



Sahara mustard as a major threat to desert biodiversity in the southwest United States and the need to integrate contemporary methods to understand its biology

Understanding the nature of biological invasions is an important grand challenge, and we share Prof. Hedrick's concern for developing good theory and mechanistic understanding of the ecological and evolutionary dynamics of invasion (Hedrick, 2020).

We understand the concern that F_{IS} cannot be calculated from the expected and observed heterozygosity values provided in the table 1 of Winkler et al. (2019) and acknowledge this discrepancy exists because of the different ways subpopulations and loci were handled by our analytical methods. Specifically, F_{IS} was calculated using the heterozygosity estimate H_S , calculated as

$$H_S = \sum_l \left(\sum_g \left(1 - \sum_{i(lg)} p_{i(lg)}^2 \right) / g \right) / l,$$

where $p_{i(lg)}$ is the i th allele frequency of the l th locus in the g th subpopulation, or the average expected proportion of heterozygote individuals within subpopulations (Nei, 1987). Calculating heterozygosity estimates with this approach is preferred because H_S specifically estimates average gene diversity within subpopulations, per our study design. This metric allows heterozygosity to be measured on an identical scale for each subpopulation by accounting for sample sizes of individuals/subpopulations and number of loci typed within subpopulations (Coltman et al., 1999; Roberts et al., 2006). This is also the standard method for calculating F_{IS} per the popular R packages *adegenet* and *hierfstat*, calculated as $F_{IS} = 1 - H_O / H_S$ (Goudet, 2005; Jombart, 2008). The alternative heterozygosity estimate H_E , suggested by the author, provides the average genetic diversity of loci within a population and does not account for the number of subpopulations or number of loci like H_S does. Given that our sampling approach was continuous and we expected high selfing rates within subpopulations (Winkler et al., 2019), we deemed H_S the more appropriate metric for calculating F_{IS} in this study. Thus, it is inappropriate to calculate F_{IS} values as in table 2 of Phillip Hedrick (2020). This method has been used to explore a diversity of population-level phenomena and processes in many biological systems (e.g., van Boheemen et al., 2017; Cruz et al., 2020; Villate et al., 2010). Recent studies that use this metric have been published in *Ecology and Evolution*. For example, Andriollo et al. (2018) used F_{IS} to examine diversity across bat species and Wogan et al. (2020) used this same metric to

examine subpopulation differentiation in a generalist bird. A methodological clarification to the original manuscript would have appropriately addressed these concerns.

Prof. Hedrick cites supplementary materials from a dissertation chapter (Winkler, 2017) to make claims about data and analyses in the related article published in *Ecology and Evolution* at the center of this discussion (Winkler et al., 2019). These claims are unsupported because the data mentioned were written in 2017, almost two years before Winkler et al. (2019) was accepted for publication in *Ecology and Evolution*. Methods were revised, data were scrutinized, and supplementary materials were all updated during the peer review process at two journals before being accepted by *Ecology and Evolution*.

We have confidence in the sequence data used in analyses as they were quality-controlled through standard, rigorous checks. Sequencing was carried out on an Illumina HiSeq2000 platform at the University of Oregon, and nextRAD methodology is detailed in Russello et al. (2015). Prior to analyses, raw sequence data were quality-filtered. We retained data with at least 15 \times coverage in at least 10% of samples, removed paralogs, and ensured samples were free of contamination per the methods section of Winkler et al. (2019). All resulting data in Winkler et al. (2019) are publicly available via the National Center for Biotechnology Information (NCBI) as a BioProject under accession number PRJNA534338 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA534338>). We encourage others to utilize these data and hope our contributions advance our understanding of the biological mechanisms enabling species like Sahara mustard to invade with the speed and force we have witnessed in recent decades across the US Southwest.

We should also note that the biology of novel species invasion might reveal patterns that are unexpected given traditional approaches, as exploring new phenomena can challenge the assumptions of the techniques we have used to study population dynamics. For example, we show Sahara mustard has been introduced at least three separate times in the United States, each of which have either spread beyond their initial introduction sites or remain isolated, at least up to the season we sampled individuals (Winkler et al., 2019).

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Ecology and Evolution* published by John Wiley & Sons Ltd.

We show this rapid, multi-introduction spread lacks the lag phase seen in other invasive species (e.g., Bock et al., 2018; Crooks, 2005; Crooks & Soulé, 1999; Pannell, 2015; Parker, 2004). We also demonstrated substantial phenotypic variation in key functional traits that align with climatic gradients across Sahara mustard's invaded US range (Winkler et al., 2018). Together, these results illustrate the invasiveness of the species while highlighting that we cannot assume typical expansion patterns as invasive species spread through increasingly disturbed habitats while simultaneously responding to human-induced climate change.

We appreciate that our study has grabbed the attention of an esteemed scientist so soon after publication. We are confident we are helping to push invasion biology forward by adding to an ever-growing discussion of species introductions, adaptation, inbreeding, and spread that will facilitate deeper understanding of invasions while simultaneously providing valuable information to managers seeking to curtail and prevent further spread.

ACKNOWLEDGMENTS

This research was supported by the Tubb Canyon Desert Conservancy, the Robert Lee Graduate Student Research Grant through the Joshua Tree National Park Association, the Howie Wier Memorial Conservation Grant through the Anza-Borrego Foundation, the Forrest Shreve Student Research Fund through the Ecological Society of America, the Mildred E. Mathias Graduate Student Research Grant through the UC Natural Reserve System, the Mayhew Graduate Research Award through the Boyd Deep Canyon Desert Research Center, a Graduate Assistance in Areas of National Need Research Grant (GAANN), UCI's Department of Ecology & Evolutionary Biology, the UCI Data Science Initiative at the University of California, Irvine, and the Victor and Virginia Voth Family Trust. Plant material was collected under the following permits from the National Park Service: DEVA-2015-SCI-0015, JOTR-2015-SCI-0015, LAKE-2015-SCI-0005, MOJA-2015-SCI-0021, and ORPI-2015-SCI-0004. Thanks to M. Barker, A. Collins, C. Bell, K. Dlugosch, J. Dong, G. Ferguson, R. Gaut, M. Goulden, P. Holm, S. Hug, D. Hughson, A. Kaiser, M. Kao, A. Kearns, M. Li, K. Lund, R. Massatti, K. Mooney, V. Olson, J. Ross-Ibarra, K. Roessler, A. Sakai, T. Scott, L. Smith, R. Staehle, C. Vagnier, S. Weller, and W. Yang. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

CONFLICT OF INTEREST

The authors declare there are no competing interests.

AUTHOR CONTRIBUTIONS

Daniel E. Winkler: Methodology (lead); Project administration (lead); Writing-original draft (lead). **Kenneth James Chapin:** Methodology (supporting); Writing-review & editing (supporting). **J. David Garmon:** Methodology (supporting); Writing-review & editing (supporting). **Brandon S. Gaut:** Methodology (supporting); Writing-review & editing (supporting). **Travis E. Huxman:** Methodology (supporting); Writing-review & editing (supporting).

DATA AVAILABILITY STATEMENT

Molecular data used in these analyses are available as a NCBI's sequence read archive (BioProject for *B. tournefortii*: PRJNA534338).

Daniel E. Winkler¹ 
 Kenneth J. Chapin^{2,3} 
 J. David Garmon⁴
 Brandon S. Gaut¹ 
 Travis E. Huxman¹ 

¹Department of Ecology & Evolutionary Biology, University of California, Irvine, CA, USA

²Department of Ecology & Evolutionary Biology, University of California, Los Angeles, USA

³Department of Ecology & Evolutionary Biology, University of Arizona, Tucson, USA

⁴Tubb Canyon Desert Conservancy, Borrego Springs, CA, USA

Present address

Daniel E. Winkler, U.S. Geological Survey, Southwest Biological Science Center, Moab, UT, USA

Correspondence

Daniel E. Winkler, Department of Ecology & Evolutionary Biology, University of California, Irvine, CA, USA.
 Email: dwinkler@usgs.gov

ORCID

Daniel E. Winkler  <https://orcid.org/0000-0003-4825-9073>
 Kenneth J. Chapin  <https://orcid.org/0000-0002-8382-4050>
 Brandon S. Gaut  <https://orcid.org/0000-0002-1334-5556>
 Travis E. Huxman  <https://orcid.org/0000-0002-0801-3442>

REFERENCES

- Andriollo, T., Ashrafi, S., Arlettaz, R., & Ruedi, M. (2018). Porous barriers? Assessment of gene flow within and among sympatric long-eared bat species. *Ecology and Evolution*, 8, 12841–12854. <https://doi.org/10.1002/ece3.4714>
- Bock, D. G., Kantar, M. B., Caseys, C., Matthey-Doret, R., & Rieseberg, L. H. (2018). Evolution of invasiveness by genetic accommodation. *Nature Ecology & Evolution*, 2, 991–999. <https://doi.org/10.1038/s41559-018-0553-z>
- Coltman, D. W., Pilkington, J. G., Smith, J. A., & Pemberton, J. M. (1999). Parasite-mediated selection against Inbred Soay sheep in a free-living island population. *Evolution*, 53, 1259–1267. <https://doi.org/10.1111/j.1558-5646.1999.tb04538.x>
- Crooks, J. A. (2005). Lag times and exotic species: The ecology and management of biological invasions in slow-motion. *Ecoscience*, 12, 316–329.
- Crooks, J. A., & Soulé, M. E. (1999). Lag times in population explosions of invasive species: Causes and implications. In O. T. Sandlund, P. J. Schei, & Å. Viken (Eds.), *Invasive species and biodiversity management* (pp. 103–125). Kluwer Academic Publishers.
- Cruz, M. V., Mori, G. M., Oh, D. H., Dassanayake, M., Zucchi, M. I., Oliveira, R. S., & Souza, A. P. D. (2020). Molecular responses to freshwater limitation in the mangrove tree *Avicennia germinans* (Acanthaceae). *Molecular Ecology*, 29, 344–362.

- Goudet, J. (2005). Hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes*, 5, 184–186. <https://doi.org/10.1111/j.1471-8286.2004.00828.x>
- Hedrick, P. W. (2020). Heterozygosity levels and estimation of self-fertilization in an invasive species. *Ecology and Evolution*, <http://doi.org/10.1002/ece3.6885>
- Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>
- Nei, M. (1987). *Molecular evolutionary genetics*. Columbia University Press.
- Pannell, J. R. (2015). Evolution of the mating system in colonizing plants. *Molecular Ecology*, 24, 2018–2037. <https://doi.org/10.1111/mec.13087>
- Parker, I. M. (2004). Mating patterns and rates of biological invasion. *Proceedings of the National Academy of Sciences of the USA*, 101, 13695–13696.
- Phillip Hedrick (2020). Heterozygosity levels and estimation of self-fertilization in an invasive species.
- Roberts, S. C., Hale, M. L., & Petrie, M. (2006). Correlations between heterozygosity and measures of genetic similarity: Implications for understanding mate choice. *Journal of Evolutionary Biology*, 19, 558–569. <https://doi.org/10.1111/j.1420-9101.2005.01003.x>
- Russello, M. A., Waterhouse, M. D., Etter, P. D., & Johnson, E. A. (2015). From promise to practice: Pairing non-invasive sampling with genomics in conservation. *PeerJ*, 3, e1106. <https://doi.org/10.7717/peerj.1106>
- van Boheemen, L. A., Lombaert, E., Nurkowski, K. A., Gauffre, B., Rieseberg, L. H., & Hodgins, K. A. (2017). Multiple introductions, admixture and bridgehead invasion characterize the introduction history of *Ambrosia artemisiifolia* in Europe and Australia. *Molecular Ecology*, 26, 5421–5434.
- Villate, L., Esmenjaud, D., Van Helden, M., Stoeckel, S., & Plantard, O. (2010). Genetic signature of amphimixis allows for the detection and fine scale localization of sexual reproduction events in a mainly parthenogenetic nematode. *Molecular Ecology*, 19, 856–873. <https://doi.org/10.1111/j.1365-294X.2009.04511.x>
- Winkler, D. E. (2017). *Effects of climate change on protected and invasive plant species*. Ph.D. dissertation, University of California, .
- Winkler, D. E., Chapin, K. J., François, O., Garmon, J. D., Gaut, B. S., & Huxman, T. E. (2019). Multiple introductions and population structure during the rapid expansion of the invasive Sahara mustard (*Brassica tournefortii*). *Ecology and Evolution*, 9, 7928–7941.
- Winkler, D. E., Gremer, J. R., Chapin, K. J., Kao, M., & Huxman, T. E. (2018). Rapid alignment of functional trait variation with locality across the invaded range of Sahara mustard (*Brassica tournefortii*). *American Journal of Botany*, 105, 1188–1197.
- Wogan, G. O., Voelker, G., Oatley, G., & Bowie, R. C. (2020). Biome stability predicts population structure of a southern African aridland bird species. *Ecology and Evolution*, 10, 4066–4081. <https://doi.org/10.1002/ece3.6175>