**Supplementary Appendix 2**

For leaf tissue, we collected 12 leaf discs from three pairs of opposite leaves using a ¼ inch diameter hole punch. Six discs were placed into 1 mL of 95% methanol and were held at 4°C until processing. The remaining six discs were placed into a coin envelope and were dried at 60°C, then weighed. For monarch wing tissue, butterflies were given ~6 hours for their wings to fully expand and dry post-eclosion. They were then placed into glassine envelopes and frozen at -20°C until processing. After approximately 2 months at -20°C, each of the four monarch wings was removed from the thorax, and the right hindwing was placed into a coin envelope and dried for ~48 hours at 60°C, then weighed. In total, we collected data from 183 leaf samples and 451 wing samples (Table S2).

To extract cardenolides, we added two stainless steel grinding beads to tubes containing leaf tissue and methanol, or to a 1.5 mL tube containing an oven-dried hindwing. Wing tissue was ground at 50 Hz for 2 minutes using a tissue lyser (Qiagen: 69980), and then 1 mL of 95% methanol was added to each tube. Leaf and wing tissue in methanol was ground for an additional 2 minutes until thoroughly homogenized, after which samples were added to a sonicator (Thomas Scientific: 1207K36) for 1 hour. Next, samples were centrifuged at 14,000 RPM for 5 minutes, and 800 µL of the supernatant was pipetted into a new tube. These samples were then vacuum-evaporated for 3 hours or until dry and stored at -20°C until analysis. Samples were reconstituted in 500 µL of a 0.15 mg/mL solution of digitoxin (Sigma-Aldrich) in methanol, and 200 µL of each sample was added to a 96-well filter plate (AcroPrep™ Advance, 0.2 μm PFTE membrane), which was centrifuged at 1500 RPM for 1 minute. Samples were injected at a volume of 2 μL onto Accucore™ C18 column (2.6 μm x 2.1 mm x 30 mm) (ThermoFisher Scientific) and separated using high performance liquid chromatography on a Thermo (Dionex) UHPLC 3000 instrument. We used the following solvent gradient of acetonitrile (ACN) and methanol that separated cardenolides according to their polarity, with the most polar compounds eluting first: 15% ACN for 5 minutes, ramping linearly to 30% for 3.5 min, then to 55% for 3 min, then ramping to 100% for 1 minute with a hold at 100% ACN for 10 min, then equilibration at initial conditions (15% ACN) for 5 minutes. Peaks were quantified at 218 nm using a diode array detector (DAD).

For wild-caught butterflies from Guam and Rota, cardenolide extraction and quantification were conducted using the methods described in Zehnder and Hunter (2007) and were similar to the methods described above. However, because of differences between instruments and non-corresponding retention times between methods, we do not directly compare these datasets from greenhouse-reared butterflies versus wild-caught Guam and Rota butterflies.