybes pylades</i>	547	16	20	210	<b>-0.095</b>	<b>0.033</b>	<b>0.004</b>
Coral Hairstreak	<i>Satyrium titus</i>	607	15	21	217	<b>-0.094</b>	<b>0.025</b>	<b>&lt;0.001</b>
Juvenal's Duskywing	<i>Erynnis juvenalis</i>	3,838	38	21	487	<b>-0.083</b>	<b>0.020</b>	<b>&lt;0.001</b>
Common Wood Nymph	<i>Cercyonis pegala</i>	21,603	77	21	788	<b>-0.073</b>	<b>0.013</b>	<b>&lt;0.001</b>
Common Sooty Wing	<i>Pholisora catullus</i>	1,142	34	20	398	<b>-0.072</b>	<b>0.015</b>	<b>&lt;0.001</b>
Sleepy Duskywing	<i>Erynnis brizo</i>	811	13	18	156	<b>-0.071</b>	<b>0.032</b>	<b>0.027</b>
Monarch	<i>Danaus plexippus</i>	46,070	104	21	1,005	<b>-0.070</b>	<b>0.023</b>	<b>0.002</b>
Red-spotted Purple	<i>Limenitis arthemis</i>	6,226	87	21	913	<b>-0.064</b>	<b>0.019</b>	<b>&lt;0.001</b>
Bronze Copper	<i>Lycaena hyllus</i>	656	23	21	254	-0.063	0.039	0.103
Northern Broken-Dash	<i>Wallengrenia egeremet</i>	5,959	49	21	528	<b>-0.062</b>	<b>0.018</b>	<b>&lt;0.001</b>
Tawny-edged Skipper	<i>Polites themistocles</i>	2,322	48	21	541	<b>-0.058</b>	<b>0.016</b>	<b>&lt;0.001</b>
West Virginia White	<i>Pieris virginianensis</i>	214	5	16	63	-0.058	0.059	0.329
Fiery Skipper	<i>Hylephila phyleus</i>	3,917	57	19	646	-0.057	0.061	0.351
Meadow Fritillary	<i>Boloria bellona</i>	5,447	55	21	598	<b>-0.056</b>	<b>0.027</b>	<b>0.040</b>

Orange Sulphur	<i>Colias eurytheme</i>	62,160	101	21	996	<b>-0.055</b>	<b>0.021</b>	<b>0.008</b>
Long Dash	<i>Polites mystic</i>	1,317	21	21	219	<b>-0.047</b>	<b>0.020</b>	<b>0.022</b>
American Lady	<i>Vanessa virginiensis</i>	2,029	54	21	637	-0.045	0.033	0.179
Black Swallowtail	<i>Papilio polyxenes</i>	12,410	92	21	941	<b>-0.044</b>	<b>0.015</b>	<b>0.004</b>
Gray Hairstreak	<i>Strymon melinus</i>	2,418	49	19	587	-0.044	0.026	0.089
Painted Lady	<i>Vanessa cardui</i>	5,564	80	21	873	-0.042	0.054	0.440
Great Spangled Fritillary	<i>Speyeria cybele</i>	33,573	90	21	904	<b>-0.041</b>	<b>0.020</b>	<b>0.047</b>
Hobomok Skipper	<i>Poanes hobomok</i>	6,863	51	21	576	<b>-0.040</b>	<b>0.014</b>	<b>0.005</b>
Viceroy	<i>Limenitis archippus</i>	16,079	85	21	896	<b>-0.039</b>	<b>0.016</b>	<b>0.014</b>
Cabbage White	<i>Pieris rapae</i>	304,105	104	21	1,005	<b>-0.038</b>	<b>0.010</b>	<b>&lt;0.001</b>
Hackberry Emperor	<i>Asterocampa celtis</i>	9,992	42	20	467	<b>-0.037</b>	<b>0.017</b>	<b>0.033</b>
Striped Hairstreak	<i>Satyrium liparops</i>	155	14	18	211	-0.028	0.067	0.682
Variegated Fritillary	<i>Euptoieta claudia</i>	956	17	19	204	-0.027	0.052	0.603
Little Wood Satyr	<i>Megisto cymela</i>	76,612	87	21	878	<b>-0.026</b>	<b>0.009</b>	<b>0.005</b>
American Snout Butterfly	<i>Libytheana carinenta</i>	1,007	36	18	418	-0.025	0.050	0.612
Hickory Hairstreak	<i>Satyrium caryaevorum</i>	196	12	20	170	-0.023	0.053	0.656
Mourning Cloak	<i>Nymphalis antiopa</i>	3,214	85	21	905	-0.021	0.018	0.256
Clouded Sulphur	<i>Colias philodice</i>	49,267	102	21	998	-0.014	0.014	0.286
Spicebush Swallowtail	<i>Papilio troilus</i>	25,322	82	21	858	-0.014	0.014	0.324
Dun Skipper	<i>Euphyes vestris</i>	1,684	49	21	585	-0.014	0.012	0.224
Question Mark	<i>Polygonia interrogationis</i>	6,564	88	21	915	-0.012	0.025	0.640
Delaware Skipper	<i>Atrytone logan</i>	1,086	30	21	313	-0.011	0.029	0.697
Horace's Duskywing	<i>Erynnis horatius</i>	2,885	31	21	376	-0.011	0.023	0.633
Eastern Tiger Swallowtail	<i>Papilio glaucus</i>	29,299	101	21	996	-0.010	0.015	0.483
Pearl Crescent	<i>Phyciodes tharos</i>	180,631	104	21	1,005	-0.010	0.014	0.461
Little Yellow	<i>Eurema lisa</i>	1,681	24	18	287	-0.008	0.073	0.917
Eastern Comma	<i>Polygonia comma</i>	6,222	92	21	944	-0.007	0.011	0.561
Giant Swallowtail	<i>Papilio cresphontes</i>	1,109	28	21	322	0.002	0.019	0.912
Banded Hairstreak	<i>Satyrium calanus</i>	1,107	36	21	468	0.004	0.031	0.896
Silver-spotted Skipper	<i>Epargyreus clarus</i>	54,462	102	21	996	0.005	0.012	0.672
Red Admiral	<i>Vanessa atalanta</i>	28,637	97	21	969	0.008	0.044	0.865
Red-banded Hairstreak	<i>Calycopis cecrops</i>	795	7	17	91	0.009	0.057	0.879
Crossline Skipper	<i>Polites origenes</i>	1,087	27	21	347	0.009	0.020	0.636
Sachem	<i>Atalopedes campestris</i>	1,445	19	18	231	0.013	0.058	0.823
Peck's Skipper	<i>Polites peckius</i>	23,702	90	21	905	0.014	0.014	0.306
Northern Eyed Brown	<i>Satyroides eurydice</i>	1,342	13	21	174	0.016	0.035	0.651
Eastern Tailed Blue	<i>Everes comyntas</i>	56,137	99	21	974	0.016	0.010	0.113
Henry's Elfin	<i>Callophrys henrici</i>	330	7	17	76	0.017	0.055	0.752
Little Glassy Wing	<i>Pompeius verna</i>	8,658	56	21	632	0.019	0.019	0.307
Silvery Checkerspot	<i>Chlosyne nycteis</i>	2,049	20	19	224	0.039	0.022	0.074

Spring/Summer Azure	<i>Celastrina ladon/neglecta</i>	63,947	103	21	1,002	<b>0.047</b>	<b>0.021</b>	<b>0.022</b>
Common Buckeye	<i>Junonia coenia</i>	15,771	73	19	834	0.050	0.067	0.459
Pipevine Swallowtail	<i>Battus philenor</i>	703	23	18	279	0.053	0.033	0.110
Least Skipper	<i>Ancyloxypha numitor</i>	27,506	84	21	844	<b>0.053</b>	<b>0.015</b>	<b>&lt;0.001</b>
Appalachian Eyed Brown	<i>Satyroides appalachia</i>	2,118	12	18	118	0.060	0.045	0.181
Zabulon Skipper	<i>Poanes zabulon</i>	10,960	71	21	747	<b>0.061</b>	<b>0.022</b>	<b>0.004</b>
Northern Pearly-Eye	<i>Enodia anhedon</i>	2,785	37	21	434	<b>0.071</b>	<b>0.020</b>	<b>&lt;0.001</b>
Zebra Swallowtail	<i>Eurytides marcellus</i>	1,349	18	18	224	<b>0.075</b>	<b>0.030</b>	<b>0.011</b>
Cloudless Sulphur	<i>Phoebis sennae</i>	1,840	27	19	355	0.088	0.057	0.121
Common Checkered-Skipper	<i>Pyrgus communis</i>	3,089	33	18	357	<b>0.092</b>	<b>0.046</b>	<b>0.046</b>
Wild Indigo Duskywing	<i>Erynnis baptisiae</i>	15,209	51	19	570	<b>0.106</b>	<b>0.020</b>	<b>&lt;0.001</b>
Harvester	<i>Feniseca tarquinius</i>	341	11	20	143	<b>0.122</b>	<b>0.061</b>	<b>0.046</b>
Sleepy Orange	<i>Eurema nicippe</i>	2,028	6	17	63	0.146	0.134	0.276
Gemmed Satyr	<i>Cyllopsis gemma</i>	1,059	6	16	81	<b>0.228</b>	<b>0.052</b>	<b>&lt;0.001</b>



294 **Fig 2: The relative abundance of counted butterflies in Ohio declined by 33% over 1996-**  
295 **2016.** Plotted are model predictions for each year based on the fixed effects of year (solid line)  
296 and annual random effects (dots) to show annual variation about the trend line. Shading shows  
297 95% confidence interval based on bootstrapped model fits for the temporal trend.

298

299 Both in the total trend in abundance and in the proportion of species with declines, these  
300 results are comparable to our review of three European butterfly monitoring schemes (Table 3).  
301 Although the longer-running programs show larger cumulative declines, the annual rate of  
302 change ranges from -2.0% to -2.6% for Ohio, Catalonia, and the Netherlands, respectively, with  
303 the United Kingdom total trends split between generalist species (-0.8%) and specialist species (-  
304 2.4%). Across monitoring programs, declining species outnumber increasing species by a factor  
305 of two to three (Table 3).

306

307 Table 3: Comparison of estimated annualized and cumulative rates of change in total abundance and species trends in regional  
 308 butterfly monitoring programs in Europe compared to this study. Number of sites represents those reported to be analyzed in total, but  
 309 may no longer be active. Number of butterflies counted per year is based on the most recent years of monitoring described in the  
 310 references.

Region (km <sup>2</sup> )	Years	Sites	Counted/year (x 1000)	Annualized trend in total abundance (cumulative)	Species' trends			Reference
					Positive	Negative	Stable/ not signif.	
United Kingdom (242,500)	41 (1976-2017)	3,164	1,700	-0.8% (-28%) countryside -2.4% (-63%) specialist	11	22	24	(50)
Netherlands (42,508)	25 (1992-2017)	600	250	-2.0% (-40%)	11	23	13	(51)
Catalonia, Spain (32,108)	22 (1994-2016)	116	122	-2.6% (-44%)	15	46	5	(39,52)
<b>Ohio, USA (116,100)</b>	<b>20 (1996-2016)</b>	<b>104</b>	<b>80</b>	<b>-2.0% (-33%)</b>	<b>9</b>	<b>32</b>	<b>40</b>	<b>this study</b>

311

312 In general, traits associated with species' responses to climate were more important,  
313 based on the predictive ability (adjusted  $R^2$ ) of univariate models, than traits associated with  
314 habitat and host plant restrictions (Fig 3, Tables A and B in S1 Appendix). Phylogenetic signal  
315 was included in most traits, so we focus on the phylogenetic generalized least squares results.  
316 Migratory species to Ohio had stable population trends on average compared to resident species  
317 and the four naturalized species. The Monarch (*Danaus plexippus*) was the only migratory  
318 species in decline. Multivoltine species with more annual generations had more positive  
319 population trends. Species with more northern geographic ranges were associated with more  
320 negative population trends. Species eating forb host plants had negative trends on average, but  
321 there was no effect of host plant specialization on population trends.

322

323 **Fig 3: Species' traits are associated with variation in the statewide trends in abundance.** We  
324 plot each trend compared to the six most important traits for the 78 species included in the  
325 phylogenetic GLS models with full results in Table A in S1 Appendix. Squares represent the  
326 regression coefficients with 95% confidence intervals shown in lines. Dots for each species are  
327 jittered for visualization.

328

329 Our choice of modeling approach did not change the overall evidence of defaunation.  
330 Generalized linear mixed models with Poisson-distributed errors and generalized additive mixed  
331 models estimated declines in total abundance similar in magnitude at -1.83% and -2.13% annual  
332 rates, respectively. The annual trend estimate from TRIM, without sampling covariates, was half  
333 the magnitude at -0.96%. Species' trends had high correlations between pairwise comparisons,

334 but TRIM models had notably more positive trends compared to the other three approaches  
335 (Table C in S1 Appendix).

336

## 337 **Discussion**

338 We show that the total butterfly abundance has declined by 33% over 20 years in Ohio.  
339 This rate is faster than the global abundance trend estimated for Lepidoptera (35% over 40 years)  
340 and corresponds more closely to the steeper declines (45% over 40 years) estimated for all  
341 insects (1). The Ohio butterfly monitoring program, judged by the weekly frequency, 20-year  
342 time period, and statewide spatial extent of its surveys, is the most extensive systematic insect  
343 survey in North America and comparable to three exemplary European butterfly monitoring  
344 schemes. The annualized 2% rate of decline in this study aligns closely with trends from  
345 European butterfly monitoring, confirming the decline of the most closely monitored group of  
346 insects in both Europe and North America (Table 3). With less known about other insect taxa,  
347 butterflies provide a necessary, if imperfect, surrogate to understand the trajectory and potential  
348 mechanisms behind broader insect trends (13). Extensive in both time and space, the decline in  
349 butterfly abundance is the current best estimate for the rate of insect defaunation in North  
350 America.

351 The proportion of butterfly species with population declines compared to population  
352 increases is similar between Ohio (negative trends three times more numerous) and European  
353 studies (negative trends 2-3 times more numerous) (Table 3). In other taxa, moths in the United  
354 Kingdom show a similar proportion of species declines (67). Long-term monitoring in protected  
355 areas, although less extensive in space, shows more positive species trends for moths in Finland  
356 (at 67.7° latitude) and across pollinators in Spain (at 850-1750 m. elevations) (68,69). These

357 counterexamples show how insect communities may shift at high-latitude or high-elevation sites  
358 with anthropogenic climate warming (21) or may persist in more remote areas. However,  
359 butterfly monitoring in populated areas show a consistency in observed declines (Table 3) that  
360 we argue would generalize to other landscapes dominated by human use.

361 We demonstrate declines in species that are generalist, widespread, and not considered  
362 vulnerable to extinction (25,70). The four species introduced to Ohio (*Pieris rapae*, *Lycaena*  
363 *phlaeus*, and *Thymelicus lineola* from Europe and *Colias eurytheme* from the western USA) are  
364 declining more rapidly than native species (Fig 3). Although few may share concern for the most  
365 widespread, invasive butterfly in the world's agricultural and urban settings (71), declines in  
366 *Pieris rapae* could be indicative of persistent environmental stressors that would affect other  
367 species as well. We would expect negative environmental changes to disproportionately affect  
368 rare species prone to the demographic dangers of small populations or specialist species that rely  
369 on a narrow range of resources or habitat (UKBMS in Table 3, Swengel et al. 2011). This pattern  
370 of species declines would lead to biotic homogenization as rarer species are lost and common,  
371 disturbance-tolerant species remain (72,73). However, our study adds another example of  
372 declines in common butterfly species thought to be well-suited to human-modified habitat  
373 (11,21,74).

374 The Eastern North American migratory Monarch (*Danaus plexippus*) abundance in Ohio  
375 is declining by 7% per year. The Monarch is the only declining migratory species out of 14 in  
376 our analysis. Despite disagreements about whether summer abundance trends have tracked  
377 winter colony declines (75,76), our study shows that the long-term trends correspond. However,  
378 our study's first two years have very high Monarch population indices which could be outliers  
379 (Fig B in S1 Appendix) following the two largest recorded winter population counts (16,77).

380 With these two years removed, the statewide Monarch trend is a 4% decline per year, showing  
381 that the magnitude of summer abundance trends are sensitive to the years of inclusion. Our  
382 results align with a study using Illinois systematic monitoring data that shows a summer  
383 abundance decline for monarchs over two decades, but at different rates across decades (78). A  
384 more recent study showed no decline during the summer during 2004-2016 using a population  
385 index from NABA counts (77). The trend we document comes from the sum of summer breeding  
386 and fall migratory butterflies returning to Mexico; estimates of abundance for these separate  
387 generations may be required to model how different stages of the lifecycle contribute to the long-  
388 term decline in the winter colonies (77).

389 Even with systematic monitoring, accurate estimates of insect abundance are missing  
390 from many species—a fifth of regularly observed species in did not meet our minimum data  
391 requirements to for us to estimate trends. None of these species are considered to be of  
392 conservation concern, but this also means that we would be limited in our ability to even  
393 determine if their populations have reached threatened status. Targeted surveys of selected  
394 species, non-adult life stages, or habitats can expand the monitoring to data-deficient species  
395 commonly excluded by protocols designed to monitor many species efficiently (50) and can be  
396 used to estimate demographic responses to environmental drivers not apparent from adult  
397 butterfly counts (79). Additional life-history knowledge about species in our study could inform  
398 how worried we should be about extreme population declines, like the Baltimore (*Euphydryas*  
399 *phaeton*). We noticed a multiyear population cycle, not captured by log-linear trends or  
400 generalized additive models, which reached a nadir at the end of our dataset and exaggerated the  
401 Baltimore's decline (Fig B in S1 Appendix). In other cases, density-dependent population

402 regulation may help inform whether species are resilient to temporary declines and guide  
403 predictions for future population trajectories (80).

404 Insect declines have multifaceted causes, and the relative impact of these causes is still  
405 unknown (81). Although analysis of the causes of site differences in abundance or species trends  
406 is beyond the scope of this study, we discuss three environmental drivers commonly associated  
407 with global insect declines: climate change, habitat loss and fragmentation, and agricultural  
408 intensification (81,82). If species' traits are associated with population trends, then their  
409 relationships may suggest environmental changes driving population responses in species sharing  
410 these traits (46,81,83). In this study, life-history traits were weakly predictive of population  
411 trends, but their associations provide hypotheses that could be tested further (46).

#### 412 *Habitat loss and fragmentation*

413 In Ohio, habitat loss and fragmentation plateaued well before butterfly monitoring  
414 started, with human population growth slowing by 1970. In common with other Midwestern  
415 states, Ohio had already lost tallgrass prairie species, such as the Regal Fritillary (*Speyeria*  
416 *idalia*), due to this habitat conversion to agriculture (25,26). Land-use has changed slowly over  
417 the course of the monitoring program; less than 10% of monitoring sites have had more than  
418 2.5% change in the surrounding (2.5-km radius) developed, agriculture, or forest land cover from  
419 2001-2011. The persistence of butterfly populations in a landscape of habitat fragments are  
420 mediated by species' traits that permit them to either move between more isolated resources or  
421 persist in smaller, localized populations (82,84). Wing size is one life history trait associated  
422 with dispersal ability, but it had no association with species' population trends (Tables A and B  
423 in S1 Appendix). However, defining habitat patches by land-use classes overlooks how mobile  
424 insect populations are bound by resources, varying across the lifecycle, rather than area (85,86).

425 Although there has been little wholesale habitat conversion around our study transects,  
426 degradation of the remaining habitat could be a cause of the general decline in butterfly  
427 abundance.

#### 428 *Climate change*

429 Species trends are associated with two life-history traits, voltinism and range distribution,  
430 which suggest that the butterfly community is changes with the warming climate. Species that  
431 complete more annual generations, or multivoltine species, had more positive abundance trends.  
432 This aligns with obligate univoltine species becoming less common in Massachusetts (20), but is  
433 the opposite of the findings in Spain where multivoltine species are in steeper declines with  
434 exposure to increasingly dry summers (39). Multivoltine species may be more adaptive to annual  
435 and spatial variation in seasonal temperatures as many have plasticity in the voltinism observed  
436 within Ohio (25). For most of the species with flexible voltinism in Ohio, adding an extra  
437 generation in warmer summers increases their annual population growth rates (54). Northern-  
438 distributed species have more negative population trends compared to widely-distributed or  
439 southern species. This corresponds with findings from Massachusetts and Europe that warm-  
440 adapted species are replacing cool-adapted species as range distributions shift (20,87). Even  
441 though these two traits should be increasing butterfly abundance for some species as the climate  
442 warms, it has not been enough to prevent the overall decline in butterfly abundance.

#### 443 *Agricultural intensification*

444 Cropland and pasture make up half of Ohio's land area, so we would expect agricultural  
445 practices to affect statewide insect abundance. One assessment of pollinator habitat suitability  
446 based on land-use, acres in conservation reserve programs, and crop type estimated an increase  
447 in resources in Ohio from 1982 through 2002, followed by a stable trend (88). However,



448 agricultural practices can decrease insect abundance with systemic insecticides, herbicide use on  
449 host plants or nectar resources, and nitrogen fertilization that alters the composition of  
450 surrounding plant communities.

451 In Ohio, the use of neonicotinoids rapidly increased after 2004 when they became widely  
452 used on corn and soybeans (89,90). The mechanistic link between neonicotinoid insecticides and  
453 insect declines is established and observational studies have shown widespread impacts of their  
454 use (91–93). Even though seed-coatings with neonicotinoids reduce broadcast spraying, the  
455 mechanical planting of these seeds exposes widespread areas around farms to contaminated dust  
456 that is incorporated into non-target plants and insects (94,95). In the United Kingdom and  
457 California, neonicotinoids are associated with butterfly declines (22,96) and hinder butterfly  
458 larval development on host plants (97). We did not design this study to test whether  
459 neonicotinoids affect butterfly abundance in Ohio. However, the observed declines across  
460 common, invasive species, which would typically be predicted to exploit an agricultural or  
461 human-altered landscape, would be consistent with widespread exposure to insecticides.

462 Species that eat forbs as larvae have negative population declines (Fig 3). Both herbicide  
463 use and nitrogen deposition may alter plant communities to favor grasses over forbs (98).  
464 Milkweed losses contribute to declines in Monarch butterfly abundance, as they lose host  
465 plants as a result of herbicide use (78,79). In Ohio, glyphosate use has increased linearly, and is  
466 now applied at 6 times the rate it was in 1996 (89,90). Nitrogen increases have been linked to  
467 declines in grassland species adapted to low-nitrogen environments (99–101) and to higher  
468 mortality during larval development on enriched host plants (102).

469

470 **Conclusions**

471           Systematic, long-term surveys of butterflies provide the most rigorous evidence for the  
472 rate of insect declines. This study demonstrates that defaunation is happening in North America  
473 similarly to Europe. In landscapes comprising natural areas amid heavy human land-use,  
474 butterfly total abundance is declining at 2% per year and 2-3 times more species have population  
475 trends declining rather than increasing. Additional Pollard-based monitoring programs in North  
476 America, listed in (9), will enable tracking insect trends over larger spatial extents as with efforts  
477 to integrate data across European monitoring schemes (11). The rates for other insect groups may  
478 deviate from this baseline and were previously estimated to be declining more rapidly than  
479 Lepidoptera (1). Expanded monitoring and support for taxonomists are imperative for other taxa  
480 and under sampled regions, like the Tropics where most insect diversity resides. Besides the  
481 evaluation if butterfly trends generalize to other insects, the most urgent need for science and  
482 conservation is understanding the causes of decline and testing mitigation actions. As butterflies  
483 are the best-monitored insect taxa, they are the best indicator of the baseline threat to the 5.5  
484 million insects, the most diverse group of animals on earth.

485

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494

495 **References**

- 496 1. Dirzo R, Young HS, Galetti M, Ceballos G, Isaac NJB, Collen B. Defaunation in the  
497 Anthropocene. *Science*. 2014 Jul 25;345(6195):401–6.
- 498 2. May RM. How many species are there on earth? *Science*. 1988;241(4872):1441–1449.
- 499 3. Conrad KF, Fox R, Woiwod IP. Monitoring biodiversity: measuring long-term changes in  
500 insect abundance. *Insect Conserv Biol*. 2007;203–225.
- 501 4. Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, et al. More than 75  
502 percent decline over 27 years in total flying insect biomass in protected areas. Lamb EG,  
503 editor. *PLOS ONE*. 2017 Oct 18;12(10):e0185809.
- 504 5. Lister BC, Garcia A. Climate-driven declines in arthropod abundance restructure a  
505 rainforest food web. *Proc Natl Acad Sci*. 2018 Oct 30;115(44):E10397–406.
- 506 6. Winfree R, W. Fox J, Williams NM, Reilly JR, Cariveau DP. Abundance of common  
507 species, not species richness, drives delivery of a real-world ecosystem service. Shea K,  
508 editor. *Ecol Lett*. 2015 Jul;18(7):626–35.
- 509 7. Warren MS, Hill JK, Thomas JA, Asher J, Fox R, Huntley B, et al. Rapid responses of  
510 British butterflies to opposing forces of climate and habitat change. 2001;414:5.
- 511 8. Parmesan C. Ecological and Evolutionary Responses to Recent Climate Change. *Annu*  
512 *Rev Ecol Evol Syst*. 2006 Dec;37(1):637–69.
- 513 9. Taron D, Ries L. Butterfly Monitoring for Conservation. In: Daniels JC, editor. *Butterfly*  
514 *Conservation in North America* [Internet]. Dordrecht: Springer Netherlands; 2015 [cited  
515 2018 Dec 7]. p. 35–57. Available from: [http://link.springer.com/10.1007/978-94-017-](http://link.springer.com/10.1007/978-94-017-9852-5_3)  
516 [9852-5\\_3](http://link.springer.com/10.1007/978-94-017-9852-5_3)

- 517 10. Pollard E, Yates TJ. Monitoring butterflies for ecology and conservation: the British  
518 butterfly monitoring scheme. Springer Science & Business Media; 1994.
- 519 11. Van Swaay C, Van Strien A, Aghababayan K, Astrom S, Botham M, Brereton T, et al. The  
520 European Butterfly Indicator for Grassland species: 1990-2013. 2015;
- 521 12. Schmucki R, Pe'er G, Roy DB, Stefanescu C, Van Swaay CAM, Oliver TH, et al. A  
522 regionally informed abundance index for supporting integrative analyses across butterfly  
523 monitoring schemes. Müller J, editor. *J Appl Ecol*. 2016 Apr;53(2):501–10.
- 524 13. Thomas JA. Monitoring change in the abundance and distribution of insects using  
525 butterflies and other indicator groups. *Philos Trans R Soc B Biol Sci*. 2005 Feb  
526 28;360(1454):339–57.
- 527 14. Brereton T, Roy DB, Middlebrook I, Botham M, Warren M. The development of butterfly  
528 indicators in the United Kingdom and assessments in 2010. *J Insect Conserv*. 2011  
529 Apr;15(1–2):139–51.
- 530 15. Dennis EB, Morgan BJT, Roy DB, Brereton TM. Urban indicators for UK butterflies.  
531 *Ecol Indic*. 2017 May;76:184–93.
- 532 16. Agrawal AA, Inamine H. Mechanisms behind the monarch's decline. *Science*.  
533 2018;360(6395):1294–1296.
- 534 17. Schultz CB, Brown LM, Pelton E, Crone EE. Citizen science monitoring demonstrates  
535 dramatic declines of monarch butterflies in western North America. *Biol Conserv*.  
536 2017;214:343–346.
- 537 18. Belitz MW, Hendrick LK, Monfils MJ, Cuthrell DL, Marshall CJ, Kawahara AY, et al.  
538 Aggregated occurrence records of the federally endangered Poweshiek skipperling  
539 (*Oarisma poweshiek*). *Biodivers Data J*. 2018;(6).

- 540 19. Haddad NM. Resurrection and resilience of the rarest butterflies. *PLOS Biol.* 2018 Feb  
541 6;16(2):e2003488.
- 542 20. Breed GA, Stichter S, Crone EE. Climate-driven changes in northeastern US butterfly  
543 communities. *Nat Clim Change.* 2013 Feb;3(2):142–5.
- 544 21. Forister ML, McCall AC, Sanders NJ, Fordyce JA, Thorne JH, O’Brien J, et al.  
545 Compounded effects of climate change and habitat alteration shift patterns of butterfly  
546 diversity. *Proc Natl Acad Sci.* 2010 Feb 2;107(5):2088–92.
- 547 22. Forister ML, Cousens B, Harrison JG, Anderson K, Thorne JH, Waetjen D, et al.  
548 Increasing neonicotinoid use and the declining butterfly fauna of lowland California. *Biol*  
549 *Lett.* 2016 Aug;12(8):20160475.
- 550 23. Schlicht D, Swengel A, Swengel S. Meta-analysis of survey data to assess trends of prairie  
551 butterflies in Minnesota, USA during 1979–2005. *J Insect Conserv.* 2009 Aug;13(4):429–  
552 47.
- 553 24. Swengel SR, Schlicht D, Olsen F, Swengel AB. Declines of prairie butterflies in the  
554 midwestern USA. *J Insect Conserv.* 2011 Apr;15(1–2):327–39.
- 555 25. Iftner DC, Shuey JA, Calhoun JV. Butterflies and skippers of Ohio. College of Biological  
556 Sciences, Ohio State University; 1992.
- 557 26. Shuey JA, Calhoun JV, Iftner DC. Butterflies that are endangered, threatened, and of  
558 special concern in Ohio. 1987;
- 559 27. Bigelow D, Borchers A. Major uses of land in the United States, 2012. 2017.
- 560 28. Thornton PE, Running SW, White MA. Generating surfaces of daily meteorological  
561 variables over large regions of complex terrain. *J Hydrol.* 1997 Mar;190(3–4):214–51.

- 562 29. Homer C, Dewitz J, Yang L, Jin S, Danielson P, Xian G, et al. Completion of the 2011  
563 National Land Cover Database for the conterminous United States—representing a decade  
564 of land cover change information. *Photogramm Eng Remote Sens.* 2015;81(5):345–354.
- 565 30. Diamond SE, Cayton H, Wepprich T, Jenkins CN, Dunn RR, Haddad NM, et al.  
566 Unexpected phenological responses of butterflies to the interaction of urbanization and  
567 geographic temperature. *Ecology.* 2014 Sep;95(9):2613–21.
- 568 31. Dennis EB, Freeman SN, Brereton T, Roy DB. Indexing butterfly abundance whilst  
569 accounting for missing counts and variability in seasonal pattern. O’Hara RB, editor.  
570 *Methods Ecol Evol.* 2013 Jul;4(7):637–45.
- 571 32. Opler PA, Krizek GO. *Butterflies east of the Great Plains: an illustrated natural history.*  
572 Johns Hopkins Univ Pr; 1984.
- 573 33. Dennis EB, Morgan BJT, Freeman SN, Brereton TM, Roy DB. A generalized abundance  
574 index for seasonal invertebrates: A Generalized Abundance Index for Seasonal  
575 Invertebrates. *Biometrics.* 2016 Dec;72(4):1305–14.
- 576 34. Wood SN. *Generalized Additive Models: An Introduction with R.* 2nd ed. Chapman and  
577 Hall/CRC; 2017.
- 578 35. Cordano EE& E. Interpol.T: Hourly interpolation of multiple temperature daily series  
579 [Internet]. 2013. Available from: <https://CRAN.R-project.org/package=Interpol.T>
- 580 36. Link WA, Sauer JR. Estimating Population Change from Count Data: Application to the  
581 North American Breeding Bird Survey. *Ecol Appl.* 1998 May;8(2):258.
- 582 37. Pellet J, Bried JT, Parietti D, Gander A, Heer PO, Cherix D, et al. Monitoring Butterfly  
583 Abundance: Beyond Pollard Walks. Schweiger O, editor. *PLoS ONE.* 2012 Jul  
584 30;7(7):e41396.

- 585 38. Henry EH, Anderson CT. Abundance estimates to inform butterfly management: double-  
586 observer versus distance sampling. *J Insect Conserv.* 2016 Jun;20(3):505–14.
- 587 39. Melero Y, Stefanescu C, Pino J. General declines in Mediterranean butterflies over the last  
588 two decades are modulated by species traits. *Biol Conserv.* 2016 Sep;201:336–42.
- 589 40. Haddad NM, Hudgens B, Damiani C, Gross K, Kuefler D, Pollock K. Determining  
590 Optimal Population Monitoring for Rare Butterflies: *Monitoring Rare Butterflies*. *Conserv*  
591 *Biol.* 2008 Aug;22(4):929–40.
- 592 41. Isaac NJB, Cruickshanks KL, Weddle AM, Marcus Rowcliffe J, Brereton TM, Dennis  
593 RLH, et al. Distance sampling and the challenge of monitoring butterfly populations:  
594 Distance sampling and monitoring butterflies. *Methods Ecol Evol.* 2011 Dec;2(6):585–94.
- 595 42. Knappe J. Decomposing trends in Swedish bird populations using generalized additive  
596 mixed models. Siriwardena G, editor. *J Appl Ecol.* 2016 Dec;53(6):1852–61.
- 597 43. Szabo JK, Vesk PA, Baxter PWJ, Possingham HP. Regional avian species declines  
598 estimated from volunteer-collected long-term data using List Length Analysis. *Ecol Appl.*  
599 2010;20(8):2157–69.
- 600 44. van Strien AJ, van Swaay CAM, Termaat T. Opportunistic citizen science data of animal  
601 species produce reliable estimates of distribution trends if analysed with occupancy  
602 models. Devictor V, editor. *J Appl Ecol.* 2013 Dec;50(6):1450–8.
- 603 45. Roy DB, Ploquin EF, Randle Z, Risely K, Botham MS, Middlebrook I, et al. Comparison  
604 of trends in butterfly populations between monitoring schemes. *J Insect Conserv.* 2015  
605 Apr;19(2):313–24.

- 606 46. Soykan CU, Sauer J, Schuetz JG, LeBaron GS, Dale K, Langham GM. Population trends  
607 for North American winter birds based on hierarchical models. *Ecosphere*.  
608 2016;7(5):e01351.
- 609 47. Kraemer HC, Blasey CM. Centring in regression analyses: a strategy to prevent errors in  
610 statistical inference. *Int J Methods Psychiatr Res*. 2004;13(3):141–51.
- 611 48. Harrison XA. Using observation-level random effects to model overdispersion in count  
612 data in ecology and evolution. *PeerJ*. 2014 Oct 9;2:e616.
- 613 49. Pannekoek J, Van Strien A. Trim 3 Manual (TRends & Indices for Monitoring data)–  
614 Statistics Netherlands. Voorburg; 2001.
- 615 50. Brereton T, Botham M, Middlebrook I, Randle Z, Noble D, Harris S, et al. United  
616 Kingdom Butterfly Monitoring Scheme report for 2017. Centre for Ecology & Hydrology  
617 & Butterfly Conservation; 2018.
- 618 51. Van Swaay CAM, Bos G, Van Grunsven RHA, Kok J, Huskens K, Van Deijk JR, et al.  
619 Vlinders en libellen geteld: Jaarverslag 2017 [Internet]. De Vlinderstichting, Wageningen;  
620 2018 [cited 2019 Feb 14]. Report No.: Rapport VS2018.006. Available from:  
621 <https://assets.vlinderstichting.nl/docs/6d51f174-b497-4777-b84a-362e344c3528.pdf>
- 622 52. Stefanescu C. Resum de les temporades 2015 i 2016. Cynthia Butlletí Butterfly Monit  
623 Scheme Catalunya [Internet]. 2018 [cited 2019 Feb 14];14. Available from:  
624 <http://www.catalanbms.org/es/cynthia/>
- 625 53. Rohatgi A. WebPlotDigitizer. 2011.
- 626 54. Wepprich TM. Effects of Climatic Variability on a Statewide Butterfly Community. North  
627 Carolina State University; 2017.



- 628 55. Diamond SE, Frame AM, Martin RA, Buckley LB. Species' traits predict phenological  
629 responses to climate change in butterflies. 2011;92(5):8.
- 630 56. Pagel M. Inferring the historical patterns of biological evolution. Nature. 1999  
631 Oct;401(6756):877–84.
- 632 57. Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids  
633 Res. 2016 Jan 4;44(Database issue):D67–72.
- 634 58. Ratnasingham S, Hebert PD. BOLD: The Barcode of Life Data System ([http://www.  
635 barcodinglife.org](http://www.barcodinglife.org)). Mol Ecol Notes. 2007;7(3):355–364.
- 636 59. Espeland M, Breinholt J, Willmott KR, Warren AD, Vila R, Toussaint EFA, et al. A  
637 Comprehensive and Dated Phylogenomic Analysis of Butterflies. Curr Biol. 2018  
638 Mar;28(5):770-778.e5.
- 639 60. R Core Team. R: A Language and Environment for Statistical Computing [Internet].  
640 Vienna, Austria: R Foundation for Statistical Computing; 2018. Available from:  
641 <https://www.R-project.org/>
- 642 61. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using  
643 **lme4**. J Stat Softw [Internet]. 2015 [cited 2018 Dec 4];67(1). Available from:  
644 <http://www.jstatsoft.org/v67/i01/>
- 645 62. Orme D, Freckleton R, Thomas G, Petzoldt T. The caper package: comparative analysis of  
646 phylogenetics and evolution in R. R Package Version. 2013;5(2).
- 647 63. Paradis E, Schliep K. ape 5.0: an environment for modern phylogenetics and evolutionary  
648 analyses in R. Bioinformatics. 2018;xx:xxx–xxx.
- 649 64. Knowles JE, Frederick C. merTools: Tools for Analyzing Mixed Effect Regression  
650 Models [Internet]. 2018. Available from: <https://CRAN.R-project.org/package=merTools>

- 651 65. Nakagawa S, Schielzeth H. A general and simple method for obtaining  $R^2$  from  
652 generalized linear mixed-effects models. O'Hara RB, editor. *Methods Ecol Evol.* 2013  
653 Feb;4(2):133–42.
- 654 66. Barton K. MuMIn: Multi-Model Inference [Internet]. 2018. Available from:  
655 <https://CRAN.R-project.org/package=MuMIn>
- 656 67. Conrad KF, Woiwod IP, Parsons M, Fox R, Warren MS. Long-term population trends in  
657 widespread British moths. *J Insect Conserv.* 2004;8(2–3):119–136.
- 658 68. Hunter MD, Kozlov MV, Itämies J, Pulliainen E, Bäck J, Kyrö E-M, et al. Current  
659 temporal trends in moth abundance are counter to predicted effects of climate change in an  
660 assemblage of subarctic forest moths. *Glob Change Biol.* 2014 Jun;20(6):1723–37.
- 661 69. Herrera CM. Complex long-term dynamics of pollinator abundance in undisturbed  
662 Mediterranean montane habitats over two decades. *Ecol Monogr.* 2019 Feb  
663 1;89(1):e01338.
- 664 70. Cech R, Tudor G, others. *Butterflies of the east coast*. Princeton University Press; 2005.
- 665 71. Ryan SF, Lombaert E, Espeset A, Vila R, Talavera G, Dincă V, et al. Global invasion  
666 history of the world's most abundant pest butterfly: a citizen science population genomics  
667 study. *bioRxiv.* 2018 Dec 26;506162.
- 668 72. McKinney ML, Lockwood JL. Biotic homogenization: a few winners replacing many  
669 losers in the next mass extinction. *Trends Ecol Evol.* 1999;14(11):450–453.
- 670 73. Clavel J, Julliard R, Devictor V. Worldwide decline of specialist species: toward a global  
671 functional homogenization? *Front Ecol Environ.* 2011;9(4):222–8.

- 672 74. Van Dyck H, Van Strien AJ, Maes D, Van Swaay CAM. Declines in Common,  
673 Widespread Butterflies in a Landscape under Intense Human Use. *Conserv Biol.* 2009  
674 Aug;23(4):957–65.
- 675 75. Ries L, Oberhauser K, Taron D, Battin J, Rendon-Salinas E, Altizer S, et al. Connecting  
676 eastern monarch population dynamics across their migratory cycle. *Monarchs Chang*  
677 *World Biol Conserv Iconic Insect Cornell Univ Press Ithaca NY.* 2015;268–281.
- 678 76. Pleasants JM, Williams EH, Brower LP, Oberhauser KS, Taylor OR. Conclusion of No  
679 Decline in Summer Monarch Population Not Supported. *Ann Entomol Soc Am.* 2016 Mar  
680 1;109(2):169–71.
- 681 77. Saunders SP, Ries L, Neupane N, Ramírez MI, García-Serrano E, Rendón-Salinas E, et al.  
682 Multiscale seasonal factors drive the size of winter monarch colonies. *Proc Natl Acad Sci.*  
683 2019 Mar 18;201805114.
- 684 78. Saunders SP, Ries L, Oberhauser KS, Thogmartin WE, Zipkin EF. Local and cross-  
685 seasonal associations of climate and land use with abundance of monarch butterflies  
686 *Danaus plexippus*. *Ecography.* 2018 Feb;41(2):278–90.
- 687 79. Pleasants JM, Oberhauser KS. Milkweed loss in agricultural fields because of herbicide  
688 use: effect on the monarch butterfly population: *Herbicide use and monarch butterflies.*  
689 *Insect Conserv Divers.* 2013 Mar;6(2):135–44.
- 690 80. Oliver TH, Roy DB. The pitfalls of ecological forecasting. *Biol J Linn Soc.* 2015  
691 Jul;115(3):767–78.
- 692 81. Fox R. The decline of moths in Great Britain: a review of possible causes: *The decline of*  
693 *moths in Great Britain.* *Insect Conserv Divers.* 2013 Jan;6(1):5–19.
- 694 82. Thomas JA. Butterfly communities under threat. *Science.* 2016 Jul 15;353(6296):216–8.

- 695 83. Wong MKL, Guénard B, Lewis OT. Trait-based ecology of terrestrial arthropods. *Biol*  
696 *Rev* [Internet]. 2018 [cited 2018 Dec 14];0(0). Available from:  
697 <https://onlinelibrary.wiley.com/doi/abs/10.1111/brv.12488>
- 698 84. Habel JC, Schmitt T. Vanishing of the common species: Empty habitats and the role of  
699 genetic diversity. *Biol Conserv*. 2018 Feb;218:211–6.
- 700 85. Dennis RLH, Shreeve TG, Dyck HV. Towards a Functional Resource-Based Concept for  
701 Habitat: A Butterfly Biology Viewpoint. *Oikos*. 2003;102(2):417–26.
- 702 86. Curtis RJ, Brereton TM, Dennis RLH, Carbone C, Isaac NJB. Butterfly abundance is  
703 determined by food availability and is mediated by species traits. Diamond S, editor. *J*  
704 *Appl Ecol*. 2015 Dec;52(6):1676–84.
- 705 87. Parmesan C, Ryrholm N, Stefanescu C, Hill JK, Thomas CD, Descimon H, et al. Poleward  
706 shifts in geographical ranges of butterfly species associated with regional warming.  
707 *Nature*. 1999 Jun;399(6736):579–83.
- 708 88. Hellerstein D. Land Use, Land Cover, and Pollinator Health: A Review and Trend  
709 Analysis. :47.
- 710 89. Baker NT, Stone WW. Estimated annual agricultural pesticide use for counties of the  
711 conterminous United States, 2008–12: U.S. Geological Survey Data Series 907 [Internet].  
712 US Geological Survey; p. 9. (U.S. Geological Survey Data Series 907). Available from:  
713 <https://dx.doi.org/10.3133/ds907>.
- 714 90. Stone WW. Estimated annual agricultural pesticide use for counties of the conterminous  
715 United States, 1992–2009. US Geological Survey; 2013. (U.S. Geological Survey Data  
716 Series 752).

- 717 91. Goulson D. REVIEW: An overview of the environmental risks posed by neonicotinoid  
718 insecticides. Kleijn D, editor. *J Appl Ecol*. 2013 Aug;50(4):977–87.
- 719 92. Hallmann CA, Foppen RPB, van Turnhout CAM, de Kroon H, Jongejans E. Declines in  
720 insectivorous birds are associated with high neonicotinoid concentrations. *Nature*. 2014  
721 Jul;511(7509):341–3.
- 722 93. Pisa L, Goulson D, Yang E-C, Gibbons D, Sánchez-Bayo F, Mitchell E, et al. An update  
723 of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 2: impacts  
724 on organisms and ecosystems. *Environ Sci Pollut Res [Internet]*. 2017 Nov 9 [cited 2019  
725 Feb 1]; Available from: <https://doi.org/10.1007/s11356-017-0341-3>
- 726 94. Douglas MR, Tooker JF. Large-Scale Deployment of Seed Treatments Has Driven Rapid  
727 Increase in Use of Neonicotinoid Insecticides and Preemptive Pest Management in U.S.  
728 Field Crops. *Environ Sci Technol*. 2015 Apr 21;49(8):5088–97.
- 729 95. Krupke CH, Holland JD, Long EY, Eitzer BD. Planting of neonicotinoid-treated maize  
730 poses risks for honey bees and other non-target organisms over a wide area without  
731 consistent crop yield benefit. Diamond S, editor. *J Appl Ecol*. 2017 Oct;54(5):1449–58.
- 732 96. Gilburn AS, Bunnefeld N, Wilson JM, Botham MS, Brereton TM, Fox R, et al. Are  
733 neonicotinoid insecticides driving declines of widespread butterflies? *PeerJ*. 2015 Nov  
734 24;3:e1402.
- 735 97. Basley K, Goulson D. Effects of Field-Relevant Concentrations of Clothianidin on Larval  
736 Development of the Butterfly *Polyommatus icarus* (Lepidoptera, Lycaenidae). *Environ Sci*  
737 *Technol*. 2018 Apr 3;52(7):3990–6.

- 738 98. Kleijn D, Snoeiijing GIJ. Field Boundary Vegetation and the Effects of Agrochemical  
739 Drift: Botanical Change Caused by Low Levels of Herbicide and Fertilizer. *J Appl Ecol.*  
740 1997;34(6):1413–25.
- 741 99. Weiss SB. Cars, cows, and checkerspot butterflies: nitrogen deposition and management  
742 of nutrient-poor grasslands for a threatened species. *Conserv Biol.* 1999;13(6):1476–1486.
- 743 100. Öckinger E, Hammarstedt O, Nilsson SG, Smith HG. The relationship between local  
744 extinctions of grassland butterflies and increased soil nitrogen levels. *Biol Conserv.* 2006  
745 Apr 1;128(4):564–73.
- 746 101. WallisDeVries MF, van Swaay CAM. A nitrogen index to track changes in butterfly  
747 species assemblages under nitrogen deposition. *Biol Conserv.* 2017 Aug;212:448–53.
- 748 102. Kurze S, Heinken T, Fartmann T. Nitrogen enrichment in host plants increases the  
749 mortality of common Lepidoptera species. *Oecologia.* 2018 Dec;188(4):1227–37.

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751 **S1 Appendix. Supplementary methods and results.** Includes detailed methods for phenology  
752 models and phylogenetic trees, one figure of species trends plotting on a cladogram, three figures  
753 showing population trends and annual variation for 81 species, two tables of model results from  
754 the trait analysis, and a table comparing our trend estimates with three other approaches.

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