**Appendix S3.** Comparison of Ex*D*FOIL with TreeMix

**Introduction**

To create a comparison for our Ex*D*FOIL analyses with an existing methodology, we carried out analyses of our targeted dataset using the program TreeMix (Pickrell & Pritchard 2012). The TreeMix method is aimed at inferring population networks. First, a maximum-likelihood population-level tree is constructed. Next, migration edges representing gene flow events can be added, with the number of migration events specified by the user. The TreeMix algorithm adds migration edges in a directed fashion by identifying populations that provide the worst fit to the tree model. These migration edges infer directionality as well as an indication of the strength of gene flow (Pickrell & Pritchard 2012).

**Methods**

For our TreeMix analysis, we chose to use our targeted dataset with full-individual sampling. We chose to use the targeted dataset as it was our preferred dataset for detecting introgression using Ex*D*FOIL, and we used full-individual sampling to help maximize the information available to TreeMix. We also treated each individual as its own population. We acknowledge that an ideal dataset for analysis in TreeMix might differ somewhat. For instance, more outgroup taxa could be included, as TreeMix scales relatively well with more individuals. Furthermore, it might be preferable to first determine the number and composition of populations in our dataset, rather than using individuals as populations. However, our goal was to provide as direct a comparison as possible to our Ex*D*FOIL analysis. In addition, we consider the determination of population structure in our dataset to be non-trivial and beyond the scope of the present work. In theory, we could have treated each locality as a separate population. However, our sample sizes per locality were quite variable and in several cases this would have required us to again treat individuals as a separate populations (see Table S2). Moreover, it is not clear that sampling localities correspond to biologically meaningful populations (e.g., some localities might belong to the same population).

To generate a dataset in TreeMix format, we used vcftools v1.1.15 (Danacek et al., 2011) and custom shell scripts. The necessary scripts are available at <https://www.github.com/SheaML/ExDFOIL/AppendixS3_Materials>. For executing TreeMix, we used the -root option to root the maximum-likelihood tree using the outgroup (minor1\_EPR743). We also used the -noss option to turn off sample-size correction, as we noticed many branches with length zero in our results with sample-size correction left on. This issue is mentioned in the TreeMix manual as likely to occur when single individuals comprise a population. We specified the addition of migration edges using the -m option, evaluating a range in the number of events from 0 to 5. To visualize the results of our TreeMix analysis, we used the plot\_tree provided with the TreeMix software, executed in R v3.4.1 (R Core Team, 2017)

To choose the number of migration events (*m*) to include, we examined both the log-likelihoods and cumulative variance explained by each model, from 0 to 5 migration events. Specifically, we examined the second-order derivative of the likelihood as a function of *m,* choosing the value of *m* where the absolute value of second-order derivative reached its maximum value. This method generally follows that of Evanno et al. (2005), widely used for selecting the number of genetic clusters in analyses with structure (Pritchard et al., 2000). The method attempts to identify a “break in slope” (as described by Evanno et al., 2005) in the likelihood plot. Beyond this point, the rate of change in likelihood experiences its largest reduction, indicating that the additional events are providing substantially diminished returns on model fit. We repeated this method using cumulative variance explained as a function of *m.* We also repeated these analyses while not considering likelihood or cumulative variance explained at *m*=0, so as to strictly compare models that include introgression (but different numbers of events).

**Results**

Raw output files for all TreeMix analyses are available at <https://www.github.com/SheaML/ExDFOIL/AppendixS3_Materials>, and in the Dryad digital repository. Log-likelihood for each number of allowed migration events was as follows: 20417.4 (*m*=0); 20657.4 (*m*=1); 20765.2 (*m*=2); 20790.9 (*m*=3); 20827.9 (*m*=4); and 20847.8 (*m*=5). The cumulative variance explained by each model was as follows: 97.64% (*m*=0); 97.98% (m=1); 98.15% (*m*=2); 98.19% (*m*=3); 98.25% (*m*=4); and 98.30% (*m*=5). Following the methodology of Evanno et al. (2005), we calculated the first order derivative of likelihood and cumulative variance explained, obtaining values of 240 (*m*=1), 107.8 (*m*=2), 25.7 (*m*=3), 37 (*m*=4), and 19.9 (*m*=5) for likelihood and 0.34 (*m*=1), 0.17 (*m*=2), 0.04 (*m*=3), 0.06 (*m*=4), and 0.06 (*m*=5) for cumulative variances. We then calculated the absolute values of the second-order derivatives, obtaining 132.2 (*m*=1), 82.1 (*m*=2), 11.3 (*m*=3), 17.1 (*m*=4) and 19.9 (*m*=5) for likelihood and 0.17 (*m*=1), 0.13 (*m*=2), 0.02 (*m*=3), <0.01 (*m*=4), and 0.06 (*m*=5) for cumulative variance. Given that the absolute value of the second-order derivative of likelihood and the cumulative variance explained as a function of *m* both reached their maxima at *m*=1, we favored the use of 1 migration event. However, when not considering *m*=0, these values reached their maxima at *m*=2, so we also choose to visualize the results using 2 migration events.

The results of our TreeMix analyses using 1 and 2 migration edges are visualized in Figure S8. These analyses both indicate introgression from within the *oberon*-black clade into the ancestors of *S. ornatus*, with a fractional contribution of alleles of ~0.16. At *m*=2, an additional migration event from within *oberon*-black into *oberon*-red is indicated, with the same fractional contribution of alleles.

**Discussion**

Overall, our results using TreeMix are broadly concordant with those from Ex*D*FOIL. The direction of introgression (from *oberon*-black into *S. ornatus*) also matches our expectations from analysis of the mtDNA data. Further comparison of the results of TreeMix and Ex*D*FOIL reveals some potential advantages and disadvantages of each method.

TreeMix has an important advantage over Ex*D*FOIL, which is that the inferred migration edges always provide information on the directionality of introgression and the fractional contribution of alleles from one species to another. In comparison, *D*FOIL can potentially infer directionality, but provides no assessment of the total genomic contribution of introgression. However, in some ways, the information provided by TreeMix is relatively coarse, as migration events can be added as discrete events only. As the TreeMix authors point out, adding too many migration edges can hurt the readability of the resulting figures (Pickrell & Pritchard 2012). In comparison, Ex*D*FOIL provides a holistic view of the signals of introgression in the data (see our Figures 3, 4). For example, our Ex*D*FOIL analysis revealed geographic heterogeneity in the detection of introgression that is consistent with patterns inferred from the mtDNA data (Figures 2, 4). Overall, our results suggest that these two methods can yield complementary inferences, and that each has advantages and disadvantages.

Finally, we note that it would likely be preferable to provide TreeMix with allele frequencies from populations rather than individuals, but this requires information on the number and composition of populations in the dataset. Although we recovered apparently sensible results using individuals in this case, the application of TreeMix to this type of dataset is poorly explored. Another potential advantage of Ex*D*FOIL is that population structure need not be known.

**References**

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