

Ecography

**ECOG-01994**

Yang, L. H., Ostrovsky, D. M., Rogers, M. C. and Welker, J. M. 2015. Intra-population variation in the natal origins and wing morphology of overwintering western monarch butterflies *Danaus plexippus*. – Ecography doi: 10.1111/ecog.01994

**Supplementary material**

## Appendix 1

### Survey for the protozoan parasite *Ophryocystis elektroscirrha*

Two separate surveys were conducted to assess *O. elektroscirrha* prevalence among the 114 monarch butterfly specimens included in this study. The first survey was conducted in 2010 using visual inspection of posterior abdominal scales swabbed from each specimen, placed on dry microscope slides and inspected under 40 to 100X magnification, with 12.2 megapixel digital images archived (combining and modifying methods described in Altizer *et al.* 2000 and Davis *et al.* 2004). No infected butterflies were identified from this survey. Because the prevalence of *O. elektroscirrha* in this population was unexpectedly low, a second survey of these same specimens was conducted in 2015 using a visual inspection of new abdominal swab samples taken from the original butterfly specimens which had been stored in glassine envelopes at -20 C, this time archiving the physical microscope slide samples. This second survey found two butterflies with a single spore. Because previous studies (e.g., Altizer and Davis 2010) of monarch butterfly wing morphology only excluded specimens with much higher spore loads (>1000 spores in a standard assay), these extremely low spore loads seem unlikely to have had any significant effect on the conclusions of this study. A further re-analysis of the dataset excluding the two butterflies with a single spore showed no qualitative changes in the conclusions of this study, and analyses of the complete dataset are presented here.

Altizer, S. M., K. S. Oberhauser, and L. P. Brower. 2000. Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. *Ecological Entomology* 25:125–139.

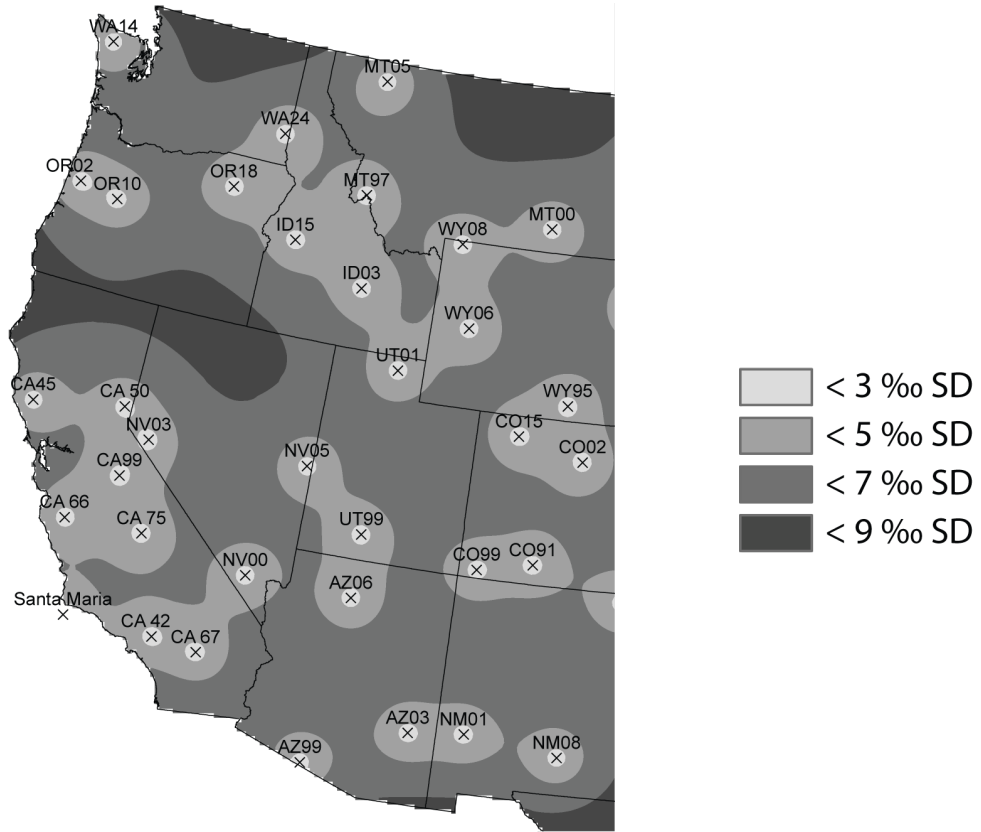
Altizer, S., and A. K. Davis. 2010. Populations of monarch butterflies with different migratory behaviors show divergence in wing morphology. *Evolution* 64:1018–1028.

Davis, A. K., S. Altizer, and E. Friedle. 2004. A non-destructive, automated method of counting spores of *Ophryocystis elektroscirrha* (Neogregarinorida: Ophryocystidae) in infected monarch butterflies (Lepidoptera: Nymphalidae). *Florida Entomologist* 87:231–234.

**Table A1.** Table of 35 precipitation collection sites in western North America, drawn from the USNIP data base of weekly precipitation  $\delta^2\text{H}$  values from 75 sites across the contiguous US.

<i>USNIP Code</i>	<i>Site Name</i>	<i>State</i>	<i>Latitude</i>	<i>Longitude</i>	<i>Elevation (m)</i>
AZ03	Grand Canyon National Park	AZ	33.07	-109.86	1173
AZ06	Organ Pipe Cactus National Monument	AZ	36.07	-112.15	2152
AZ99	Oliver Knoll	AZ	31.95	-112.80	506
CA42	Tanbark Flat	CA	34.21	-117.76	853
CA45	Hopland	CA	39.00	-123.08	253
CA50	Sagehen Creek	CA	39.43	-120.24	1931
CA66	Pinnacles National Monument-Bear Valley	CA	36.48	-121.16	317
CA67	Joshua Tree National Park-Black Rock	CA	34.07	-116.39	1239
CA75	Sequoia National Park-Giant Forest	CA	36.57	-118.78	1921
CA99	Yosemite National Park	CA	37.79	-119.85	1408
CAMR	Santa Maria	CA	34.20	-120.45	66
CO02	Niwot Saddle	CO	40.05	-105.59	3520
CO15	Sand Spring	CO	40.50	-107.70	1998
CO91	Wolf Creek Pass	CO	37.47	-106.79	3292
CO99	Mesa Verde National Park	CO	37.19	-108.49	2172
ID03	Craters of the Moon National Monument	ID	43.46	-113.55	1807
ID15	Smith's Ferry	ID	44.29	-116.06	1442
MT00	Little Bighorn Battlefield National Monument	MT	45.57	-107.43	957
MT05	Glacier National Park - Fire Weather Station	MT	48.51	-113.10	980
MT97	Lost Trail Pass	MT	45.69	-113.97	2414
NM01	Gila Cliff Dwellings National Monument	NM	33.22	-108.23	1772
NM08	Mayhill	NM	32.91	-105.47	2009
NV00	Red Rock Canyon	NV	36.13	-115.42	1137
NV03	Smith Valley	NV	38.79	-119.25	1501
NV05	Great Basin National Park - Lehman Caves	NV	39.00	-114.21	2067
OR02	Alsea Guard Ranger Station	OR	44.38	-123.61	104
OR10	H.J. Andrews Experimental Forest	OR	44.21	-122.25	436
OR18	Starkey Experimental Forest	OR	45.22	-118.51	1253
UT01	Logan	UT	41.66	-111.90	1370
UT99	Bryce Canyon National Park	UT	37.62	-112.17	2477
WA14	Olympic National Park - Hoh Ranger Station	WA	47.856	-123.93	176
WA24	Palouse Conservation Farm	WA	46.76	-117.18	766
WY06	Pinedale	WY	42.92	-109.79	2388
WY08	Yellowstone National Park Tower Falls	WY	44.92	-110.42	1912
WY95	Brooklyn Lake	WY	41.37	-106.24	3212

**Figure A1.** Map of standard deviations from kriging interpolation of the monarch wing hydrogen isoscape ( $\delta^2\text{H}_m$ ). Mean SD across the map was 5.5 ‰.



**Figure A2.** Partial correlations of PC-size and PC-shape by  $\delta D_m$  for each site controlling for sex; males are represented as blue points, females are represented as orange points. In these figures, high PC-size values represent larger wings, and high PC-shape values represent wings with higher aspect ratios (i.e., lower roundness). Significant correlations (assessed at  $P < 0.05$  with Pearson's partial correlation statistic) are shown with a bivariate normal ellipse representing 95% of the total probability area.

