Supplementary Figures S1-S4

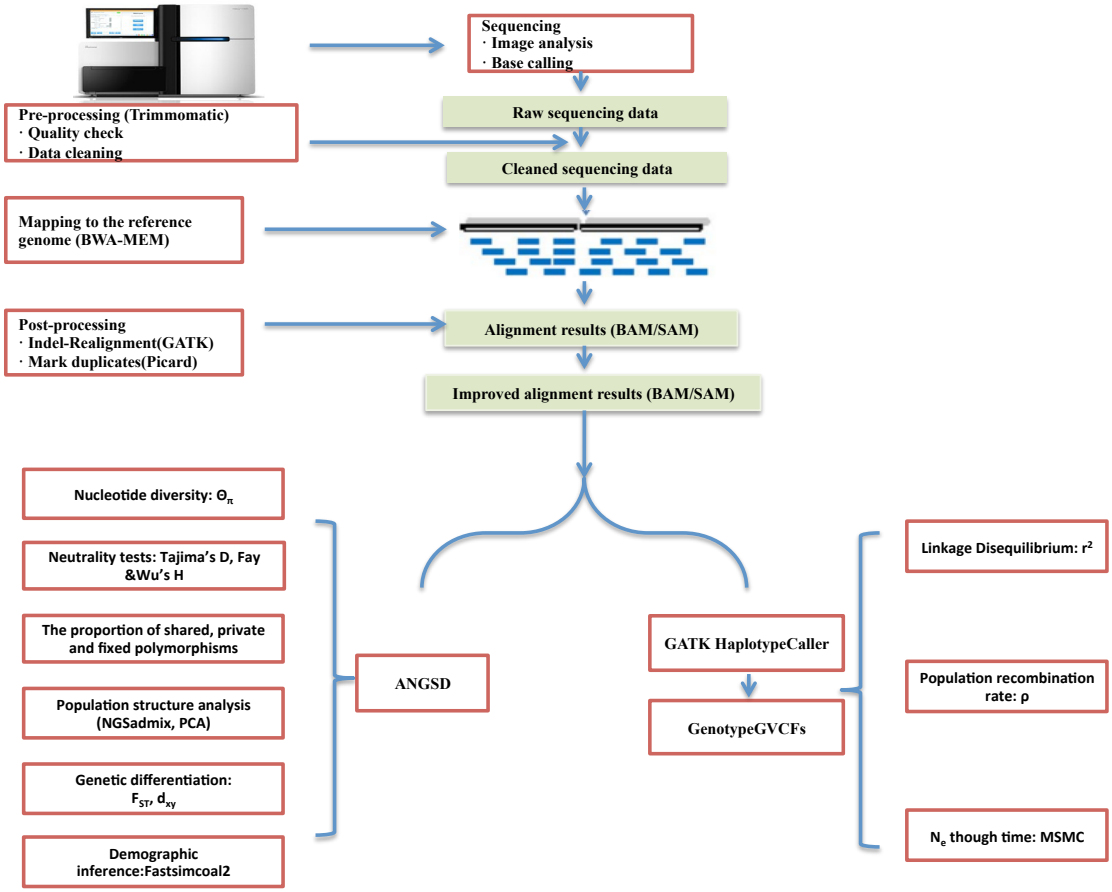
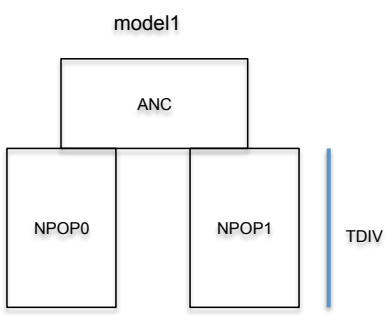


Figure S1. Analysis workflow in this study.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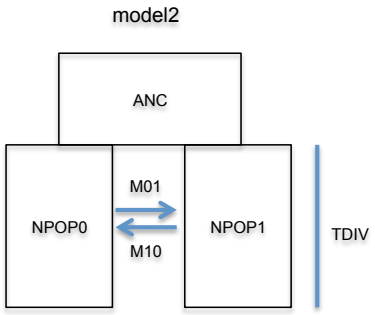
NPOP1
TDIV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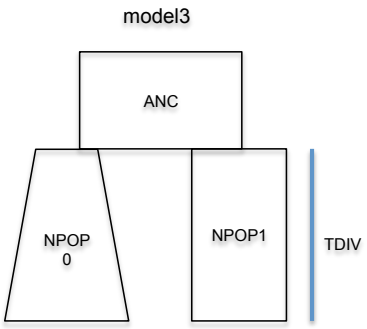
NPOP0
M01
M10
NPOP1
TDIV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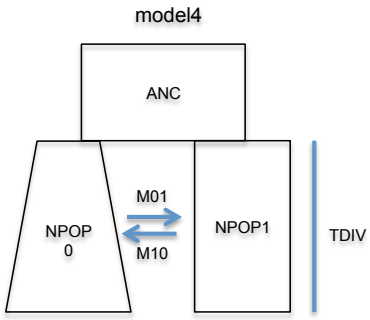
NPOP
0
NPOP1
TDIV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M01
M10
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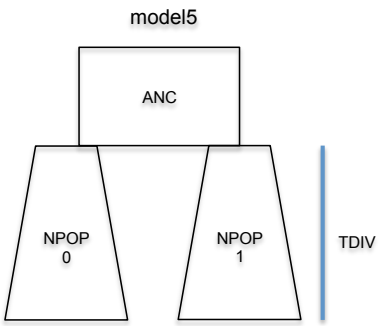
TDIV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NPOP
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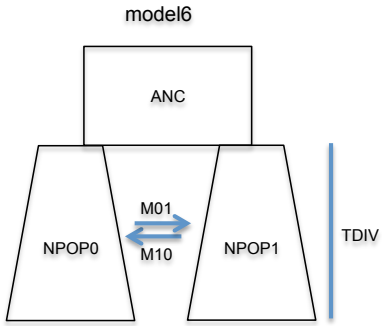
NPOP
1
TDIV


model6
ANC
NPOP0
M01
M10
NPOP1
TDIV

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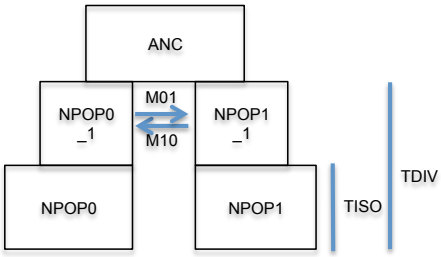
model7



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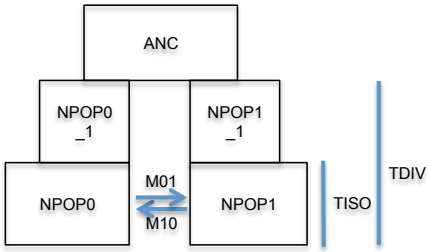

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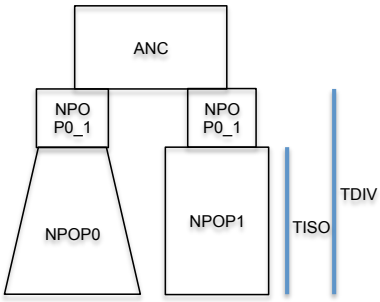

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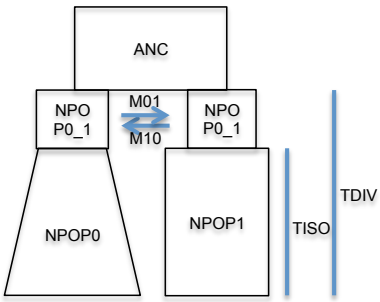

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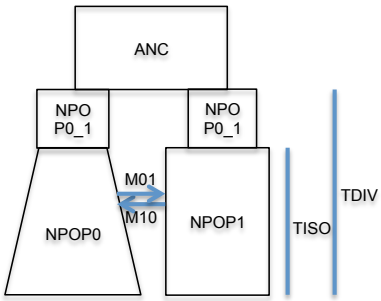

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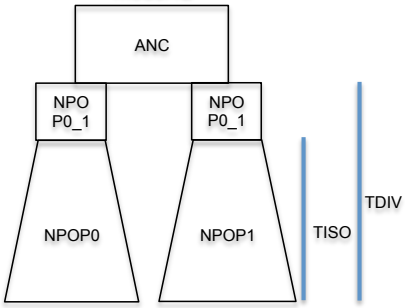

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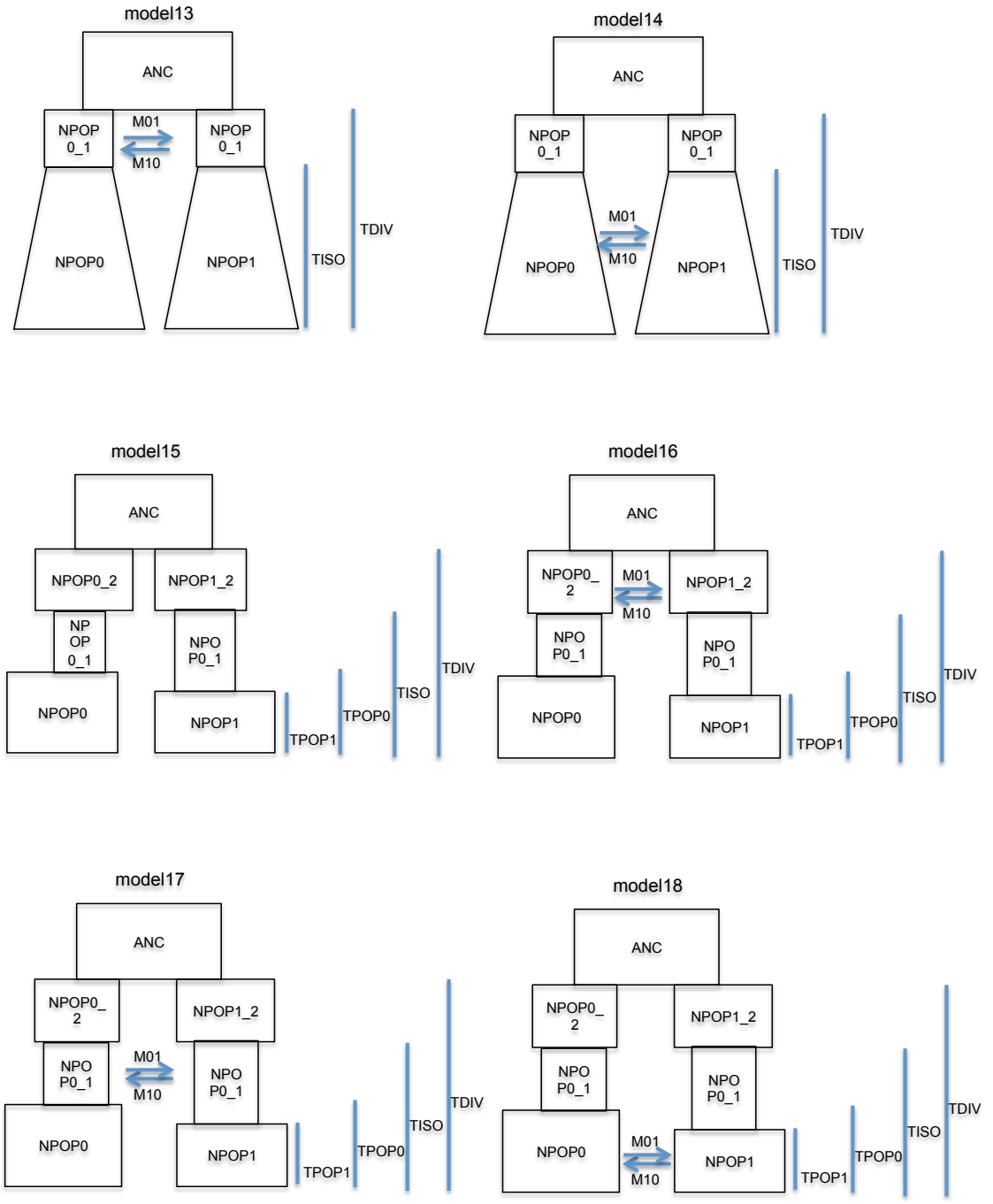


Figure S2. Tested demographic models. Model1, isolation of two species without gene flow; model2, isolation of two species with asymmetric gene flow; model3, isolation of two species with exponential population size change in *P. tremuloides* and stepwise population size change in *P. tremula*, no gene flow; model4, isolation of two species with exponential population size change in *P. tremuloides* and stepwise population size change in *P. tremula*, with asymmetric gene flow; model5, isolation

of two species with exponential population size changes in both species, no gene flow; model6, isolation of two species with exponential population size changes in both species, with asymmetric gene flow; model7, isolation of two species with stepwise population size changes in both species, asymmetric gene flow in the early stage of species divergence until the time of T_{ISO}, no gene flow afterwards; model8, isolation of two species with stepwise population size changes in both species, no gene flow in the early stage of species divergence until the time of T_{ISO}, asymmetric gene flow afterwards; model9-model11, isolation of two species with two steps of population size changes in both species, both species experienced stepwise population size changes until the time of T_{ISO}, afterwards, *P. tremuloides* experienced exponential population size change, and *P. tremula* experienced another stepwise change, the difference between models is the occurrence and the time of gene flow between species; model12-model14, isolation of two species with two steps of population size changes in both species, both species experienced stepwise population size changes until the time of T_{ISO}, afterwards, both species experienced exponential population size changes, the difference between models is the occurrence and the time of gene flow between species ; model 15-model18, isolation of two species with three steps of stepwise population size changes in both species, the difference between models is the occurrence and the time of gene flow between species.

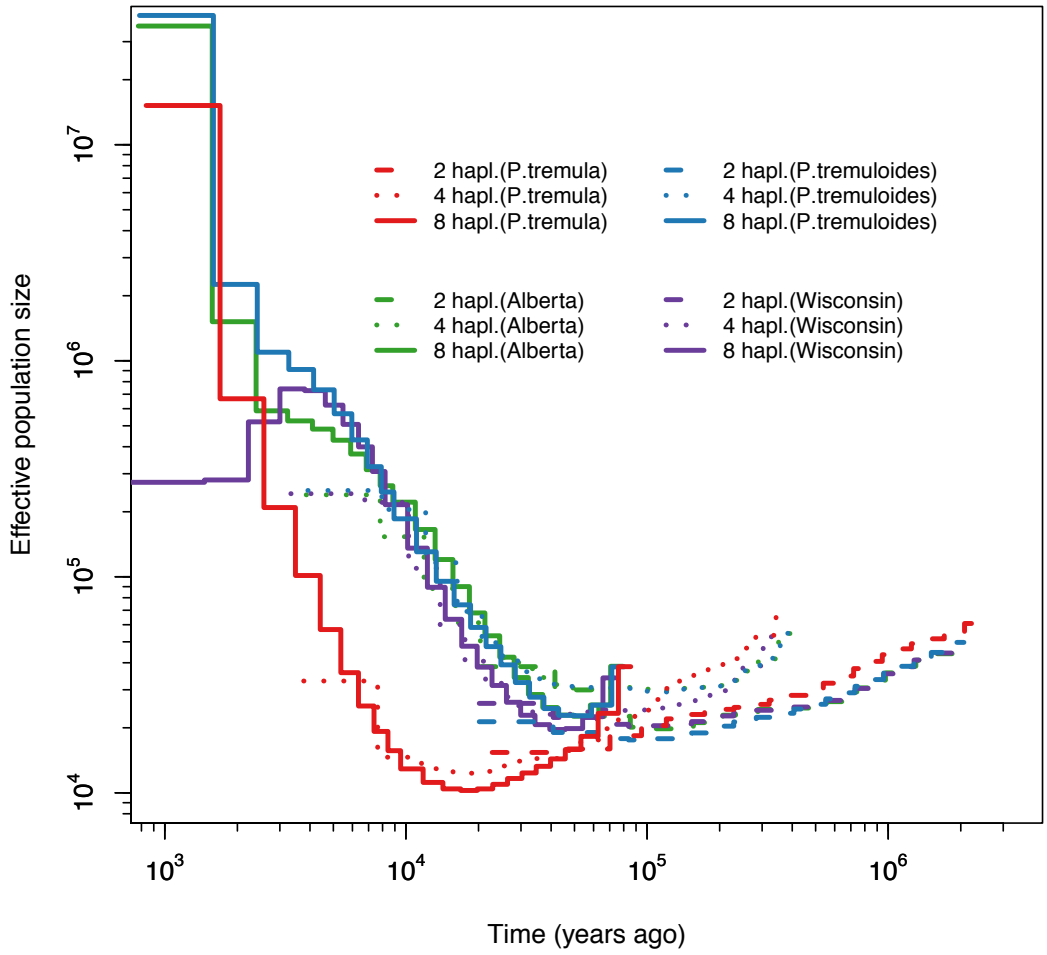
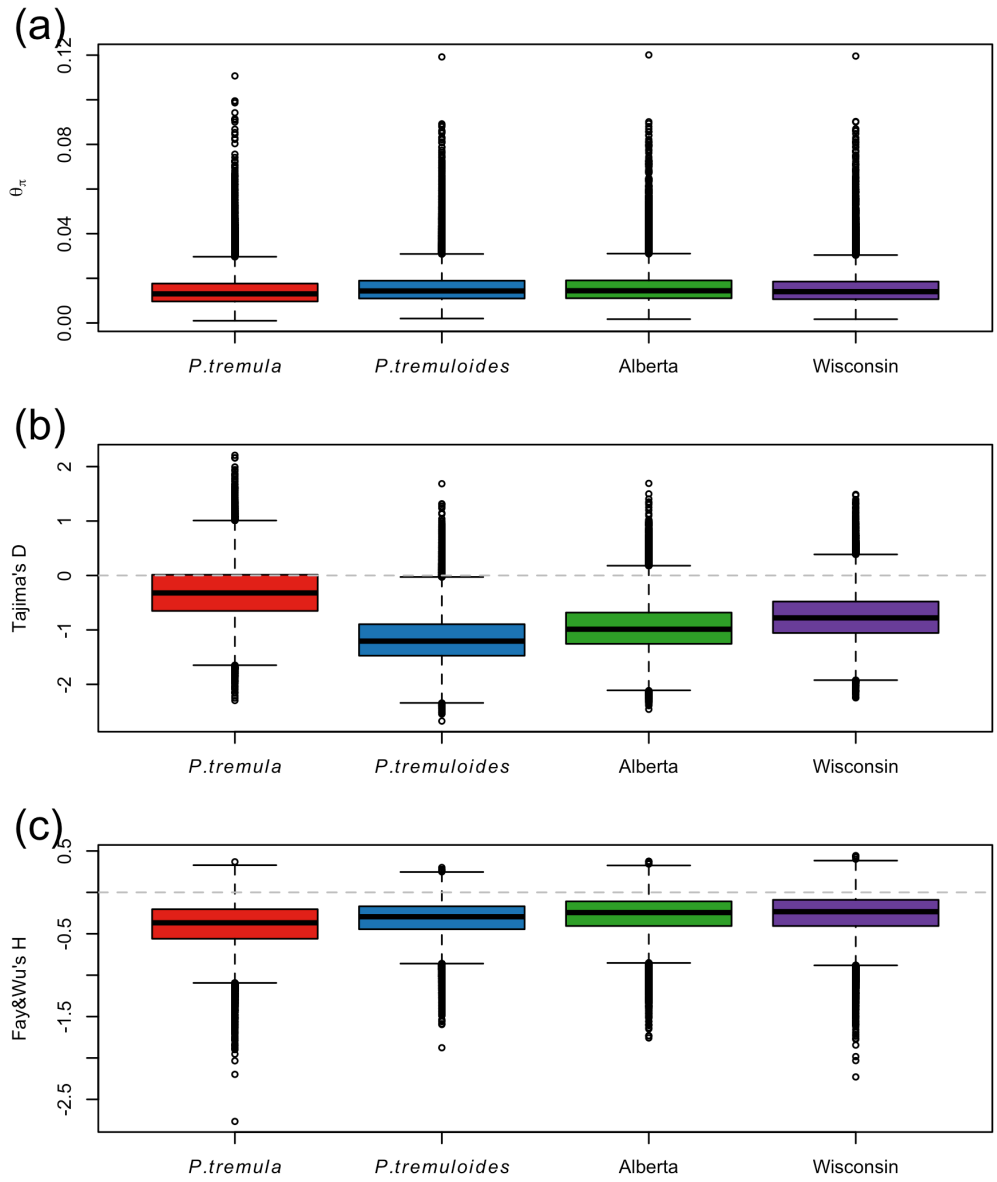


Figure S3. Comparative estimates of the effective population size (N_e) changes for subpopulations of Alberta (green line) and Wisconsin (purple line) of *P. tremuloides* from Multiple Sequential Markovian Coalescent (MSMC) analyses, which were then compared to the pooled samples of *P. tremuloides* (blue line) and *P. tremula* (red line). All estimations were based on the inference from two (dashed), four (dotted) and eight (solid) phased haplotypes in the four groups of populations, respectively. Time scale on the x-axis is calculated assuming a neutral mutation rate per generation (μ) = 3.75×10^{-8} and generation time (g) = 15 years.



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72 **Figure S4.** The distributions of estimates of (a) nucleotide diversity (θ_π), (b) Tajima's
73 D and (c) Fay &Wu's H in *P. tremula*, *P. tremuloides* and population of Alberta and
74 Wisconsin in *P. tremuloides* over 10 Kbp non-overlapping windows. The dashed grey
75 line in (b) and (c) indicate the expected values of Tajima's D and Fay&Wu's H from
76 neutral expectations.

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