***New Phytologist* Supporting Information**

Article title: Introgression from *Populus balsamifera* underlies adaptation and range boundaries in *P. trichocarpa*
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Fig. S1 Geographic distribution of admixed and reference individuals used in local ancestry analysis across the contact zones between *P. trichocarpa* and *P. balsamifera*. Ranges of *P. trichocarpa* and *P. balsamifera* are shown in red and blue, respectively (Little 1971).

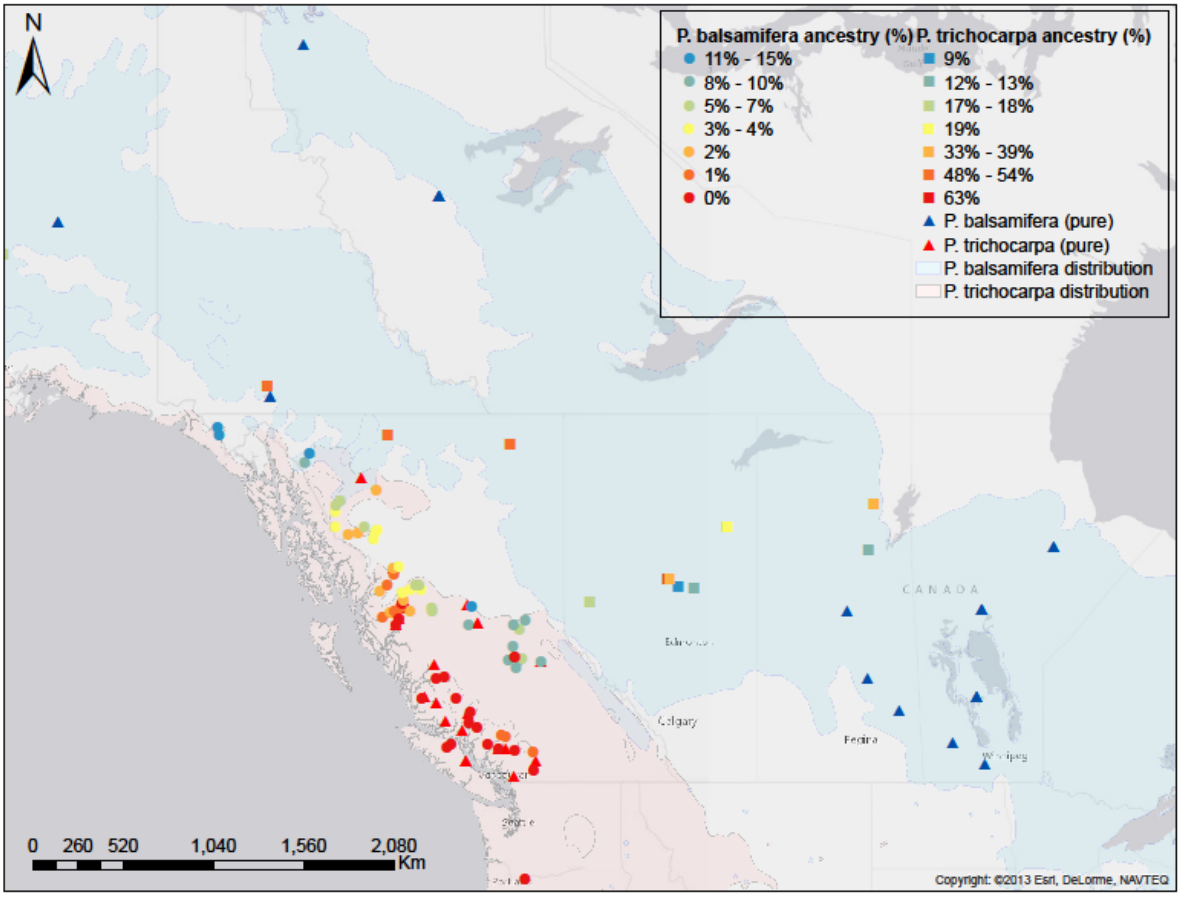

Fig. S2 Proportion of *P. balsamifera* admixed ancestry in admixed *P. trichocarpa* individuals across 19 chromosomes showing unusually high levels of introgression (sliding window analysis: size: 100-kb, step: 20-kb). Regions with unusually high levels of introgression – peaks above broken line - have ancestry higher than 3 standard deviations from the weighted mean across the whole genome based on SNP density per window (broken line). Chromosomes in gray did not show unusually high levels of introgression. Putative centromeres are shown in gray (Pinosio et al 2016).

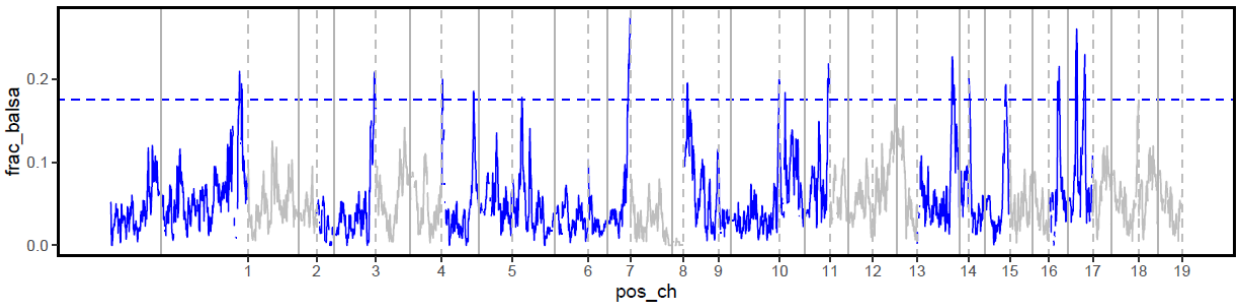

Fig. S3 NJ trees based on SNPs that had both fixed differences in the pure species and displayed associations with traits on the BMIX analysis revealing well-defined clusters. The cluster of *P. balsamifera* haplotypes (cluster with filled blue circles) and cluster of *P. trichocarpa* haplotypes (cluster with filled red circles) had strong support in chromosome 9 and 15 (bootstrap values: 90 and 80 for chromosome 9 and 15 respectively). The NJ trees were used to determined individuals that were homozygote for *P. balsamifera* haplotypes (individuals with both haplotypes in the *P. balsamifera* cluster), homozygote for *P. trichocarpa* haplotypes (individuals with both haplotypes in the *P. trichocarpa* cluster) or heterozygote. These trees include admixed *P. trichocarpa* individuals (red open circles) as well as admixed *P. balsamifera* individuals (blue open circles). The latter were not used in the admixture mapping analysis but were included in the whole genome local ancestry analysis. The NJ analyses were conducted in MEGA6.

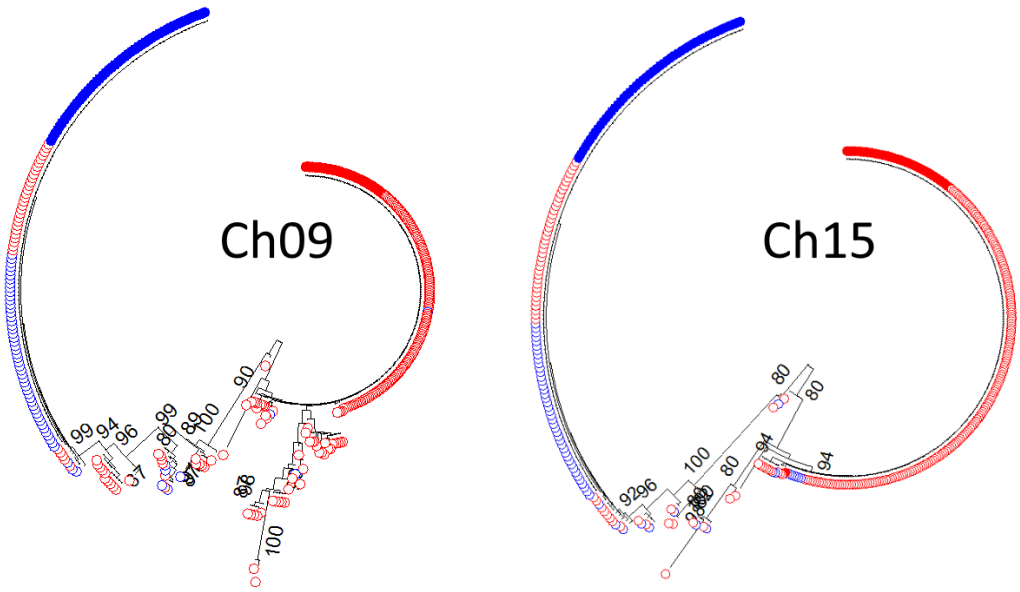

Table S1 List of traits used in the admixture mapping analysis in P. trichocarpa.

Trait code	Trait details	Trait category	Source
AB_density	Abaxial density (# mm^{-2})	ecophysiology	McKown et al 2014
AB_pore_length	Abaxial pore length (µm)	ecophysiology	McKown et al 2014
AB_SPI	Abaxial SPI - stomatal pore index.	ecophysiology	McKown et al 2014
Activegrowthratecmday_2009	Active growth rate cm day	biomass	McKown et al 2013
Activegrowthratecmday_2010	Active growth rate cm day	biomass	McKown et al 2013
AD_density	Adaxial density (# mm^{-2})	ecophysiology	McKown et al 2014
AD_pore_length	Adaxial pore length (µm)	ecophysiology	McKown et al 2014
AD_SPI	Adaxial SPI - stomatal pore index.	ecophysiology	McKown et al 2014
Ad_STM_distribution	Adaxial stomata distribution	ecophysiology	McKown et al 2014
Ad_STM_presence	Adaxial stomata presence	ecophysiology	McKown et al 2014
Ad_StomataNUM1	Adaxial stomata numbers	ecophysiology	McKown et al 2014
ADAB	AD:AB density ratio-AD:AB pore ratio	ecophysiology	McKown et al 2014
ADAB_PL	AD:AB density ratio-AD:AB pore ratio	ecophysiology	McKown et al 2014
Amax_2009	Maximum photosynthetic rate	ecophysiology	McKown et al 2013
Amax_2010	Maximum photosynthetic rate	ecophysiology	McKown et al 2013
Amax_mass_2009	Photosynthetic rate per unit dry mass	ecophysiology	McKown et al 2013
Amax_mass_2010	Photosynthetic rate per unit dry mass	ecophysiology	McKown et al 2013
AUDPC_2010	Area under the disease progress curve	disease	McKown et al 2014
AUDPC_2011	Area under the disease progress curve	disease	McKown et al 2014
Boledensitykgm_3_2012	Bole density kgm3	biomass	McKown et al 2013
Bolemasskg_2012	Bole mass kg	biomass	McKown et al 2013
Branch_2009	Branch	biomass	McKown et al 2013
Budbreakday_2010	Bud break day	phenology	McKown et al 2013
Budbreakday_2011	Bud break day	phenology	McKown et al 2013
Budsetday_2008	Bud set day	phenology	McKown et al 2013
Budsetday_2009	Bud set day	phenology	McKown et al 2013
Budsetday_2010	Bud set day	phenology	McKown et al 2013
C_N_2009	Carbon nitrogen	ecophysiology	McKown et al 2013
C_N_2010	Carbon nitrogen	ecophysiology	McKown et al 2013
Canopydurationdays_2009	Canopy duration days	phenology	McKown et al 2013
Canopydurationdays_2010	Canopy duration days	phenology	McKown et al 2013
CCI2015_ap29	Chlorophyll concentration index summer	ecophysiology	This study
CCI2015_au7	Chlorophyll concentration index summer	ecophysiology	This study
CCI2015_jl1	Chlorophyll concentration index summer	ecophysiology	This study
CCI2015_jl15	Chlorophyll concentration index summer	ecophysiology	This study

Trait code	Trait details	Trait category	Source
CCI2015_ju10	Chlorophyll concentration index summer	ecophysiology	This study
CCI2015_ju16	Chlorophyll concentration index summer	ecophysiology	This study
CCI2015_ju24	Chlorophyll concentration index summer	ecophysiology	This study
CCI2015_ma15	Chlorophyll concentration index summer	ecophysiology	This study
CCI2015_ma22	Chlorophyll concentration index summer	ecophysiology	This study
CCI2015_ma7	Chlorophyll concentration index summer	ecophysiology	This study
Chlpost_budsetCCI_2009	Chlorophyll concentration index post bud set	ecophysiology	McKown et al 2013
Chlpost_budsetCCI_2011	Chlorophyll concentration index post bud set	ecophysiology	McKown et al 2013
ChlspringCCI_2009	Chlorophyll concentration index spring	ecophysiology	McKown et al 2013
ChlsummerCCI_2009	Chlorophyll concentration index summer	ecophysiology	McKown et al 2013
ChlsummerCCI_2011	Chlorophyll concentration index summer	ecophysiology	McKown et al 2013
d13Cwood_2012	Stable carbon isotope ratio wood	ecophysiology	McKown et al 2013
d15N_2009	Stable nitrogen isotope ratio	ecophysiology	McKown et al 2013
d15N_2010	Stable nitrogen isotope ratio	ecophysiology	McKown et al 2013
DENS	Total density (# mm^{-2})	ecophysiology	McKown et al 2014
Dleaf_2009	Net discrimination leaf	ecophysiology	McKown et al 2013
Dleaf_2010	Net discrimination leaf	ecophysiology	McKown et al 2013
Growthperioddays_2009	Growth period days	biomass	McKown et al 2013
Growthperioddays_2010	Growth period days	biomass	McKown et al 2013
gsmol_2009	Stomatal conductance	ecophysiology	McKown et al 2013
gsmol_2010	Stomatal conductance	ecophysiology	McKown et al 2013
H_Dcm_cm_2009	Height diameter cm	biomass	McKown et al 2013
H_Dcm_cm_2010	Height diameter cm	biomass	McKown et al 2013
H_Dcm_cm_2011	Height diameter cm	biomass	McKown et al 2013
Heightcm_2008	Height cm	biomass	McKown et al 2013
Heightcm_2009	Height cm	biomass	McKown et al 2013
Heightcm_2010	Height cm	biomass	McKown et al 2013
Heightcm_2011	Height cm	biomass	McKown et al 2013
Heightgaincm_2009	Height gain cm	biomass	McKown et al 2013
Heightgaincm_2010	Height gain cm	biomass	McKown et al 2013
Heightgaincm_2011	Height gain cm	biomass	McKown et al 2013
Heightgrowthcessationday_2009	Height growth cessation day	biomass	McKown et al 2013
Leafdropday_2008	Leaf drop day	phenology	McKown et al 2013
Leafdropday_2009	Leaf drop day	phenology	McKown et al 2013
Leafdropday_2010	Leaf drop day	phenology	McKown et al 2013
Leafflushday_2010	Leaf flush day	phenology	McKown et al 2013
Leafflushday_2011	Leaf flush day	phenology	McKown et al 2013
Leafflushday_2012	Leaf flush day	phenology	McKown et al 2013
Leaflifespandays_2010	Leaf life span days	phenology	McKown et al 2013
Leafshapelength_width_2009	Leaf shape length width	ecophysiology	McKown et al 2013
Leavesperbud_2011	Leaves per bud	ecophysiology	McKown et al 2013
Leavesperbud_2012	Leaves per bud	ecophysiology	McKown et al 2013
LMApost_budset_2010	Leaf mass per unit area post budset	ecophysiology	McKown et al 2013
LMApost_budset_2011	Leaf mass per unit area post budset	ecophysiology	McKown et al 2013
LMAspring_2010	Leaf mass per unit area spring	ecophysiology	McKown et al 2013
LMAspring_2011	Leaf mass per unit area spring	ecophysiology	McKown et al 2013
LMAsummer_2009.1	Leaf mass per unit area summer	ecophysiology	McKown et al 2013
LMAsummer_2010.1	Leaf mass per unit area summer	ecophysiology	McKown et al 2013
LMAsummer_2011.1	Leaf mass per unit area summer	ecophysiology	McKown et al 2013
Logheightgrowthlogcmday_2009	Log height growth log cm day	biomass	McKown et al 2013
Logvolumegrowthlogcm3day_2009	Log volume growth log cm3 day	biomass	McKown et al 2013
Narea_2009	Nitrogen area	ecophysiology	McKown et al 2013
Narea_2010	Nitrogen area	ecophysiology	McKown et al 2013
Nmass_2009	Nitrogen mass	ecophysiology	McKown et al 2013
Nmass_2010	Nitrogen mass	ecophysiology	McKown et al 2013
NUE_2009	Photosynthetic nitrogen-use efficiency	ecophysiology	McKown et al 2013
NUE_2010	Photosynthetic nitrogen-use efficiency	ecophysiology	McKown et al 2013

Trait code	Trait details	Trait category	Source
Post_budsetperioddays_2009	Post bud set period days	phenology	McKown et al 2013
Post_budsetperioddays_2010	Post bud set period days	phenology	McKown et al 2013
SPI	Stomatal pore index.	ecophysiology	McKown et al 2014
Tannins	Tannins (µg mg DW-1)	ecophysiology	McKown et al 2014
Volumecm3_2009	Volume cm3	biomass	McKown et al 2013
Volumecm3_2010	Volume cm3	biomass	McKown et al 2013
Volumecm3_2011	Volume cm3	biomass	McKown et al 2013
Volumegaincm3_2010	Volume gain cm3	biomass	McKown et al 2013
Volumegaincm3_2011	Volume gain cm3	biomass	McKown et al 2013
Wholetreemasskg_2012	Whole tree mass kg	biomass	McKown et al 2013
WUE_2009	Instantaneous water-use efficiency	ecophysiology	McKown et al 2013
WUE_2010	Instantaneous water-use efficiency	ecophysiology	McKown et al 2013
Yellowing100_2010	Yellowing 100	phenology	McKown et al 2013
Yellowing25_2010	Yellowing 25	phenology	McKown et al 2013
Yellowing50_2010	Yellowing 50	phenology	McKown et al 2013
Yellowing75_2010	Yellowing 75	phenology	McKown et al 2013

Table S2 List of environmental variables used in a principal component analysis. Twenty-three climate variables were compiled from ClimateNA (Wang et al. 2012) based on 1971–2000.

Type of variable	Abbreviation	Variable
temperature	MAT	mean annual temperature (°C)
temperature	MWMT	mean warmest month temperature (°C)
temperature	MCMT	mean coldest month temperature (°C)
temperature	TD	temperature difference between MWMT and MCMT or continentality (°C)
temperature	DD<0	degree-days below 0°C chilling degree-days
temperature	DD>5	degree-days above 5°C growing degree-days
temperature	DD<18	degree-days below 18°C heating degree-days
temperature	DD>18	degree-days above 18°C cooling degree-days
temperature	NFFD	the number of frost-free days
temperature	FFP	frost-free period
temperature	bFFP	the day of the year on which FFP begins
temperature	eFFP	the day of the year on which FFP ends
temperature	EMT	extreme minimum temperature over 30 years
temperature	EXT	extreme maximum temperature over 30 years
temperature	MAR	mean annual solar radiation (MJ m-2 d-1)
humidity	MAP	mean annual precipitation (mm)
humidity	MSP	May to September precipitation (mm)
humidity	AHM	annual heat-moisture index (MAT+10)/(MAP/1000))
humidity	SHM	summer heat-moisture index ((MWMT)/(MSP/1000))
humidity	PAS	precipitation as snow (mm) between August in previous year and July in current year
humidity	Eref	Hargreaves reference evaporation (mm)
humidity	CMD	Hargreaves climatic moisture deficit (mm)
humidity	RH	mean annual relative humidity (%)

Table S3 P-values of ANOVAs of 16 traits in pure and admixed P. trichocarpa

Comparisons	Yellowing100_2010	Heightcm_2010	Heightgaincm_2009	Heightcm_2011
ch09_tb-Ch09_bb	0.4086	0.2839	0.6642	0.1928
Ch09_tt-Ch09_bb	0.0074	0.0148	0.0181	0.0142
Purenorth-Ch09_bb	0.8026	0.8933	0.7922	0.8791
Puresouth-Ch09_bb	0.8955	0.9978	1.0000	0.9847
Ch09_tt-ch09_tb	0.3503	0.7591	0.2562	0.9060
Purenorth-ch09_tb	0.0195	0.0193	0.0606	0.0088
Puresouth-ch09_tb	0.0323	0.1014	0.5749	0.0305
Purenorth-Ch09_tt	0.0000	0.0001	0.0000	0.0001
Puresouth-Ch09_tt	0.0000	0.0012	0.0079	0.0004
Puresouth-Purenorth	0.9993	0.9701	0.8193	0.9913
	Growthperioddays_2010	**Leaflifespandays_2010**	**Yellowing75_2010**	**Heightgaincm_2010**
ch09_tb-Ch09_bb	0.2790	0.5959	0.4603	0.3796
Ch09_tt-Ch09_bb	0.0131	0.0253	0.0213	0.0320
Purenorth-Ch09_bb	1.0000	0.9803	0.7427	0.9560
Puresouth-Ch09_bb	0.9870	1.0000	0.9993	0.9990
Ch09_tt-ch09_tb	0.7376	0.3909	0.5527	0.8003
Purenorth-ch09_tb	0.2148	0.2024	0.0174	0.0626
Puresouth-ch09_tb	0.0577	0.5050	0.2489	0.1791
Purenorth-Ch09_tt	0.0060	0.0012	0.0000	0.0008
Puresouth-Ch09_tt	0.0004	0.0089	0.0029	0.0044
Puresouth-Purenorth	0.9913	0.9815	0.8375	0.9897
	Budsetday_2010	**Yellowing25_2010**	**AUDPC_2010**	**Yellowing50_2010**
ch09_tb-Ch09_bb	0.2706	0.7051	0.3129	0.4867
Ch09_tt-Ch09_bb	0.0210	0.0351	0.0108	0.0208
Purenorth-Ch09_bb	0.9029	0.9443	1.0000	0.8652
Puresouth-Ch09_bb	0.9205	0.9251	0.9998	0.9815
Ch09_tt-ch09_tb	0.8616	0.3252	0.6139	0.5013
Purenorth-ch09_tb	0.0194	0.1870	0.3034	0.0434
Puresouth-ch09_tb	0.0188	0.9943	0.1608	0.8164
Purenorth-Ch09_tt	0.0002	0.0007	0.0101	0.0001
Puresouth-Ch09_tt	0.0001	0.2649	0.0014	0.0776
Puresouth-Purenorth	1.0000	0.4817	0.9998	0.5027
	Volumecm3_2011	**Activegrowthratecmday_2010**	**Leafdropday_2009**	**Budsetday_2009**

ch09_tb-Ch09_bb	0.6619	0.5519	0.4029	0.8804
Ch09_tt-Ch09_bb	0.8374	0.0611	0.2255	0.2710
Purenorth-Ch09_bb	0.0063	0.9796	0.2144	0.6552
Puresouth-Ch09_bb	0.6837	1.0000	0.4192	0.6958
Ch09_tt-ch09_tb	0.9466	0.7359	1.0000	0.7409
Purenorth-ch09_tb	0.0000	0.1734	0.0003	0.0841
Puresouth-ch09_tb	0.0276	0.4331	0.0013	0.0912
Purenorth-Ch09_tt	0.0000	0.0039	0.0000	0.0010
Puresouth-Ch09_tt	0.0263	0.0227	0.0000	0.0009
Puresouth-Purenorth	0.1548	0.9851	0.9909	1.0000

Notes S1: Script for BMIX (Shriner *et al.,* 2011)

```
#script to run IBMIX Shriner et al 2011 - published script only for one SNP one trait
#here SNPs and traits are looped and each chromosome is run independently
#input files:
#data1 (phen=traits)
#data2 (globalanc= global ancestries average of local ancestries from RASPberry across chromosomes)
#data3 (localanc = local ancestries from RASPberry for each chromosome)
#data4 (geno = genotype data using .raw files from plink)
#admixture_burden and association_burden should be changed accordingly, based on AR model

for(i in 3:ncol(data1)){
 y=data.matrix(data1[,i])
 globalanc<-data.matrix(data2[,2])
 resF<-NULL
 for(j in 2:ncol(data3)){ tryCatch({
  localanc<-data.matrix(data3[,j])
  geno<-data.matrix(data4[,j])

  posterior <- function(x,prior,lambda) {(dchisq(x,1,lambda)*prior)/((dchisq(x,1,lambda)*prior)+(dchisq(x,1,0)*(1-
prior)))}
  admixture_burden <- 474.59372 #order Max
  association_burden <- 149467.2837 #order Max

  result <- summary(glm(y~localanc+globalanc,family=gaussian))
  admixture_p <- result$coefficients[2,4]
  admixture_lambda <- (qnorm(1-0.05/admixture_burden/2)+qnorm(0.8))^2
  admixture_prior <- 1/admixture_burden
  admixture_test <- qchisq(admixture_p,1,0,lower.tail=FALSE)
  admixture_posterior <- posterior(x=admixture_test,prior=admixture_prior,lambda=admixture_lambda)

 res<-paste((j-1),admixture_p,admixture_test,admixture_prior,admixture_posterior,admixture_lambda,sep=" ")
 resF<-rbind(resF,res) }, error=function(e){cat("ERROR :",conditionMessage(e), "\n")})
}
write.table(resF,file=paste("/home/adriana/IBMX/results_admi_oc2015/ch01_trait",(i),"-
results.txt",sep=""),row.names=F,col.names=T,quote=F)
}
```

Reference

McKown, A.D., R.D. Guy, J. Klápště, A. Geraldes, M. Friedmann, Q.C.B. Cronk, Y. El-Kassaby, S.D. Mansfield and C.J. Douglas. 2013. Geographical and environmental gradients shape phenotypic trait variation and genetic structure in *Populus trichocarpa*. New Phytol. 201:1263-1276.

McKown, A.D., J. Klápště, R.D. Guy, A. Geraldes, I. Porth, J. Hannemann, M. Friedmann, W. Muchero, G.A. Tuskan, J. Ehlting, Q.C.B. Cronk, Y. El-Kassaby, S.D. Mansfield and C.J. Douglas. 2014. Genome-wide association implicates numerous genes underlying ecological trait variation in natural populations of *Populus trichocarpa*. New Phytol. 203:535-553.

Little, E.L. 1971. Conifers and important hardwoods. p. 1146-1155. *In* U.S. Department of Agriculture Miscellaneous Publication (ed.) Atlas of United States trees Volume 1. U.S. Department of Agriculture Miscellaneous Publication, Washington D. C.

Pinosio, S., S. Giacomello, P. Faivre-Rampant, G. Taylor, V. Jorge, M. Le Paslier, G. Zaina, C. Bastien, F. Cattonaro, F. Marroni and M. Morgante. 2016. Mol. Biol. Evol. 33: 2706-2719.

Shriner, D., A. Adeyemo and C.N. Rotimi. 2011. Joint ancestry and association testing in admixed individuals. PLOS Computational Biology 7:e1002325.

Wang, T., A. Hamann, D.L. Spittlehouse and T.Q. Murdock. 2012. ClimateWNA – high-resolution spatial climate data for western North America . J. Appl. Meteor. Climatol. 51:16-29.