Lyside sulphur (Lepidoptera: Pieridae): origin and possible modes of transport of an Ontario, Canada specimen

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Abstract—A dead lyside sulphur (*Kricogonia lyside* (Godart, 1819): Lepidoptera: Pieridae) was found in Guelph, Ontario, Canada, on 4 April 2011. DNA barcode analysis indicates it likely originated in Texas, United States of America or northern Mexico. The occurrence of this specimen coincided with a very strong weather system extending from southern Texas into eastern Canada on 3–4 April 2011. All possible means of it reaching Ontario are unlikely: natural dispersal, natural transportation on the jet stream, transportation on a vehicle, importation of a pupa on produce, and direct human transport. Unfortunately, there was no way to differentiate between these possibilities.

In the late morning of 4 April 2011, Jon Spero found a dead male lyside sulfur (*Kricogonia lyside* (Godart, 1819): Lepidoptera: Pieridae) beside Gordon Street in Guelph, Ontario, Canada, ~1.4 km NW of the centre of the University of Guelph campus. The individual had died with its wings folded ventrally. Its wings were in reasonably good condition but its head was missing (Fig. 1). It was kept frozen until a leg was removed for DNA analysis, after which it was pinned.

This is an extremely unusual record for this species, especially for early spring. *Kricogonia lyside* adults winter in southern Texas, United States of America and typically disperse northward into the Great Plains in mid to late summer, but not in April. In the spring of 2011, lyside sulphurs were relatively scarce in southern Texas and were not reported further north until later in the year (L. Gilbert, University of Texas, Austin, Texas, United States of America, personal communication). Historically they have been recorded in summer as far north as Nebraska, Missouri, and Kentucky, United States of America (Opler and Krizek 1984; Opler 1992) and possibly Illinois, United States of America (Klots 1951), but never in Canada.

We sequenced 433 bp of cytochrome b (cytB) from the specimen (GenBank accession KC473944). We searched the cytB sequence of the Guelph specimen in GenBank (Benson *et al.* 2011) using the blastn algorithim (Altschul *et al.* 1997) of the nucleotide collection (nr). There was no exact match, for as of January 2013 there were no *K. lyside* cytB sequences in GenBank. The fragment most closely matched specimens from five Lepidoptera families, with the most similar sequences from other species within Pieridae (GenBank accessions – HQ177611, EF584877, JQ305693, EF584873, JN246082, DQ008110, EU622524, FJ435433, FJ435434, EF545675).

To make a more precise species identification, we sequenced the barcode region of cytochrome *c* oxidase I (658 bp of COI; GenBank accession KC473943) and compared it to sequences maintained in the reference library of the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007). In July 2013, BOLD contained 44 publicly available COI DNA sequences of *K. lyside* from Texas, Arizona, Mexico, and Caribbean islands (http://www.barcodinglife.org/index.php/Public_BarcodeCluster?clusterguid=BOLD:AAB8209). *Kricogonia lyside* displayed significant isolation by distance within this single mitochondrial marker (Mantel $R = 0.8$, $P = 0.002$, Fig. 2), suggesting that the Guelph

Received 7 August 2013. Accepted 19 November 2013. First published online 7 August 2014.

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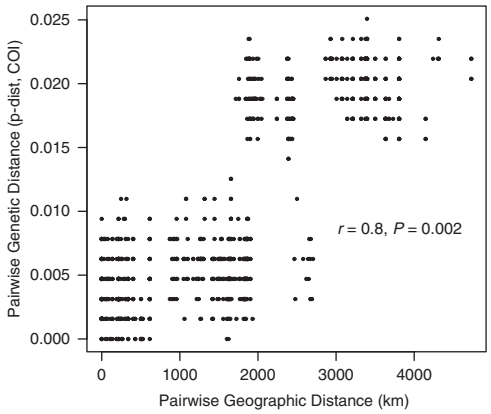
Subject editor: Chris Schmidt
doi:10.4039/tce.2014.18

Fig. 1. Dorsal (left) and ventral (right) photographs of the *Kricogonia lyside* specimen found dead in Guelph, Ontario, Canada, on 4 April 2011.

Fig. 2. Mantel test of geographic distance (km) and genetic distance (COI p-dist) for the 44 *Kricogonia lyside* publicly available on Barcode of Life Data System (BOLD). Pairwise geographic distance calculated using GDMGv1.2.3 (Ersts 2013) and p-dist calculated using MEGA5 (Tamura *et al.* 2011).



specimen might be identifiable to both species and originating region. It was most similar to the cluster of *K. lyside* individuals from northern Mexico and Texas (Fig. 3). It has, on average, 3 bp differences with specimens collected in the United States of America (Texas and Arizona) and Mexico and 13 bp differences with specimens collected in the Caribbean region. The most similar specimens (1 bp difference) were collected in southwestern Texas (Fort Clark Springs: 29.305°N, 100.41°W; Pecos River: 30.788°N, 101.835°W). These analyses strongly suggest that the butterfly originated in southern Texas or northern Mexico and not the Caribbean islands.

There are several possible explanations for the occurrence of this butterfly in Ontario in early spring of 2011.

1. Natural dispersal of the butterfly from southern Texas to Ontario

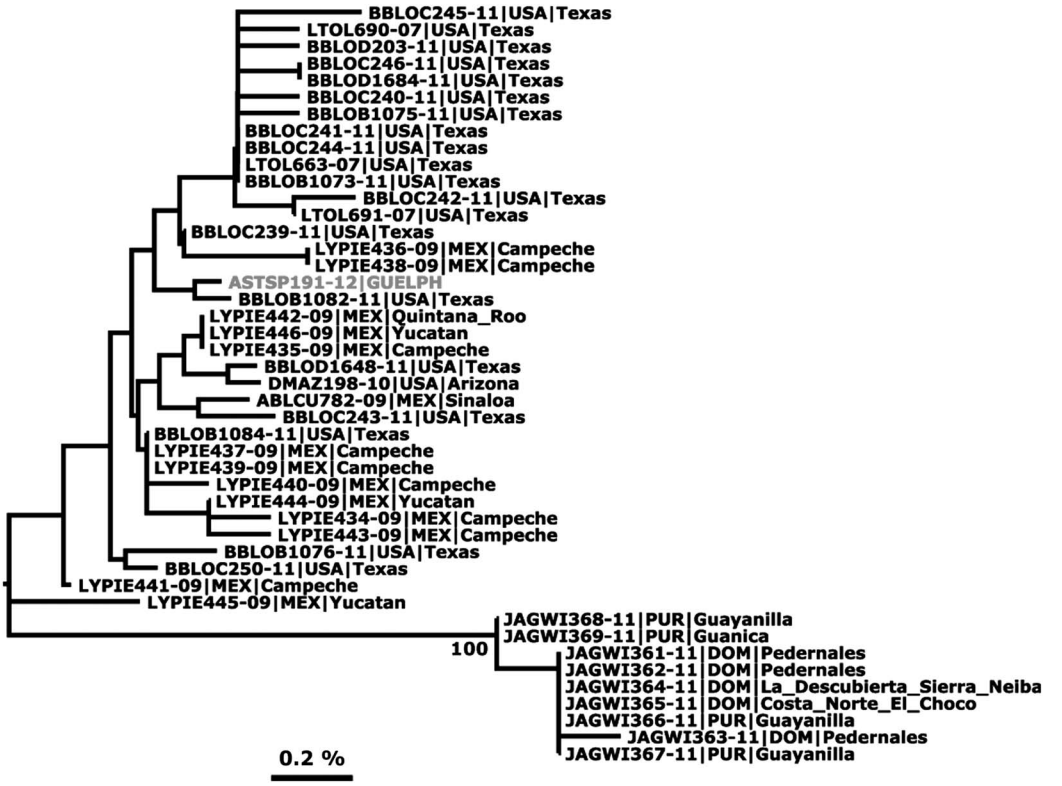
There is no evidence to suggest that lyside sulphurs were dispersing northward prior to summer of 2011. Because of the drought in southern Texas in early 2011, lyside sulphurs were uncommon there in spring and were largely absent in central Texas at that time (L. Gilbert, University of Texas, Austin, Texas, United States of America, personal communication). Guelph is ~1000 km from the most northeasterly locality (Kentucky) ever recorded for this species (see map distributional records, Butterflies and moths of North America 2013), making natural dispersal as typically occurs in this species later in summer unlikely.

2. Weather system transport of the adult butterfly

Storms and strong climatic fronts often transport butterflies outside of their normal range (Johnson 1969). This may have happened in this instance because a strong weather event developed over eastern United States of America between 3 and 5 April 2011 (Panovich 2013; Unisys Weather 2013). On the morning of 3 April 2011, the eastern half of Texas experienced high temperatures. For example, at Del Rio the temperature at 7 am was 24–28 °C at 1500 m elevation, and intense thunderstorms with cloud tops in the 8–12 km range developed during the day. High positive Convective Available Potential Energy (Unisys Weather 2013) in southern Texas created strong

https://doi.org/10.4039/tce.2014.18 Published online by Cambridge University Press

Fig. 3. Genetic distances of the Guelph specimen (labelled "Guelph") displayed with the 44 publicly available *Kricogonia lyside* individuals as a neighbour-joining tree (Saitou and Nei 1987) computed based on p-distance (Nei and Kumar 2000), where the units are the number of base differences per site. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein 1985). The neighbour-joining tree was conducted in MEGA5 (Tamura *et al.* 2011). Tip labels are BOLD process identifications pipe-separated from collection nationality.



updrafts capable of lifting a butterfly into the jet stream. The meeting of a stationary region of high pressure over southeastern United States of America and a strong cold front (low pressure cell) approaching from the Great Plains created a line of severe weather watches from eastern Kansas to Chicago by evening of 3 April 2011. The jet stream, with wind speeds of up to 550 km/hour, bent southward to Texas, then extended northeastwards in the direction of southwestern Ontario. Cooling and low convection over Ontario by the morning of 4 April 2011 would have allowed the dead butterfly to drop to the ground.

3. Transportation of butterfly specimen on or in a vehicle

Our specimen may have been struck by a vehicle in southern Texas and fallen off in Guelph. Many Canadian "snowbirds", who spend the winter in the southern United States of America, return home in early spring. If the specimen reached Guelph in this way, it is surprising that its wings remained in reasonably good condition after being buffeted at highway speeds for nearly 3000 km.

A live adult lyside sulphur inside a vehicle would have had likely flown to a window and should have had several opportunities to escape before reaching Canada.

4. Importation of a pupa on imported produce

Insects and spiders are often transported with produce (Office of Technology Assessment 1993). However, in this case, the host plant of larval *K. lyside* in Texas and northern Mexico is *Guaiacum angustifolium* Engelmann (Zygophyllaceae), a

https://doi.org/10.4039/tce.2014.18 Published online by Cambridge University Press

common shrub that inhabits shrubby grasslands dominated by mesquite (*Prosopis* Linnaeus species (Fabaceae)) and *Opuntia* Miller (Cactaceae) cacti (Gilbert 1985), not croplands. A larva would have had to crawl away from its host plant and pupate in a nearby crop that was then shipped to Canada for sale. Two grocery stores are relatively close (~1.0 km NW (270°) and ~1.6 km S (70°)) to where the specimen was found. After emergence, the adult would have likely flown to the brightest areas of the store (*i.e.*, a window, not a doorway). If it managed to exit the store, northwest surface winds on 1 and 2 April 2011 and southerly winds on 3 April 2011 (historical weather data from Environment Canada 2011) could have transported the individual from one of these stores to the site where it was collected. However, temperatures on those dates were ⩽8 °C (Environment Canada 2011) and not conducive to flight of a subtropical butterfly.

5. Specimen dropped by an insect collector

This scenario is very unlikely. Before pinning, collectors usually store butterflies with their wings folded dorsally over the thorax, unlike the position this specimen was found in. The only people known to have been collecting in regions this species inhabits in the weeks before this specimen was found were one of us (M.A.S.) and Claudia Bertrand, neither of whom had collected this species.

6. April Fool's Day joke

This scenario is very unlikely. Someone would have had to be willing to destroy a specimen of this butterfly species by setting it out along a busy street more than 1.0 km from the university campus with a low probability it would be noticed.

7. Escapee from a live butterfly exhibit

Impossible. This species is not one imported by either of the two local live butterfly exhibits (Cambridge Butterfly Conservatory or Greenway Nursery), ruling out this possibility.

In summary, it is highly likely that the lyside sulfur individual found in Ontario originated in southern Texas or northern Mexico. Unfortunately, all possible modes of transport of this specimen are unlikely, and the way in which this unusual specimen reached Ontario remains a mystery.

Acknowledgements

The authors thank Paulson Des Brisay for conducting the cytB sequencing. Jeff Skevington and Alan Wormington provided useful discussions about the ways this butterfly could have been transported to Ontario. Jess Howell and Jon Warland condensed detailed weather data into language and explanations understandable to biologists. This study was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants to Alex Smith and Gard Otis. Some of the publically available specimens of *Kricogonia lyside* on BOLD were collected by the BIObus. The support of Texas State Parks in the collection program that produced the other lyside sulphur specimens in BOLD is acknowledged. Comments of C. Schmidt, L. Gilbert, J. Kerr, and an anonymous reviewer helped to improve the manuscript.

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., *et al.* 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, **25**: 3389–3402.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., and Sayers, E.W. 2011. GenBank. Nucleic Acids Research, **39**: D32–D37.

Butterflies and moths of North America 2013. Lyside sulphur *Kricogonia lyside* (Godart, 1819) [online]. Available from http://www.butterfliesandmoths.org/species/Kricogonia-lyside [accessed 17 October 2013].

Environment Canada. 2011. Weather records [online]. Available from http://www.climate.weather.gc.ca [accessed 29 July 2013].

Ersts, P.J. 2013. Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation [online]. Available from http://biodiversityinformatics.amnh.org/open_source/gdmg [accessed 2 January 2013].

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**: 783–791.

Gilbert, L.E. 1985. Ecological factors which influence migratory behavior in two butterflies of the semi-arid shrublands of South Texas. *In* Migration: mechanisms and adaptive significance. Contributions in Marine Science, Supplementary Volume 27, *Edited by* M.A. Rankin. Marine Science Institute, University of Texas at Austin, Port Aransas, Texas, United States of America. Pp. 724–747.

Johnson, C.G. 1969. Migration and dispersal of insects by flight. Methuen, London, United Kingdom.

https://doi.org/10.4039/tce.2014.18 Published online by Cambridge University Press

Klots, A.B. 1951. A field guide to the butterflies of North America, east of the Great Plains. Houghton-Mifflin, Boston, Massachusetts, United States of America.

Nei, M. and Kumar, S. 2000. Molecular evolution and phylogenetics. Oxford University Press, New York, New York, United States of America.

Office of Technology Assessment. 1993. Harmful non-indigenous species in the United States [online], U.S. Congress, Office of Technology Assessment, OTA-F-565. United States Government Printing Office, Washington, District of Columbia, United States of America. Available from http://www.invasive.org/publications/9325.pdf [accessed 22 April 2013].

Opler, P.A. 1992. A field guide to eastern butterflies. Houghton Mifflin, Boston, Massachusetts, United States of America.

Opler, P.A. and Krizek, G.O. 1984. Butterflies east of the Great Plains. John Hopkins University, Baltimore, Maryland, United States of America.

Panovich, B. 2013. What is a derecho and the great derecho of 3–4 April, 2011 [online]. Available from http://www.wxbrad.com/the-great-derecho-of-april-4-5th-2011 [accessed 29 July 2013].

Ratnasingham, S. and Hebert, P.D.N. 2007. BOLD: The Barcode of Life Data System. Molecular Ecology Notes, **7**: 355–364. (www.barcodinglife.org).

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, **4**: 406–425.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, **28**: 2731–2739.

Unisys Weather. 2013. Available from http://weather.unisys.com [accessed 24 April 2013].

https://doi.org/10.4039/tce.2014.18 Published online by Cambridge University Press