Supplementary Information for

Functional evidence supports adaptive plant chemical defense along a geographical cline

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This PDF file includes:

Appendix S1
Figures S1 to S11
Tables S1 to S7
References
Appendix S1

Population genetic differentiation, isolation by distance and Q_{ST}-F_{ST} comparisons

Population differentiation $F_{ST}$ was estimated using 925 putatively neutral SNPs obtained from 46 randomly sampled individuals from across 12 of the 24 sampled milkweed populations (2-10 individuals per population) (SNP details in ref. 1). Wright’s F-statistic $F_{ST}$ and confidence intervals were estimated by 1,000 bootstrap simulations with resampling over loci using the program GDA (2).

In order to explore the geographical structure of the neutral differentiation among populations, population pairwise $F_{ST}$ was estimated with Arlequin (3). Significance ($\alpha=0.05$) of the genetic distances was tested by permuting the individuals between the populations 1,000 times. Additionally, a Mantel test was performed with Genepop v. 4.7.0 (4) in order to test for isolation by distance among populations. Pairwise genetic distance ($F_{ST}/(1 - F_{ST})$ (5) and geographic distance matrices were used as input to test the null hypothesis that there is no spatial correlation between genetic samples, with 10,000 permutations of samples between geographical locations.

In order to evaluate whether neutral, directional or stabilizing selection might be contributing to the differentiation of cardenolides among milkweed populations, we estimated $P_{ST}$ (the phenotypic analog of $Q_{ST}$ for populations sampled in the wild) for each of the seed chemistry traits and compared them to the mean neutral $F_{ST}$ estimated from the SNPs. We combined the approach of Brommer (6) for the estimation of $P_{ST}$ and the parametric bootstrap method of Whitlock and Guillaume (7) (originally conceived for $Q_{ST}$–$F_{ST}$), extended to $P_{ST}$–$F_{ST}$ comparisons.

First, the $P_{ST}$ was estimated following Brommer (6):

$$P_{ST} = \frac{c \sigma_B^2}{c \sigma_B^2 + 2 \sigma_W^2}.$$  

Where $\sigma_B^2$ denotes the phenotypic variance between populations and $\sigma_W^2$ denotes the phenotypic variance within populations. The scalar $c$ expresses the proportion of the total variance that is due to additive genetic effects across population, whereas $h^2$ is the heritability of the trait (the proportion of phenotypic variance within populations that is due to additive genetic effects). Therefore, $c/h^2$ informs the differences in additive genetic variance between and within populations, which critically describes how well $P_{ST}$ approximates $Q_{ST}$ (6). Since both $c$ and $h^2$
are unknown here, deviations of $P_{ST}$ from neutrality for a given trait should be conservatively assessed in the range of $c < h^2$ (i.e., $c/h^2 < 1$). Because $P_{ST}$ is an increasing function of $c h^2$, the lower the $c/h^2$ ratio threshold is for significant deviations of $P_{ST}$ from neutrality, the stronger the inferences can be made regarding signatures of divergent selection between populations.

$P_{ST}$ was estimated for each trait by using PROC MIXED in SAS v9.4, considering ‘Population’ as a random factor. Parameters $\sigma_B^2$ and $\sigma_W^2$ were obtained from ‘Population’ and residual variance components, respectively, and $P_{ST}$ was estimated along increasing values of the $c/h^2$ ratio ranging from 0 to 2 by 0.1 increments (i.e., varying the relative contribution of population additive genetic variance over within-population additive variance). $P_{ST}–F_{ST}$ comparisons were conducted for each value of $c/h^2$ by parametric bootstrap with 10,000 simulations in SAS, following the method of Whitlock and Guillaume (7) for $Q_{ST}–F_{ST}$ comparisons. This method predicts a null distribution for $Q_{ST}–F_{ST}$ ($P_{ST}–F_{ST}$ in our case) under the null hypothesis that both the quantitative trait and neutral markers show neutral differentiation (i.e., the $P_{ST}$ equals the $F_{ST}$). Traits with significantly higher $P_{ST}$ than $F_{ST}$ are inferred to be under spatially heterogeneous divergent selection while $P_{ST} < F_{ST}$ would be indicative of stabilizing selection, and $P_{ST} = F_{ST}$ would reflect neutral evolution of the trait (8, 9). To test for departures from the null hypothesis of neutral differentiation, we tested whether the observed $P_{ST}–F_{ST}$ difference is in the tail of the neutral null distribution. For a given $c/h^2$, an observed $P_{ST}–F_{ST}$ difference in the lower tail suggests spatially stabilizing selection, while a $P_{ST}–F_{ST}$ difference in the upper tail suggests spatially divergent selection on the trait. Despite known deviations of $P_{ST}$ in comparison to $Q_{ST}$ (6, 10), our combined approach increases the robustness of the test in wild populations by simultaneously exploring multiple scenarios of selection with variable $c/h^2$ ratios and controlling for biases when estimating $P_{ST}$ through bootstrapping when $Q_{ST}$s are not available.
Fig. S1. Concentration pattern of specific cardenolides in *Asclepias syriaca* seeds across latitude.
Relative concentration quantified by HRMS. A quadratic model was the best fit in all cases except for reduced labriformin where a linear model was the best fit. * p< 0.05, ** p<0.01, *** p<0.001, ns = not significant.
Fig. S2. Labriformin’s putative biosynthesis in *Asclepias syriaca*.
Fig. S3 Correlation heat map of cardenolides in Asclepias syriaca seeds.
The heat map of pairwise Spearman’s correlations among the 21 cardenolides detected in A. syriaca seed samples across latitude (n=24 populations). The heat map uses the correlation matrix as clustering distance to sort by similarity of each cardenolide to the others. Blue squares indicate negative correlations and red squares indicate positive correlations (color intensity indicates the strength of the correlation coefficient). * p< 0.05, ** p<0.01, *** p<0.001. Note that Aspecioside C was not included in Fig. S1 because a few concentration values were missing (i.e., the ion adduct peak was not detectable) but had a sufficient number of values to be included here.
Fig. S4. Plot of Mantel test for isolation by distance across 12 milkweed populations. The plot shows no isolation by distance among pairs of populations (p > 0.05), where pairwise Slatkin’s genetic distance ($F_{ST}/(1-F_{ST})$) was regressed over geographic distance.
Fig. S5. Observed $P_{ST} - F_{ST}$ values.

Observed $P_{ST} - F_{ST}$ values for each trait (colored lines) and their simulated null distribution by parametric bootstrap with 10,000 simulations (dashed lines representing 2.5% and 97.5% confidence intervals) assuming neutrality, along increasing values of $c/h^2$ (colored dots) (i.e., relative contribution of additive variance between populations vs. additive variance within populations when estimating $P_{ST}$). The lower the $c/h^2$ ratio threshold indicates significant deviations of $P_{ST}$ from neutrality (i.e., observed $P_{ST} - F_{ST}$ outside the inside area delimited by the dashed lines). $P_{ST}$ for cardenolides syrioside B (9.5) and labriformin (15.9) fall in the upper tail of the neutral $P_{ST} - F_{ST}$ distribution for $c/h^2 >= 0.4$ under a conservative scenario ($c/h^2 < 1$), suggesting spatially divergent selection acting on those traits.
Fig. S6. Biotransformation of glycosylated aspecioside in *Oncopeltus fasciatus*
Fig. S7. Relative concentration of cardenolides in *Asclepias syriaca* seeds and *Oncopeltus fasciatus* in the labriformin degradation pathway

The box and whisker plots show the original and normalized concentration values. The mean concentration is indicated with a yellow diamond. * p< 0.05, ** p<0.01, *** p<0.001
Fig. S8. The effect of labriformin and its insect-modified end-products on the unadapted and monarch sodium pumps.

The difference in inhibitory impacts of the parent compound labriformin, oxidized labriformin (modified) and syriobioside A (breakdown product) on the unadapted and monarch sodium pumps. While syriobioside A is sequestered by monarchs, it is not known whether monarchs modify labriformin to oxidized labriformin. Data are presented as the molar concentration of plant toxin necessary to cause 50% inhibition of the animal enzyme, or IC$_{50}$. Higher values on the Y axis indicate that the enzyme is more tolerant to the cardenolide. Each bar represents the mean of 3-6 replicates (each based on a 6-concentration inhibition curve) ± SE.
Fig. S9. Structures of five N-containing cardenolides known in the genus *Asclepias* and the predicted structure of reduced labriformin and oxidized labriformin.

The structures of reduced labriformin and oxidized labriformin are anticipated for the first time herein and are supported by high resolution mass spectrometry and MS/MS fragmentation in positive and negative mode.
Fig. S10. MS/MS product ion mass spectrum from [M+H]^+ adduct of oxidized labriformin.
Several fragments with the same exact mass were found in the MS/MS profile of labriformin and support the proposed structure.
Fig. S11. MS/MS product ion mass spectrum from [M-H]⁻ adduct of oxidized labriformin. Several fragments with the same exact mass were found in the MS/MS profile of labriformin and support the proposed structure.
Table S1. Percentage of cardenolides in *Asclepias syriaca* seed extract.
Data were collected by HPLC-UV for total cardenolides. Compounds are ordered by their percentage of the total cardenolides. The seeds were collected in the Ithaca area.

| Compound                          | % total |
|----------------------------------|---------|
| Glycosylated aspecioside         | 42%     |
| Diglycosylated syriogenin        | 12%     |
| Glycosylated syriobioside        | 11%     |
| Syrioside B                      | 8%      |
| Labriformin                      | 8%      |
| Diglycosylated oxidized syriogenin| 6%      |
| Diglycosylated digitoxigenin     | 3%      |
| Syrioside A                      | 3%      |
| Aspecioside A                    | 3%      |
| Syriobioside A                   | <2%     |
| Reduced labriformin              | <2%     |
| Syriogenin                       | <2%     |
| Glycosylated syriogenin A        | <2%     |
Table S2. HRMS data of the cardenolides detected in samples.
To simplify the table, only MS data from one sample is listed for each precursor ion.

| Name                      | Sample | Retention time (min) | Precursor ion | Observed m/z | Calculated m/z | ∆m/z (ppm) | Cardenolide formula | Genin fragment (1) | Observed m/z | Calculated m/z | ∆m/z (ppm) | Genin fragment ion formula |
|---------------------------|--------|----------------------|---------------|--------------|----------------|------------|------------------|-------------------|--------------|----------------|------------|---------------------------|
| Compound 8.7 Glycosylated aspecioside | PITT   | 4.72                 | [M+H]^+       | 713.3379     | 713.3379       | 0.0        | C_{36}H_{52}O_{15} | [M-C_{12}H_{20}O_{9}+H]^+ | 405.2264     | 405.2272       | 1.9        | C_{23}H_{33}O_{6}^+       |
| Compound 7.6 Diglycosylated syriogenin | OTT    | 4.85                 | [M+H]^+       | 699.3586     | 699.3586       | 0.0        | C_{35}H_{54}O_{14} | [M-C_{12}H_{20}O_{9}+H]^+ | 391.2475     | 391.2479       | 1.0        | C_{23}H_{33}O_{5}^+       |
| Compound 8.3 Aspecioside A (2) | SLY    | 5.07                 | [M+H]^+       | 551.2851     | 551.2851       | 0.0        | C_{29}H_{42}O_{10} | [M-C_{6}H_{10}O_{4}+H]^+  | 405.2263     | 405.2272       | 2.2        | C_{23}H_{33}O_{5}^+       |
| Compound 8.9 Glycosylated syriobiocide | SLY    | 5.22                 | [M+H]^+ (3)   | 727.3177     | 727.3172       | -0.6       | C_{30}H_{50}O_{16} | [M-H_{2}O-C_{12}H_{18}O_{8}+H]^+ | 419.2059     | 419.2064       | 1.1        | C_{23}H_{31}O_{7}^+       |
| Compound 9.3 Syriosome A | AMH    | 5.27                 | [M+NH_{4}]^+ (4) | 742.3275     | 742.3286       | -1.4       | C_{35}H_{48}O_{16} | [M-2H_{2}O-C_{12}H_{18}O_{9}+H]^+ | 399.1801     | 399.1802       | 0.2        | C_{23}H_{27}O_{6}^+       |
| Compound 9.5 Syriosome B (5) | AMH    | 5.35                 | [M+NH_{4}]^+  | 742.3286     | 742.3286       | 0.0        | C_{35}H_{48}O_{16} | [M-H_{2}O-C_{12}H_{18}O_{9}+H]^+ | 417.1895     | 417.1908       | 3.1        | C_{23}H_{27}O_{7}^+       |
| Compound 11.8 Diglycosylated oxidized syriogenin | AMH  | 5.84                 | [M+H]^+ (6)   | 697.3429     | 697.3430       | 0.1        | C_{35}H_{52}O_{16} | [M-C_{12}H_{21}O_{5}+H]^+  | 389.2316     | 389.2323       | 1.7        | C_{23}H_{32}O_{5}^+       |
| Compound 12.9 Diglycosylated digitoxigenin | AMH  | 6.07                 | [M+NH_{4}]^+ (7) | 700.3897     | 700.3897       | 0.0        | C_{36}H_{54}O_{13} | [M-C_{12}H_{20}O_{9}+H]^+  | 375.2523     | 375.2530       | 1.8        | C_{23}H_{33}O_{4}^+       |
| Compound 15.9 Labriformin | OTT    | 6.65                 | [M+H]^+       | 618.2367     | 618.2367       | 0.0        | C_{31}H_{50}NO_{10}S | [M-H_{2}O-C_{6}H_{11}NO_{3}S+H]^+ | 417.1905     | 417.1908       | 0.7        | C_{23}H_{27}O_{7}^+       |
| Syribioside A (8) (9) | PHOX   | 5.60                 | [M+H]^+       | 565.2643     | 565.2643       | 0.0        | C_{29}H_{42}O_{11} | [M-H_{2}O-C_{10}H_{16}O_{5}+H]^+ | 419.2053     | 419.2064       | 2.6        | C_{23}H_{31}O_{7}^+       |
| Syriogenin (8) | SLY    | 4.84                 | [M+H]^+       | 391.2479     | 391.2479       | 0.0        | C_{23}H_{34}O_{6}  |                             |                |              |            |                           |
| Compound                        | Method | R_t  | m/z       | Intensity | Mass (ppm) | Retention Time |
|--------------------------------|--------|------|-----------|-----------|------------|----------------|
| Reduced labriformin (8)        | BISH   | 6.36 | 620.2524  | 0.0       | C_{31}H_{41}NO_{10}S   | 401.1962   |
|                                |        |      | 620.2524  |           |            | 401.1959       |
|                                |        |      |           |           |            | -0.7 C_{23}H_{29}O_{7}+ |
| Glycosylated syriogenin A (8)  | CHILL  | 5.26 | 537.3058  | 0.0       | C_{29}H_{44}O_{9}   | 391.2468   |
|                                |        |      | 537.3058  |           |            | 391.2479       |
|                                |        |      |           |           |            | 2.8 C_{23}H_{35}O_{5}+ |
| Desglucosyrioside (8)          | PHOX   | 5.27 | 563.2479  | -1.4      | C_{29}H_{38}O_{11} | 435.2017   |
|                                |        |      | 563.2479  |           |            | 435.2013       |
|                                |        |      |           |           |            | 0.9 C_{23}H_{31}O_{8}+ |
| Oxidized labriformin (8)       | SB10.4 | 5.55 | 650.2252  | -2.1      | C_{31}H_{39}NO_{12}S | 417.1913   |
|                                |        |      | 650.2266  |           |            | 417.1908       |
|                                |        |      |           |           |            | 1.1 C_{23}H_{29}O_{7}+ |

(1) Only for glycosylated cardenolides.
(2) Isomers named aspecioside B and C were detected at retention time = 4.72 and 5.22 respectively but were not isolated.
(3) Most intense peak [M-H_{2}O+H]^+; Observed m/z = 709.3066; Calculated m/z = 709.3066.
(4) Most intense peak [M-2H_{2}O-C_{6}H_{10}O_{6}+H]^+; Observed m/z = 527.2276; Calculated m/z = 527.2276.
(5) Isomers named syrioside C and D were detected at retention time = 5.09 and 5.12 but respectively but were not isolated.
(6) Most intense peak [M-C_{12}H_{22}O_{10}+H]^+; Observed m/z = 371.2219; Calculated m/z = 371.2219.
(7) Most intense peak [M-2H_{2}O-C_{12}H_{20}O_{9}+H]^+; Observed m/z = 339.2316; Calculated m/z = 339.2319.
(8) Cardenolide detected in samples but was isolated.
(9) Isomers named syriobioside B, C, and D were detected at retention time = 5.21, 5.35, 5.53 respectively but were not isolated.
(10) An isomer named glycosylated syriogenin B was detected at retention time = 4.85 but was not isolated.

Note that over 15 isomers of labriformidin were detected in the seed extract but were not included to simplify data.
### Table S3. Chemical structures of cardenolides.

| Name               | Cardenolide chemical structure | Name               | Cardenolide chemical structure |
|--------------------|--------------------------------|--------------------|--------------------------------|
| Compound 6.7       | ![Glycosylated aspecioside](image) | Syriobioside A     | ![Glycosylated aspecioside](image) |
| Compound 7.6       | ![Diglycosylated syriogenin](image) | Syriogenin         | ![Syriogenin](image)            |
| Compound 8.3       | ![Aspecioside A](image)          | Glycosylated syriogenin A | ![Glycosylated syriogenin A](image) |
| Compound 15.9      | ![Labriformin](image)           | Desglucosyriside   | ![Desglucosyriside](image)      |
| Reduced labriformin| ![Reduced labriformin](image)   | Oxidized labriformin| ![Oxidized labriformin](image)  |

The chemical structure of compounds 8.9 (glycosylated syriobioside), 9.3-9.5 (syrioside A and B), 11.8 (diglycosylated oxidized syriogenin), and 12.9 (diglycosylated digitoxigenin) will be reported in a separate manuscript.
Table S4. Population pairwise $F_{ST}$ among 12 milkweed populations.
Genetic distances significantly greater than zero ($p < 0.05$) are highlighted in bold.

| Pop | AND | EDGE | FRED | FULK | GLX | ITH | JER | KNOX | PHIL | PHOX | SLY | URB |
|-----|-----|------|------|------|-----|-----|-----|------|------|------|-----|-----|
| AND | 0   | 0.083| 0    | 0.063| 0    | 0.094| 0    | 0    | 0.001| 0    | 0    | 0.074|
| EDGE| 0    | 0.083| 0    | 0.063| 0    | 0.094| 0    | 0    | 0.001| 0    | 0    | 0.074|
| FRED| 0.134| 0.063| 0    | 0    | 0.011| 0.049| 0.020| 0    | 0.028| 0    | 0    | 0.074|
| FULK| 0.086| 0.001| 0.094| 0    | 0    | 0.094| 0    | 0    | 0    | 0    | 0    | 0.074|
| GLX | 0.125| 0.098| 0.154| 0.091| 0    | 0.094| 0    | 0    | 0    | 0    | 0    | 0.074|
| ITH | 0.146| 0.028| 0.159| 0.058| 0.128| 0.033| 0    | 0    | 0    | 0    | 0    | 0.074|
| JER | 0.146| 0.028| 0.159| 0.058| 0.128| 0.033| 0    | 0    | 0    | 0    | 0    | 0.074|
| KNOX| 0.078| 0.018| 0.078| 0.007| 0.057| 0.028| 0    | 0.048| 0    | 0    | 0    | 0.074|
| PHIL| 0.050| 0.000| 0.091| 0.037| 0.088| -0.002| 0.068| 0.024| 0    | 0    | 0    | 0.074|
| PHOX| 0.086| 0.027| 0.097| 0.004| 0.064| -0.011| 0.100| 0.028| -0.001| 0    | 0    | 0.074|
| SLY | 0.123| 0.093| 0.172| 0.118| 0.171| 0.083| 0.105| 0.090| 0    | 0.093| 0.109| 0    |
| URB | 0.097| 0.065| 0.178| 0.054| 0.139| 0.094| 0.131| 0.096| 0.043| 0.073| 0.140| 0    |
Table S5. The effect of four diet treatments on the growth and development of *Oncopeltus fasciatus*.

| Fitness parameter          | Diet                          | Mean  | SE   | Test          | p-value |
|----------------------------|-------------------------------|-------|------|---------------|---------|
| Mass at week 3 in mg       | Control                       | 40.00 | 2.29 | One-way ANOVA | 0.925   |
| (n=10 replicates/diet)     | Ouabain                       | 38.74 | 3.30 |               |         |
|                            | Labriformin                   | 43.32 | 2.65 |               |         |
|                            | Glycosylated aspecioside      | 41.71 | 2.14 |               |         |
| Days until adulthood       | Control                       | 22.80 | 0.83 | Kruskal-Wallis test | 0.874 |
| (n=10 insects/diet)        | Ouabain                       | 23.30 | 2.02 |               |         |
|                            | Labriformin                   | 24.11 | 2.08 |               |         |
|                            | Glycosylated aspecioside      | 23.40 | 1.05 |               |         |
| Adult length in mm         | Control                       | 9.90  | 0.31 | One-way ANOVA  | 0.914   |
| (n=10 insects/diet)        | Ouabain                       | 10.06 | 0.23 |               |         |
|                            | Labriformin                   | 9.91  | 0.31 |               |         |
|                            | Glycosylated aspecioside      | 10.14 | 0.22 |               |         |
| Total eggs (n=8 pairs/diet | Control                       | 331.14| 106.09| One-way ANOVA | 0.928  |
| except control n=7)        | Ouabain                       | 341.13| 59.42 |               |         |
|                            | Labriformin                   | 294.38| 39.27 |               |         |
|                            | Glycosylated aspecioside      | 294.38| 38.24 |               |         |
| Hatchlings (n=8 pairs/diet | Control                       | 92.14 | 38.03| One-way ANOVA  | 0.776   |
| except control n=7)        | Ouabain                       | 133.88| 25.88 |               |         |
|                            | Labriformin                   | 120.13| 28.09 |               |         |
|                            | Glycosylated aspecioside      | 120.63| 20.11 |               |         |
Table S6. Sequestered cardenolides (mg/g dry mass) in adult bodies of *Oncopeltus fasciatus* fed artificial diets, each spiked with one of three isolated cardenolides.

Note that *Oncopeltus fasciatus* on the control diet had one cardenolide (which may have been maternally produced or transferred (see ref. 11). Shown are mean concentrations as determined by HPLC-UV (n = 6-10). Labriformin degradation products were confirmed by mass spectrometry analysis.

| Diet          | Ouabain | Oxidized labriformin | Glycosylated aspecioside | Diglycosylated syriogenin | Aspecioside A | Syriobioside A | Desgluco-syrioside | labriformin |
|---------------|---------|----------------------|--------------------------|--------------------------|---------------|-----------------|---------------------|-------------|
| Control       | -       | -                    | -                        | -                        | -             | 0.17            | -                   | -           |
| Ouabain       | 1.09    | -                    | -                        | -                        | -             | -               | -                   | -           |
| Glycosylated aspecioside | -       | -                    | 0.07                     | 0.12                     | 2.44          | 0.04            | -                   | -           |
| Labriformin   | -       | 0.03                 | -                        | 0.12                     | 0.01          | 0.55            | -                   | -           |
Table S7. Location and key climatic variables of the 24 study populations.
Analysis of climatic correlations with latitude and longitude for these populations is provided in ref. 12.

| Population                  | Latitude    | Longitude   | Mean annual precipitation (cm) | Mean annual temperature (°C) |
|-----------------------------|-------------|-------------|--------------------------------|------------------------------|
| Amherst, MA, USA            | 42.37526    | -72.51891   | 118.29                         | 8.56                         |
| Anderson, IN, USA           | 40.10216    | -85.67869   | 101.14                         | 10.78                        |
| Bedford, VS, USA            | 37.402891   | -79.351501  | 113.8                          | 13.11                        |
| Bellbrook, OH, USA          | 39.616902   | -84.097379  | 100.43                         | 11.11                        |
| Bishop, NC, USA             | 33.81461    | -83.43533   | 127                            | 16.39                        |
| Boyce, VA, USA              | 39.09324    | -78.05992   | 99.31                          | 11.67                        |
| Chapel Hill, NC, USA        | 35.9666     | -79.094652  | 120.9                          | 14.61                        |
| Edgewater, MA, USA          | 42.73752    | -84.48381   | 78.51                          | 8.11                         |
| East Lansing, MI, USA       | 38.889071   | -76.544577  | 110.97                         | 12.56                        |
| Fredericton, NB, Canada     | 45.96064    | -66.63912   | 112.42                         | 5.61                         |
| Fulks Run, VA, USA          | 38.65947    | -78.90405   | 105.69                         | 12.67                        |
| Galax, VA, USA              | 36.659311   | -80.92991   | 111.51                         | 10.22                        |
| Hanover, NH, USA            | 43.70247    | -72.28854   | 98.27                          | 7.78                         |
| Ithaca, NY, USA             | 42.44049    | -76.49545   | 93.24                          | 7.83                         |
| Jericho, VT, USA            | 44.50549    | -72.9959    | 101.27                         | 6.78                         |
| Knoxville, TN, USA          | 35.96054    | -83.92079   | 135.46                         | 14.11                        |
| Ottawa, ON, Canada          | 45.42146    | -75.69188   | 91.41                          | 6.28                         |
| Philipsburg, PA, USA        | 40.910518   | -78.056099  | 113.94                         | 10.61                        |
| Phoenixville, PA, USA       | 40.099968   | -75.463508  | 111.43                         | 11.78                        |
| Pittsburg, PA, USA          | 40.436315   | -79.08887   | 95.96                          | 10.67                        |
| Quebec City, QC, Canada     | 46.81274    | -71.21935   | 123.03                         | 4.04                         |
| Sylvania, OH, USA           | 41.71556    | -83.705     | 85.17                          | 11.89                        |
| Urbana, IL, USA             | 40.11727    | -88.20449   | 104.29                         | 10.78                        |
| Westford, VT, USA           | 44.61194    | -73.01039   | 101.2                          | 6.8                          |
References

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