

Genomic Admixture Analysis in European *Populus* spp. Reveals Unexpected Patterns of Reproductive Isolation and Mating

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Manuscript received May 13, 2010
Accepted for publication July 21, 2010

ABSTRACT

Admixture between genetically divergent populations facilitates genomic studies of the mechanisms involved in adaptation, reproductive isolation, and speciation, including mapping of the loci involved in these phenomena. Little is known about how pre- and postzygotic barriers will affect the prospects of “admixture mapping” in wild species. We have studied 93 mapped genetic markers (microsatellites, indels, and sequence polymorphisms, ~60,000 data points) to address this topic in hybrid zones of *Populus alba* and *P. tremula*, two widespread, ecologically important forest trees. Using genotype and linkage information and recently developed analytical tools we show that (1) reproductive isolation between these species is much stronger than previously assumed but this cannot prevent the introgression of neutral or advantageous alleles, (2) unexpected genotypic gaps exist between recombinant hybrids and their parental taxa, (3) these conspicuous genotypic patterns are due to assortative mating and strong postzygotic barriers, rather than recent population history. We discuss possible evolutionary trajectories of hybrid lineages between these species and outline strategies for admixture mapping in hybrid zones between highly divergent populations. Datasets such as this one are still rare in studies of natural hybrid zones but should soon become more common as high throughput genotyping and resequencing become feasible in nonmodel species.

ADMIXTURE or hybrid zones between genetically divergent populations are increasingly being explored for their use in studies of adaptation, reproductive isolation, and speciation (RIESEBERG *et al.* 1999; MARTINSEN *et al.* 2001; WU 2001; VINES *et al.* 2003; PAYSEUR *et al.* 2004; reviewed by COYNE and ORR 2004), especially for their potential in identifying recombinants for gene mapping (otherwise known as “admixture mapping”; CHAKRABORTY and WEISS 1988; BRISCOE *et al.* 1994; RIESEBERG *et al.* 1999; REICH *et al.* 2005; SLATE 2005; ZHU *et al.* 2005; LEXER *et al.* 2007; NOLTE *et al.* 2009). In many taxa of animals and plants, recombinants are created by admixture between divergent populations or species in hybrid zones or ecotones (BUERKLE and LEXER 2008; GOMPERT and BUERKLE 2009). The growing

interest of evolutionary geneticists in admixture has its roots in both basic evolutionary genetics and breeding.

With respect to evolutionary genetics, admixed populations have been viewed as important resources for studying the genetics of adaptation and speciation, since the discovery that by fitting geographical clines of allele frequencies across hybrid zones, the strength of intrinsic and extrinsic (ecological) barriers to gene flow can be estimated (BARTON and HEWITT 1985; BARTON and GALE 1993). More recently, the genomics era has taken these concepts to a new level by providing genetic or physical genome maps for many species so that clines or introgression patterns of individual loci can be compared to their genomic background (see below; FALUSH *et al.* 2003; GOMPERT and BUERKLE 2009). Thus, hybrid zones permit the identification and study of quantitative trait loci (QTL), genes, or other genetic elements involved in reproductive isolation and speciation *in situ*, directly in natural populations, if sufficient genetic recombination has occurred (RIESEBERG and BUERKLE

Supporting information is available online at <http://www.genetics.org/cgi/content/full/genetics.110.118828/DC1>.

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2002). In applied genetics, studies of hybrid zones yield information on the genomic architecture of barriers to introgression, which is of great interest to breeders concerned with the establishment of pedigrees for tree selection and domestication (STETTLER *et al.* 1996).

Most animal or plant hybrid zones studied to date involve hybridization between parental populations that are much more divergent than the admixed human populations that have been used successfully for gene mapping in human medical genetics (*e.g.*, REICH *et al.* 2005; ZHU *et al.* 2005). Little experience exists with interpreting genomic patterns of ancestry and admixture in such highly divergent, nonhuman populations. Early genomic work on hybrid zones, based on dominant genetic markers, suggested the feasibility of mapping genome regions involved in reproductive isolation and speciation (RIESEBERG *et al.* 1999; ROGERS *et al.* 2001), but these studies did not allow tests for selection on genotypes at single loci in different genomic backgrounds. This became possible only recently due to the development of novel analytical tools suited to large numbers of codominant markers, especially linkage models of Bayesian admixture analysis (FALUSH *et al.* 2003, 2007) and methods to fit “genomic clines” of codominant marker genotypes across complete genomic admixture gradients (LEXER *et al.* 2007; GOMPERT and BUERKLE 2009; NOLTE *et al.* 2009; TEETER *et al.* 2010). Great advances also have been made in interpreting single-locus estimates of genetic divergence between populations and species (BEAUMONT 2005; FOLL and GAGGIOTTI 2008; EXCOFFIER *et al.* 2009a). Here, we bring these approaches together to yield novel insights into genomic patterns of reproductive isolation and mating in hybrid zones of two widespread and important members of the “model tree” genus *Populus*. Our goal was to infer patterns of reproductive isolation and the likely evolutionary trajectories of hybrid populations and to develop strategies for genetic mapping in admixed populations.

Populus alba (white poplar) and *P. tremula* (European aspen) are ecologically divergent (floodplain *vs.* upland habitat) hybridizing tree species related to *P. trichocarpa*, the first completely sequenced forest tree (TUSKAN *et al.* 2006). The two species are highly differentiated for neutral DNA-based markers (LEXER *et al.* 2007) and numerous phenotypic and ecological traits (LEXER *et al.* 2009). Mosaic hybrid zones between these species often form in riparian habitats (LEXER *et al.* 2005; hybrids sometimes referred to as *P. × canescens*) and have been proposed as potential “mapping populations” for identifying QTL and genes of interest in evolutionary biology (LEXER *et al.* 2007; BUERKLE and LEXER 2008) and breeding (FOSSATI *et al.* 2004; LEXER *et al.* 2004). Previous studies of these hybrid zones were conducted with a relatively small number of genetic markers and without making use of linkage information; the genomic composition of hybrid zones between these species has never been studied with a genomewide panel of

codominant markers with known linkage relationships. Specifically, we address the following questions in this contribution:

(1) What does an analysis of admixture and differentiation based on a genome-wide panel of mapped markers tell us about patterns of reproductive isolation and mating in hybrid zones of European *Populus* species? (2) What are the likely roles of pre- and postzygotic barriers *vs.* recent, localized historical factors in generating the observed genomic patterns? (3) What are the practical implications for admixture mapping in hybrid zones between highly divergent populations? We showcase where the genetic peculiarities of hybrid zones will limit their use for gene mapping and where they suggest new approaches that were perhaps not foreseen by geneticists with a focus on human medical applications.

MATERIALS AND METHODS

Sampling of admixture zones: Three interspecific “mosaic” hybrid zones and adjacent parental populations of *P. alba* and *P. tremula* were sampled. These are defined by the three river drainage systems in which they are situated: Danube (North-eastern Austria), Ticino (Northwestern Italy), and Tisza (Northeastern Hungary) (Table 1). The three hybrid or admixture zones were previously discovered with the help of genetic markers (BARTHA 1991; FOSSATI *et al.* 2004; LEXER *et al.* 2005). The main focus of this article is on the Danube hybrid zone; the Ticino and Tisza populations were used as additional “replicates” to check whether genotypic patterns observed for the Danube could be generalized to other localities.

In each zone, widely spaced trees were sampled with a minimum distance of 50 m to avoid sampling of asexually derived ramets, based on results of a previous analysis of fine-scale spatial structure (VAN LOO *et al.* 2008). Sampling was carried out without regard to morphology, and emphasis was placed on broad geographic coverage within each river valley (Table 1); sampling transects is not feasible in mosaic hybrid zones and was not required for the statistical analyses employed in this study (GOMPERT and BUERKLE 2009). Parental reference populations were sampled adjacent to each hybrid zone in lowland forest (*P. alba*) and upland habitat (*P. tremula*), with widely spaced individuals as described by LEXER *et al.* (2005).

Within-genome sampling and laboratory analyses: Ninety-three polymerase chain reaction (PCR) or DNA sequence-based molecular genetic markers were typed in the Danube hybrid zone and its parental reference populations. These included 83 microsatellites, eight sequence-based markers, and two insertion-deletion polymorphisms (supporting information, Table S1). Seventy-six of the markers were developed from genomic libraries or directly from the *P. trichocarpa* genome assembly v.1 (<http://www.ornl.gov/sci/ipgc>; VAN DER SCHOOT *et al.* 2000; SMULDERS *et al.* 2001; TUSKAN *et al.* 2004), and 17 were developed from expressed sequence tags (ESTs) (JOSEPH and LEXER 2008; DE CARVALHO *et al.* 2010). The markers were distributed across all 19 chromosomes of the *Populus* genome with an average of 5 ± 1 (SE) markers per linkage group (Table S1). Different sets of markers were used at various stages of the work, depending on the requirements of each statistical method (see below).

To facilitate marker genotyping, genomic DNA was extracted from silica-dried leaves with the Dneasy plant mini kit (QIAGEN). Subsequently, all microsatellites and indel poly-

morphisms were PCR amplified using the protocols described previously by LEXER *et al.* (2005) and precisely sized using an Applied Biosystems (ABI) 3100 Genetic Analyzer and associated fluorescent dyes and software. All sequence-based polymorphisms were examined by PCR amplification and direct sequencing, using a Biomek NX S8 Laboratory Automated Workstation (Beckman Coulter) and an ABI 3730 Genetic Analyzer with accompanying chemistry, following precisely the protocols of JOSEPH and LEXER (2008).

Statistical analyses: *Descriptive statistics:* Since European hybrid zones of *P. alba* and *P. tremula* were characterized for their genetic diversity and structure elsewhere (FOSSATI *et al.* 2004; LEXER *et al.* 2005), descriptive population genetic analyses were kept to a minimum here: populations were characterized for their gene diversity equivalent to expected (H_E) heterozygosity, observed (H_O) heterozygosity (for fully codominant markers), inbreeding coefficient (F_{IS}) and allelic richness corrected for sample size by rarefaction, and the following measures of genetic divergence: F_{ST} , HEDRICK'S (2005) G'_{ST} , and the allele frequency differential (δ). These calculations were carried out using the FSTAT software (GOUDET 1995) or manually on the basis of the output of this program.

Bayesian admixture analysis with linkage model: Bayesian admixture analysis of the focal hybrid zone (Danube/Austria) was carried out with Structure 2.2, using a linkage model (FALUSH *et al.* 2003) that uses genotype and linkage information for each marker to estimate admixture parameters. Admixture analysis can reveal information regarding patterns of reproductive isolation (RI) and mating in recent generations. For example, mating events involving F_1 's may be rare or absent (MILNE *et al.* 2003), or F_1 's may mate frequently with one or both parental species (MINDER *et al.* 2007). Alternatively, mating may happen preferentially among F_1 's or other early hybrid classes, potentially leading to partial or complete reproductive isolation between hybrids and their parents (RIESEBERG *et al.* 2003; JIGGINS *et al.* 2008). These aspects are of great interest in both speciation genetics and admixture mapping, and each scenario leaves a characteristic signature in admixture coefficients obtained by a linkage model.

Since 12 of the 80 microsatellites used at this step were scored as band present/absent (Table S1; dominant markers), we used the Structure module of FALUSH *et al.* (2007) that is able to resolve incomplete or ambiguous marker information. The analysis was carried out for a $K = 2$ gene pool model based on previous results for this hybrid zone (LEXER *et al.* 2005; 2007) using a burn-in of 50,000 and 100,000 subsequent iterations, assuming unphased genotypic data. Then, the site-by-site output of Structure 2.2 was used to estimate the probabilities of *interspecific heterozygosity* or *homozygosity* for each locus in each individual. With unphased data, Structure estimates two probabilities informative regarding heterozygosity (ss2 and ss3; assignment probabilities of *each* allele at an unphased locus in a diploid individual), and two probabilities informative regarding homozygosity (ss1 and ss4; probabilities of assignment of *both* alleles of a locus to one or the other parental species). The following linear combinations of these probabilities were used to plot and explore hetero- and homozygosity in the Danube hybrid zone: (ss2 + ss3) – (ss1 + ss4), which quantifies evidence for interspecific heterozygosity for each locus and individual, with values ranging from -1 to $+1$; and ss1-ss4, which summarizes the evidence for interspecific homozygosity for alleles from *P. tremula* (-1) vs. *P. alba* ($+1$).

Effect of null alleles: Allele nonamplification is a potential source of error when interpreting homo- and heterozygosity of PCR-based markers. Commonly used methods for estimating null allele frequencies are not easily applicable to hybrid

zones. Thus, we assessed the role of null alleles by comparing interspecific heterozygosities from Structure to the actual marker heterozygosities in the raw data. Interspecific heterozygosity as examined by Structure refers to the ancestry of alleles in each individual, not to actual marker heterozygosities. For example, an individual may present a heterozygous marker genotype at a focal locus but may nevertheless be classified as an interspecific homozygote by Structure, because both alleles of that locus are derived from the same parental species. This scenario should be encountered fairly frequently for highly variable microsatellites. It can be used to rule out null alleles as the cause of interspecific homozygosity for the individuals in question.

Genome scan for interspecific divergence: Admixture analysis can only reveal the effects of very recent gene flow across hybrid zones because admixture proportions Q from the donor species will decrease by approximately one-half with each new backcross generation. Thus, gene flow further back in the past is better captured by estimating divergence between hybridizing populations. A genome scan for interspecific divergence was carried out for the two parental populations of *P. alba* and *P. tremula* sampled adjacent to the focal (Danube) hybrid zone, using all 93 marker loci (Table S1). Interspecific divergence was estimated using F_{ST} and HEDRICK'S (2005) G'_{ST} . Wright's F_{ST} offers the advantage that plenty of experience is available for it (including potentials and pitfalls; JOST 2008) and that it can be estimated for different kinds of markers within an analysis-of-variance framework (WEIR and COCKERHAM 1984). HEDRICK'S (2005) G'_{ST} , on the other hand, is useful because it takes within-population heterozygosity into account. Thus, a comparative analysis of F_{ST} and G'_{ST} for the same set of loci should be informative regarding the amount of interspecific differentiation at each locus considering its within-population diversity.

To complete the interspecific divergence analysis, a standard neutrality test was carried out that compares F_{ST} to neutral coalescent simulations, using the frequentist approach of BEAUMONT and NICHOLS (1996), also discussed by BEAUMONT and BALDING (2004). This test is often used to detect candidate loci for divergent selection, *i.e.*, loci that are more divergent between two populations or species than expected under neutrality (BEAUMONT 2005). Many aspects of population demography can affect the outcome of this type of test, *e.g.*, hierarchical population structure (EXCOFFIER *et al.* 2009a), thus highly divergent "outlier" loci from F_{ST} -based tests need not necessarily indicate the action of selection (FOLL and GAGGIOTTI 2008; EXCOFFIER *et al.* 2009a). Nevertheless, the test allowed us to compare the pattern of divergence between our study species to published work on other hybridizing taxa. It was carried out with two rounds of simulations, one to obtain "neutral" F_{ST} and a second round to identify outliers. This was done for two datasets, one including all 93 loci genotyped for the Danube hybrid zone, including microsatellites, indels, and sequence polymorphisms and the other one comprising microsatellites only. Since the distributions were very similar in shape, only the results of the full analysis are discussed below.

Heterozygosity vs. hybrid index in observed and simulated data: Our interest in speciation genetics and admixture mapping led us to explore patterns of reproductive isolation (RI) and mating in the Danube hybrid zone more deeply. This was achieved by examining interspecific heterozygosity vs. genomic admixture for each individual, using the 68 fully codominant microsatellites typed for the Danube hybrid zone (Table S3). We were also interested in checking the generality of our results, and thus we analyzed two additional replicate hybrid zones, Ticino (Italy) and Tisza (Hungary), for 18 widely spaced,

fully codominant microsatellites (Table S3). Our rationale was as follows: if patterns of RI and mating are primarily determined by ecological differences among localities or by local history (*e.g.*, human influence), then we may expect genotypic patterns to vary across localities. Conversely, if RI and mating are determined primarily by intrinsic organismal features shared by all populations, then we would expect genotypic patterns to be similar across localities.

For all three zones, microsatellite alleles were first combined into two allelic classes (converted into biallelic markers) with frequency differentials between species equal to the observed differential based on all alleles, following GOMPERT and BUERKLE (2009). These biallelic data were then used to estimate interclass heterozygosity, which corresponds to a measure of interspecific heterozygosity, similar to but simpler than that obtained by Structure (above). This was plotted against genomic admixture estimated by a maximum likelihood hybrid index, all calculations carried out with the R package Introgress (GOMPERT and BUERKLE 2010). This two-dimensional representation of genomic composition for each individual (heterozygosity *vs.* hybrid index) was then compared to simulated data for parental genotypes and F_1 's. Our rationale was to check whether observed, genetically intermediate hybrid genotypes in nature were more compatible with the simulated F_1 's (maximum heterozygosity with intermediate hybrid index) or with recombinant hybrids of subsequent generations (reduced interspecific heterozygosity compared to simulated F_1 's). In simulations, 500 individuals were sampled from the allele frequency distributions for each locus for each genotypic class (parentals and F_1 's). Note that this analysis follows similar principles to the method by ANDERSON and THOMPSON (2002) implemented in their NewHybrids software. Compared to their approach, ours makes fewer assumptions, because we do not attempt to assign hybrids to artificial categories such as F_1 , F_2 , backcross (BC), or "parental-like."

Tests for epistatic interactions: The presence of genetic discontinuities between recombinant hybrids and their parental species (steep clines despite fertile F_1 's) can be explained by several competing hypotheses, some of which can be addressed by available data for these species. A plausible hypothesis for which no data are currently available involves epistasis, *i.e.*, negative genomic interactions among loci (GAVRILETS 1997). Thus we used a recently developed method (TEETER *et al.* 2010) to test for epistatic interactions in the Danube hybrid zone, the focal population with the best genomic coverage. To keep the number of pairwise tests manageable, the analysis was restricted to those 29 fully codominant microsatellites with the greatest differentiation between the parental species ($\delta \geq 0.8$).

In a first step, single locus genomic clines of genotype frequencies at each locus were fitted against genomic admixture gradients (*i.e.*, against the genetic background) using multinomial logistic regression, as in LEXER *et al.* (2007) but using the methodological improvements by GOMPERT and BUERKLE (2009). Comparisons of observed clines against simulated neutral expectations allow the detection of *under-* or *overrepresentation* of interspecific homo- and heterozygotes, respectively (GOMPERT and BUERKLE 2009). However, those were not at the center of attention here. Instead, two-locus interactions were explored by adding a second *predictor locus* to each regression model, and the two models for each focal locus (with or without the predictor) were compared using the Aikake information criterion ($AIC = -2L + 2p$, where L is the log-likelihood of the model and p is the number of parameters in the model). Since smaller AICs represent higher likelihoods and better fits, ΔAIC was calculated as $AIC_{\text{basic model}} - AIC_{\text{with predictor}}$, so that large positive values of

ΔAIC indicate loci for which the model fit is greatly improved by including the predictor locus, consistent with epistatic interactions. Since differences in AIC are used for interpretation of the results, rather than formal statistical tests of null hypotheses, there is no adjustment for multiple tests. The results can be interpreted on the basis of the magnitudes of ΔAIC for each pairwise locus combination. Particular pairwise interactions may be studied further in the future, on the basis of candidate gene information, the magnitude of ΔAIC , or other analyses.

Use of different types of genetic markers in statistical analyses: All 93 marker loci were used for estimating interspecific genomic divergence (F_{ST} and G_{ST}) between *P. alba* and *P. tremula* in the focal hybrid zone (Danube/Austria; Table S1), and a subset of 80 microsatellites was used for Bayesian admixture analysis in Structure 2.2 (Table S2). These 80 loci comprised all those for which information on genomic location was regarded adequate for analysis on the basis of a linkage model. The 80 loci included 12 microsatellites for which allele calling was difficult and that were thus scored conservatively via the presence/absence of a well-defined focal band. The 68 remaining, easily scorable and fully codominant microsatellites were used for analyzing heterozygosity *vs.* hybrid index and for epistasis tests in the Danube population. Out of these, 18 widely spaced, codominant markers were used to assess the generality of the results using the replicate localities Ticino (Italy) and Tisza (Hungary) (Table S3).

RESULTS

Genetic diversity in admixture zones: All three hybrid zones exhibited increased diversity compared to the parental species, measured in the form of expected (H_E) and observed (H_O) heterozygosity and allelic richness (Table 1). Inbreeding coefficients (F_{IS}) were significantly positive, consistent with previously observed Wahlund effects arising from wide local sampling of the parental reference populations (DE CARVALHO *et al.* 2010) and widespread departures from random mating due to assortative mating in hybrid zones (VAN LOO *et al.* 2008).

Patterns of genomic ancestry inferred by linkage-based Bayesian analysis: Bayesian-based admixture analysis based on 80 mapped microsatellites indicated an admixed ancestry for 31 (16%) individuals ($0.05 < Q < 0.95$) out of 192 sampled from the Danube hybrid zone of *P. alba* and *P. tremula*. Locus-by-locus analysis of these intermediate genotypes revealed "genomic mosaics" consisting of heterozygous loci and loci homozygous for alleles derived from one or another of the parental species (Figure 1, A and B), as expected for advanced recombinant hybrids derived from crosses of F_1 's. Pronounced gaps in the genotype distribution were uncovered between these recombinant hybrids and the two parental species (Figure 1, A and B; genotype data in File S1). The results are unlikely to be systematically biased by null alleles, as on average 44% of "interspecific homozygotes" classified by Structure were indeed true heterozygous marker genotypes in the raw data. Thus, classification as interspecific homozygotes at most loci was due to allelic ancestry rather than null alleles mimicking homozygosity.

TABLE 1
Interspecific hybrid zones of *P. alba* and *P. tremula* sampled in the present study

Locality	Country	Coordinates	Geography	Population	<i>N</i>	H_E	H_O	F_{IS}^a	<i>As</i>
Danube	Austria	48.26°N, 16.23°E	~90 km of river valley	Hybrid zone	192	0.596	0.489	0.201	8.581
			Riverine lowland forest	<i>P. alba</i>	48	0.508	0.436	0.114	6.770
			Eastern Alps	<i>P. tremula</i>	48	0.533	0.425	0.177	6.757
Ticino	Italy	45.28°N, 8.98°E	~95 km of river valley	Hybrid zone	138	0.590	0.517	0.129	6.694
			Riverine lowland forest	<i>P. alba</i>	40	0.428	0.355	0.169	4.609
			Alps/Lago Maggiore	<i>P. tremula</i>	40	0.479	0.351	0.197	5.021
Tisza	Hungary	48.32°N, 22.26°E	~85 km of river valley	Hybrid zone	106	0.610	0.470	0.227	7.331
			Riverine lowland forest	<i>P. alba</i>	40	0.462	0.401	0.151	5.074
			Zemplen mountains	<i>P. tremula</i>	40	0.547	0.450	0.167	5.568

Included are coordinates and additional geographic information, number of individuals sampled (*N*), and the following genetic diversity summary statistics estimated from 68 mapped, codominant microsatellites: expected (H_E) and observed heterozygosity (H_O), inbreeding coefficient F_{IS} , and allelic richness (*As*).

^aAll F_{IS} values significant at $P < 0.001$.

Genome scan for species divergence: Between-locus variation for genetic divergence (F_{ST}) between the parental populations of the Danube hybrid zone was great (0.369 ± 0.289 SD), and the same was true for G'_{ST} , which takes within-population variation into account (0.632 ± 0.354 SD) (Figure 2). At the 99% level, 33 loci (35%) were more divergent than expected under a neutral model following BEAUMONT and NICHOLS (1996). This number is not readily interpretable in terms of divergent natural selection maintaining the species barrier, but it facilitates comparisons to recent studies of other groups of hybridizing species. The large proportion of loci with low interspecific divergence (low F_{ST} or G'_{ST} ; lower left corner of Figure 2) indicates extensive allele sharing, which is most plausibly attributed to past gene flow between these ecologically divergent, parapatric species (discussed below).

Genomic composition of replicate hybrid zones: Analysis of interspecific heterozygosity *vs.* hybrid index in the focal (Danube/Austria) and two additional replicate hybrid zones of the same two species (Ticino/Italy and Tisza/Hungary) revealed a similar genomic composition of all three hybrid populations (Figure 3; genotype data in File S2 and File S3). In all three localities, most hybrids had intermediate hybrid index (horizontal axis) with interspecific heterozygosities (vertical axis) that fell below the 95% confidence intervals of simulated F_1 's (Figure 3). The increased variance in the observed and simulated data for the Ticino and Tisza populations stems from the lower number of loci genotyped for these localities ($N = 18$ loci) compared to the Danube ($N = 68$ codominantly scored loci in this analysis; see MATERIALS AND METHODS). The reduced interspecific heterozygosity in hybrids indicates their recombinant nature, beyond the F_1 generation, as also visible in the linkage-based Structure analysis for the Danube population (above; Figure 1) and from initial inspection of the raw data.

Conspicuous gaps in the genotype distributions between hybrids and parental species were present in all three hybrid zones (Figure 3), which was not expected from previous studies of these species and hybrids with smaller genomic coverage and less powerful analytical tools (discussed below). In each locality, many advanced generation introgressants had a hybrid index that was distinguishable from the simulated "pure" parental populations (hybrid index further away from 0.0 or 1.0 than expected of parental genotypes in the simulations). This is consistent with the locus-to-locus variation in marker ancestry seen in the Structure graph (Figure 1) and with the high proportion of weakly differentiated loci detected by F_{ST} and G'_{ST} (Figure 2).

Tests for epistatic interactions: Tests for epistatic interactions were informative regarding the possible origins of the genomic patterns seen in hybrid zones of *P. alba* and *P. tremula* (Figures 1 and 3). Among 29 loci with allele frequency differentials >0.8 in the Danube hybrid zone, a high proportion showed evidence for two-locus interactions (Figure 4). This is visible from the great number of two-locus comparisons for which an *epistasis model* for predicting the genotypes at a focal locus was strongly preferred over a simple *one-locus model* (see MATERIALS AND METHODS for the details to these models). The results indicate that epistasis needs to be considered explicitly when discussing the origin of genotypic discontinuities between these two ecologically divergent forest trees despite the presence of fertile F_1 's. In this study we were interested primarily in interactions between loci, not in individual genomic clines. Nevertheless, basic cline parameters for all loci are shown in Table 2 and Table S3.

DISCUSSION

Despite a longstanding interest of evolutionary geneticists in using hybrid zones as natural laboratories for studying the evolutionary process (BARTON and HEWITT

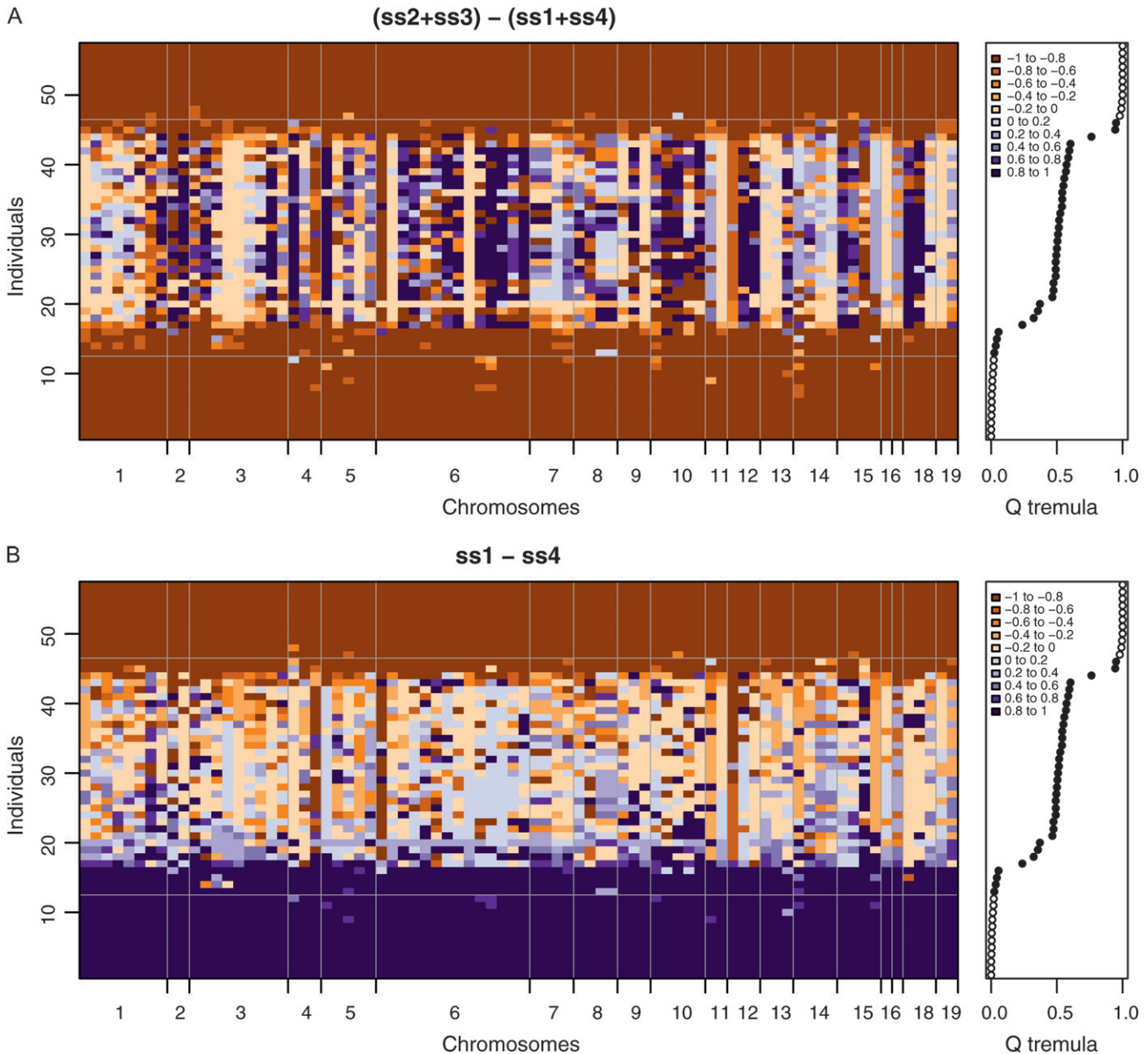


FIGURE 1.—Representative set of genetically intermediate and some parental individuals (along the vertical axis) from the Austrian Danube hybrid zone of *P. alba* and *P. tremula*, analyzed with 80 mapped microsatellites distributed across all 19 chromosomes of the *Populus* genome (horizontal axis). A linkage model (FALUSH *et al.* 2003) was used to infer probabilities of interspecific hetero- and homozygosity for each locus in each individual. (A) Evidence for *interspecific heterozygosity*, calculated from Structure site-by-site output as described in the text. High positive values for interspecific heterozygosity ($ss2 + ss3 > ss1 + ss4$) are indicated by blue, whereas brown colors indicate evidence for homozygosity for alleles from one or the other parental species ($ss2 + ss3 < ss1 + ss4$). (B) Evidence for *interspecific homozygosity*, calculated as described in the text, corresponding to the evidence ($ss1 - ss4$) that an individual is homozygous for alleles from *P. tremula* (brown color, $ss4$) vs. *P. alba* (blue color, $ss1$) at each locus. The recombinant, mosaic-like nature of genetically intermediate individuals is clearly visible, as are some introgressed individuals of *P. alba*. Overall admixture proportions (Q) of each tree are shown to the right. See text and Table S1, Table S2, and Table S3 for details.

1985; BARTON and GALE 1993; RIESEBERG *et al.* 1999; MARTINSEN *et al.* 2001; WU 2001; VINES *et al.* 2003; COYNE and ORR 2004; PAYSEUR *et al.* 2004; MINDER *et al.* 2007; BUERKLE and LEXER 2008), there is little experience in the interpretation of genomic patterns of admixture and ancestry in terms of reproductive isolation and speciation (but see PAYSEUR *et al.* 2004; LEXER *et al.*

2007; NOLTE *et al.* 2009; TEETER *et al.* 2010). The results of our study allow us to address this topic and to comment on the utility of gene mapping in hybrid zones of wild species in animals and plants.

Genomic patterns of ancestry and admixture: Analysis of a genome-wide panel of mapped markers (Figures 1–4) yields a different picture of patterns of

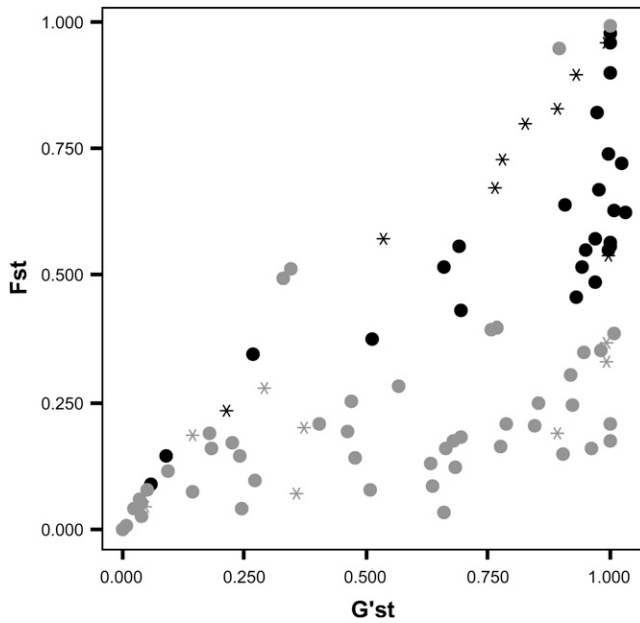


FIGURE 2.—Results of a genomic scan for interspecific divergence between *P. alba* and *P. tremula* for the Austrian Danube hybrid zone, based on 93 DNA-based genetic markers. HEDRICK'S (2005) G'_{ST} is shown on the x -axis and F_{ST} on the y -axis. Marker loci with greater-than-expected F_{ST} (99% level) relative to neutral expectations are solid; all other markers are shaded. Circles indicate markers derived from genomic DNA sequence (genomic libraries or *P. trichocarpa* shotgun genome sequence), stars stand for markers sourced from expressed sequence tags (ESTs). Marker loci with maximum interspecific divergence for their heterozygosity are visible along the diagonal.

reproductive isolation and mating in hybrid zones of *P. alba* and *P. tremula* compared to previous work based on much smaller numbers of codominant markers and less powerful analytical tools (RAJORA and DANCİK 1992; FOSSATI *et al.* 2004; LEXER *et al.* 2005). Early phenotype- and isozyme-based studies suggested that hybrid zones between these species consisted primarily of backcrosses to one parental species, namely *P. alba* (BARTHA 1991; RAJORA and DANCİK 1992). Previous DNA-based studies (LEXER *et al.* 2005, 2007) did not allow rejection of this hypothesis, most likely due to the large error associated with individual-based analysis of a small number of markers. Here, linkage-, F_{ST} -, and genomic cline-based analyses of a genome-wide panel of mapped markers reveal the presence and positioning (in genotypic space) of unexpectedly strong reproductive barriers between these species and hybrids and suggest hypotheses on their origin and future fate.

The Bayesian analysis of genotypic ancestry (using Structure) indicates that early generation hybrids detected in the Danube hybrid zone of *P. alba* and *P. tremula* (Table 1) are genetically intermediate, recombinant hybrid genotypes (a genomic mosaic, VIA and WEST 2008) separated from their parental species by sharp genomic discontinuities (Figure 1), equivalent to steep

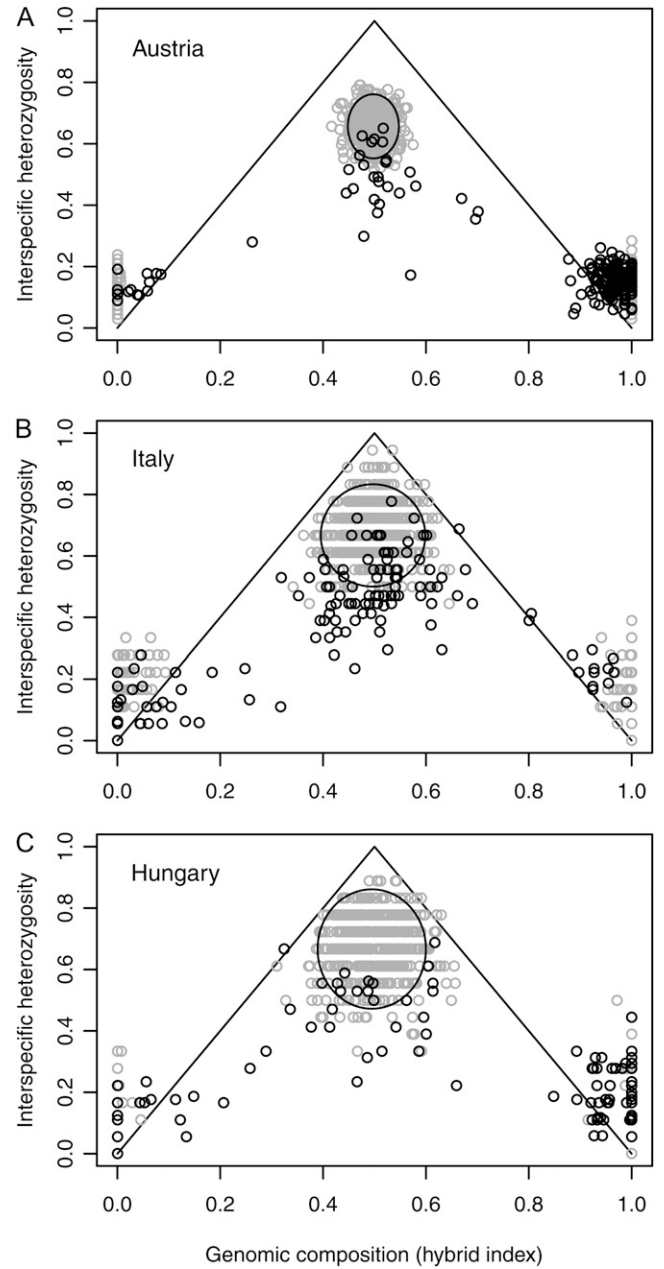


FIGURE 3.—Interspecific heterozygosity (vertical axis) *vs.* hybrid index (horizontal axis) in three interspecific hybrid zones of *P. alba* and *P. tremula*: Danube (Austria), Ticino (Italy), and Tisza (Hungary). Solid circles indicate observed values for individual genotypes sampled in each population; shaded circles indicate values for simulated F_1 's (center of each graph) and parents (left and right end of each graph). Univariate 95% confidence intervals for simulated F_1 's (ellipses) allow detection of observed hybrid genotypes with reduced interspecific heterozygosity (individuals below the ellipses), indicative of recombinant hybrid generations. The graphs are based on 68 codominant microsatellites in the case of the Danube hybrid zone (Austria) and 18 codominant loci for the Ticino (Italy) and Tisza (Hungary) hybrid zones, respectively.

genomic clines *sensu* GOMPERT and BUERKLE (2009) and similar to steep clines seen in simulations of hybrid zones with strong postzygotic barriers (GAVRILETS 1997). Note that most trees from the Danube hybrid

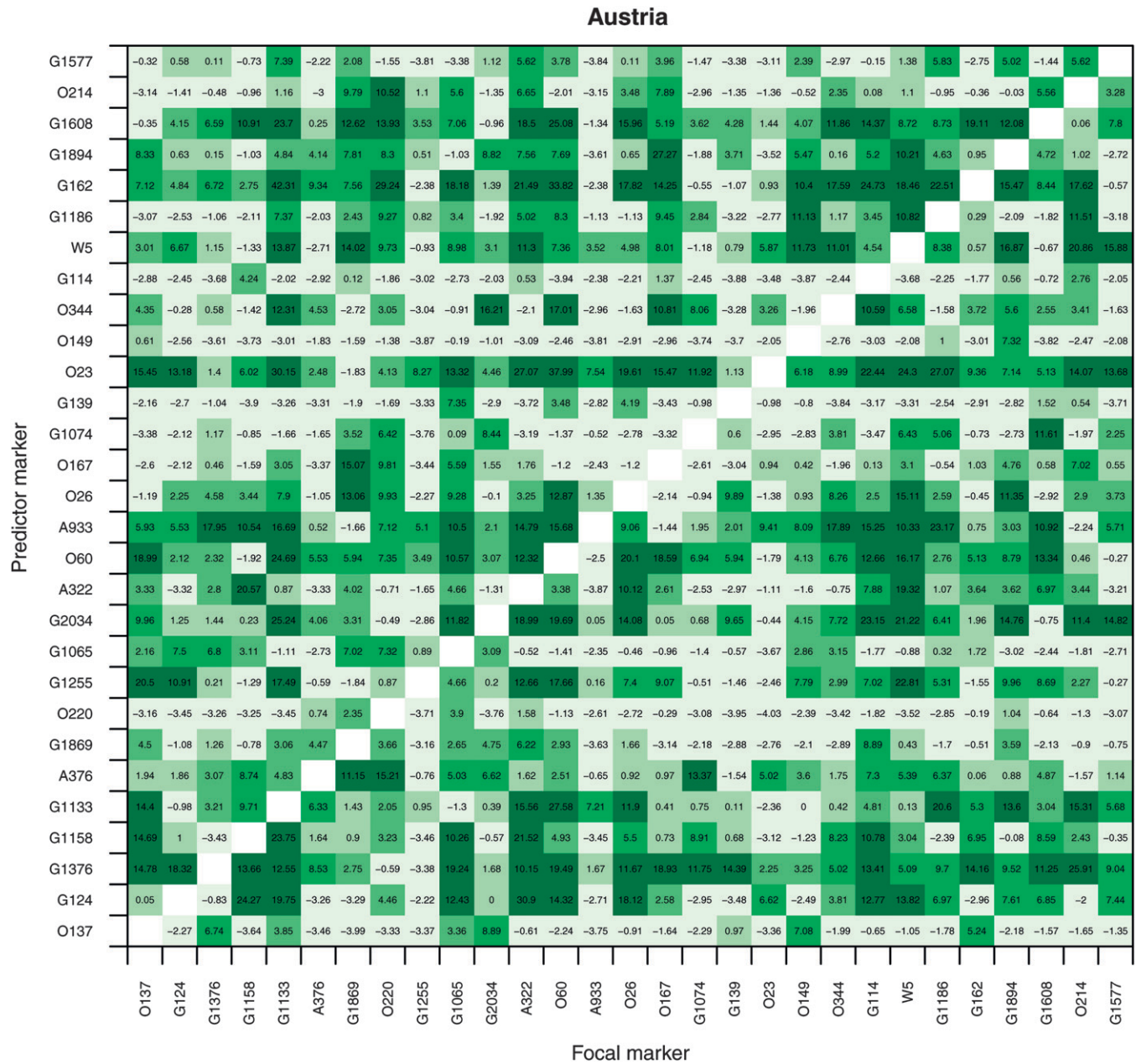


FIGURE 4.—Results of tests for epistatic interactions between pairs of loci in the Danube hybrid zone, carried out for those 29 loci with the greatest interspecific divergence (allele frequency differential $\delta \geq 0.8$). Shown are differences between AIC values (ΔAIC) for logistic regression models that include *vs.* models that exclude a second predictor locus to predict genotypes at a focal locus (i.e., epistasis *vs.* single-locus models). Large values of ΔAIC indicate that the model fit is substantially greater for the epistasis model. The cells with the darkest green indicate a positive AIC difference of 10–50 (natural logarithmic scale) for the epistasis model relative to the one-locus model and the next shade of green an AIC difference of 5–10, and then 2–5, and then 0–2. See text for details of regression models.

zone were *P. alba*-like (very low admixture coefficients Q ; see genotypic data in [File S1](#)); only genetically intermediate individuals are shown in Figure 1, ordered by their Q_s . Blocks of LD along the chromosomes are clearly visible in these genetically intermediate hybrids on the basis of the similarity of colors (probability of homo- or heterozygosity) along the horizontal axes (Figure 1, A and B). Also, occasional introgression of particular genome segments or loci across the barrier in either direction can be inferred from Figure 1. These

observations are mirrored by characteristic patterns of genomic differentiation visible in our F_{ST} - and G'_{ST} -based genome scan (Figure 2).

The genomic scan for pairwise species differentiation (Figure 2) is informative regarding gene flow in the more distant past—on the order of dozens or hundreds of generations (WHITLOCK 1992). Our pairwise genome scan reveals many loci with weak interspecific differentiation (F_{ST} or G'_{ST} ; Figure 2), despite the fact that the proportion of highly differentiated loci (F_{ST} greater

TABLE 2

Interspecific genetic divergence and results of genomic clines for 68 mapped codominant microsatellites studied in the Austrian Danube hybrid zone of *P. alba* and *P. tremula*

Locus	Chrom	F_{ST}	G'_{ST}	Delta	lnL ratio	P	Selection ^a		
G1719	1	0.182	0.694	0.625	7.486	0.004	TT: +	TA:	AA: -
A302	1	0.070	0.358	0.448	1.237	0.648	TT:	TA:	AA:
P2852	1	0.042	0.245	0.490	2.105	0.341	TT:	TA:	AA:
O30_2	1	0.085	0.636	0.677	26.601	0.000	TT: +	TA:	AA: -
G1568	1	0.033	0.660	0.740	46.820	0.000	TT: +	TA:	AA: -
O137 ^b	1	0.488	0.968	0.969	20.551	0.000	TT:	TA: -	AA: +
G124 ^b	1	0.350	0.947	0.885	6.276	0.015	TT:	TA:	AA:
G1376 ^b	2	0.669	0.979	0.906	4.488	0.049	TT: +	TA: -	AA:
A376 ^b	2	0.366	0.994	0.948	6.696	0.008	TT:	TA:	AA:
G1158 ^b	2	0.897	1.001	0.979	10.200	0.000	TT:	TA:	AA:
G1133 ^b	3	0.306	0.920	0.802	49.745	0.000	TT: +	TA: -	AA:
G1629	3	0.124	0.681	0.729	7.098	0.007	TT: +	TA:	AA:
O30_1	3	0.189	0.180	0.292	3.757	0.060	TT:	TA:	AA:
G1416	3	0.000	0.000	0.083	15.482	0.000	TT:	TA:	AA: +
O203	3	0.173	0.225	0.406	6.808	0.009	TT:	TA:	AA:
G1869 ^b	3	0.174	1.000	0.979	5.361	0.031	TT:	TA:	AA:
G1688	3	0.394	0.758	0.698	5.857	0.013	TT: -	TA:	AA: -
O220 ^b	4	0.740	0.996	1.000	8.684	0.001	TT: -	TA: +	AA:
O127	4	0.375	0.513	0.594	18.490	0.000	TT: +	TA: -	AA:
G1809	4	0.431	0.695	0.677	13.221	0.000	TT: +	TA:	AA:
G1255 ^b	5	0.990	1.000	1.000	11.093	0.001	TT:	TA: +	AA:
G1192	5	0.146	0.243	0.458	5.418	0.018	TT:	TA:	AA:
G1838	5	0.208	0.788	0.781	12.455	0.000	TT: -	TA: +	AA: -
G20	5	0.558	0.691	0.615	8.600	0.001	TT: +	TA:	AA: -
W15	5	0.141	0.479	0.542	5.944	0.010	TT: -	TA:	AA:
G1065 ^b	6	0.628	1.006	0.938	31.029	0.000	TT: +	TA: -	AA:
O369	6	0.026	0.039	0.219	9.428	0.000	TT:	TA: +	AA: -
G2034 ^b	6	0.388	1.007	0.958	6.721	0.012	TT: -	TA:	AA:
A322 ^b	6	0.188	0.892	0.813	10.775	0.000	TT:	TA:	AA: +
O60 ^b	6	0.397	0.767	0.813	59.904	0.000	TT: -	TA:	AA: +
W12	6	0.163	0.775	0.719	16.142	0.000	TT: +	TA: -	AA: +
O190	6	0.638	0.908	0.792	4.721	0.025	TT: -	TA:	AA: +
A933 ^b	6	0.538	0.995	0.969	8.354	0.001	TT: -	TA:	AA:
G1485	6	0.061	0.036	0.083	27.080	0.000	TT: +	TA: -	AA:
O26 ^b	6	0.548	0.950	0.875	13.139	0.000	TT: +	TA: -	AA:
O167 ^b	6	0.959	1.000	0.990	13.543	0.002	TT:	TA: +	AA: -
G1831	6	0.247	0.853	0.667	1.524	0.462	TT:	TA:	AA:
G1074 ^b	6	0.978	0.999	0.938	7.469	0.004	TT: -	TA:	AA:
G139 ^b	6	0.624	1.033	1.000	0.248	0.940	TT:	TA:	AA:
G1260	7	0.208	0.404	0.469	9.752	0.000	TT: +	TA:	AA: -
W17	7	0.194	0.461	0.521	15.861	0.000	TT: +	TA:	AA: -
O312	7	0.159	0.663	0.677	2.663	0.231	TT:	TA:	AA:
G2062	8	0.077	0.511	0.573	7.916	0.002	TT: -	TA: +	AA:
O374	8	0.160	0.961	0.760	24.973	0.000	TT: +	TA:	AA: -
O202	8	0.283	0.567	0.594	1.524	0.487	TT:	TA:	AA:
O268	8	0.254	0.468	0.531	3.749	0.092	TT:	TA:	AA:
G1949	9	0.096	0.271	0.479	7.082	0.010	TT:	TA:	AA:
O23 ^b	9	0.209	1.001	1.000	12.457	0.000	TT: -	TA:	AA: +
O21	9	0.090	0.058	0.125	8.391	0.001	TT:	TA:	AA:
G2020	10	0.205	0.844	0.740	6.384	0.014	TT:	TA: +	AA:
O149 ^b	10	0.548	0.996	0.958	11.453	0.000	TT: +	TA:	AA: -
O344 ^b	10	0.721	1.022	1.000	5.124	0.039	TT:	TA: +	AA: -
G1574	10	0.458	0.930	0.792	26.168	0.000	TT: +	TA: -	AA:
G114 ^b	10	0.572	0.970	0.885	2.521	0.290	TT:	TA:	AA:
G1037	11	0.000	0.000	0.073	10.924	0.000	TT:	TA: +	AA: -
W05 ^b	12	0.244	0.923	0.917	6.368	0.015	TT:	TA:	AA:
G1186 ^b	12	0.353	0.980	0.865	3.504	0.132	TT:	TA: -	AA: +

(continued)

TABLE 2
(Continued)

Locus	Chrom	F_{ST}	G'_{ST}	Delta	lnL ratio	P	Selection ^a		
G1353	13	0.076	0.145	0.271	9.181	0.001	TT:	TA:	AA:
G162 ^b	13	0.148	0.902	0.823	0.629	0.741	TT:	TA:	AA:
G1812	14	0.176	0.679	0.656	5.965	0.010	TT:	TA: –	AA: +
G1894 ^b	15	0.516	0.941	0.906	3.770	0.101	TT:	TA:	AA:
G1454	15	0.131	0.632	0.708	7.272	0.008	TT:	TA: –	AA: +
G1608 ^b	15	0.558	1.000	0.896	46.011	0.000	TT:	TA: –	AA: +
O14	16	0.041	0.023	0.052	6.242	0.001	TT: +	TA: +	AA: –
O214 ^b	18	0.819	0.974	0.927	13.741	0.000	TT:	TA: +	AA: –
G1577 ^b	18	0.564	1.000	0.979	4.121	0.086	TT:	TA:	AA:
O28	18	0.161	0.183	0.333	3.382	0.119	TT:	TA:	AA:
O206	19	0.517	0.659	0.635	2.032	0.341	TT:	TA:	AA:

Included are locus name, assignment to chromosomes, WEIR and COCKERHAM'S (1984) F_{ST} , HEDRICK'S (2005) G'_{ST} , allele frequency differential delta and the following genomic cline parameters: ln likelihood, probability for departure from neutrality, and indications for significant over or underrepresentation (+ or –) of individual genotypic classes.

^aAA, interclass homozygotes for *P. alba*; AT, interclass heterozygotes for *P. alba* and *P. tremula*; TT, interclass homozygotes for *P. tremula*.

^bMarkers included in tests for epistasis.

than expected in neutral coalescent simulations) was much greater in our study (35%) than in studies of a different pair of outcrossing forest trees (12% in *Quercus*; SCOTT-SAINTAGNE *et al.* 2004) and a pair of self-incompatible annual plants (5% in *Helianthus*; YATABE *et al.* 2007). The numerous weakly differentiated loci in our genome scan (Figure 2; lower portion of graph) indicate occasional but persistent introgression over long time scales, despite strong barriers to gene exchange. This makes sense: we know that even strong barriers to gene flow cannot prevent the introgression of neutral or advantageous alleles (PIALEK and BARTON 1997; FAURE *et al.* 2008), and emerging phylogeographic data for these species suggest repeated contact upon colonization from divergent lineages (FUSSI *et al.* 2010).

Ancestral polymorphism is an unlikely explanation for allele sharing at the weakly differentiated loci seen in Figure 2. These species diverged at least several million years ago (STETTLER *et al.* 1996), and *P. alba* and *P. tremula* are not even sister species in most molecular phylogenetic analyses of the genus (HAMZEH and DAYANANDAN 2004). Accordingly, allele frequency differentials between these taxa often reach values of 0.90 or higher (Table 2; Table S3). Also, intermediate genotypes in hybrid zones often carry blocks of neighboring markers from one or the other parental species (Figure 1), which is consistent with interspecific recombination but not ancestral polymorphism.

We did not wish to interpret the highly differentiated outlier loci in our genome scan as being under divergent selection (see FOLL and GAGGIOTTI 2008 and EXCOFFIER *et al.* 2009a for a discussion of these issues). Rather, our primary interest was in comparing the overall distribution of interspecific genomic differentiation to expectations from comparable pairs of sympatric, hybridizing taxa. Nevertheless, it is noteworthy that

most EST-derived markers used in our study are on the diagonal in our F_{ST}/G'_{ST} plot (Figure 2), in contrast to numerous other markers developed from the *Populus* genome sequence or genomic libraries (Figure 2; Table S1). This indicates that the EST-derived polymorphisms have attained maximum differentiation for their within-species heterozygosity (HEDRICK 2005), which is consistent with their placement outside the 99% confidence interval of the neutral distribution of F_{ST} .

Repeatability of genomic patterns and mechanisms of reproductive isolation: Genomic discontinuities in hybrid zones of the type seen in Figure 1 are highly informative regarding the genetics of species boundaries and can be explained by several competing hypotheses: (1) assortative mating, an important prezygotic barrier in both animals and plants (COYNE and ORR 2004), easily mediated by differences in flowering time in the case of plants; (2) strong postzygotic barriers affecting hybrid generations beyond the F_1 , *i.e.*, nuclear genetic incompatibilities caused by Dobzhansky–Bateson–Muller (DBM)-type epistatic selection (GAVRILETS 1997); and (3) localized factors affecting only particular populations, *e.g.*, natural or human-mediated disturbance in a particular locality may have triggered a recent “wave” of hybridization, mimicking a steep cline. Our data allow us to address each of these hypotheses. Additional alternative hypotheses include postmating barriers involving uniparentally inherited genetic factors, but these will result in strongly asymmetric intrinsic barriers (TURELLI and MOYLE 2007) for which there is no evidence in these species to date (LEXER *et al.* 2005).

To rule out recent local disturbance (hypothesis 3), we studied two replicate hybrid zones (Tisza and Ticino; Table 1) for 18 widely spaced codominant microsatellites. Our comparative analysis of interspecific hetero-

zygosity *vs.* hybrid genomic composition in these populations (Figure 3) indicates similar patterns of reproductive isolation in all three localities: intermediate hybrids have reduced heterozygosity compared to simulated F_1 's, in agreement with their recombinant nature, backcrosses are infrequent (although possible in both directions), and many parental-like genotypes exhibit hybrid indices (horizontal axes) that suggest low levels of introgression in the distant past. These observations are consistent with those from Structure-based ancestry analysis (Figure 1) and the F_{ST} -based genome scan (Figure 2). More importantly, the repeatability of the results strongly contradicts the third hypothesis: these three river drainages have very different post-glacial histories (timing of recolonization and onset of human disturbance); thus it is unlikely that recent events would have affected all three of them in identical ways.

So, are genomic discontinuities in hybrid zones of these hybridizing forest trees caused by prezygotic isolation (assortative mating) or by postzygotic barriers? The two parental species differ in their peak timing of flowering by up to several weeks (FISCHER *et al.* 2005) and appreciable genetic variation for phenological traits exists even at the within-species level (HALL *et al.* 2007). Thus assortative mating due to phenological differences is plausible. A study of fine-scale spatial genetic structure (SGS) of the Danube hybrid zone is consistent with this hypothesis: SGS was much stronger in hybrids compared to parental-like genotypes, even after correcting for differences in the propensity of hybrids and parentals to form clones (VAN LOO *et al.* 2008). Also, hybrids tended to form larger clones (more ramets extending over larger areas) than the parental species, which can easily lead to preferential mating between adjacent hybrid genotypes with similar phenology, *i.e.*, to discontinuities in spatial and temporal patterns of flowering. A molecular marker-based study of progeny arrays from natural hybrids is currently underway to test the amount of assortative mating in these populations.

With respect to postzygotic barriers, our logistic regression-based analysis of the Danube hybrid zone indicates that epistasis is widespread (Figure 4). This is consistent with theoretical predictions under DBM-type incompatibilities among nuclear genes: strong epistatic selection will keep fit recombinants at low frequency, effectively maintaining the barrier and leading to steep clines despite the presence of fertile F_1 's (GAVRILETS 1997), which would result in genotypic patterns such as those seen in Figures 1 and 3. It is thus likely that genomic patterns of admixture in hybrid zones of *P. alba* and *P. tremula*—in particular the conspicuous genomic discontinuities between hybrids and their parental species—are due to both assortative mating and DBM incompatibilities. Note that the presence of isolated adaptive peaks (*e.g.*, strong ecological divergence between hybridizing species) may affect the dynamics and

outcome of epistatic selection, compared to hybrid zones maintained by a simple balance of selection and migration (GAVRILETS 1997).

Potential evolutionary trajectories of hybrid zones: Several possible trajectories have been proposed for the evolution of hybrid populations between diploid taxa, including swamping of the species with the smaller effective population size (N_e) by gene flow from the more abundant species (WOLF *et al.* 2001), massive introgression from the earlier colonizing (“native”) into the later colonizing species (EXCOFFIER *et al.* 2009b), homoploid hybrid speciation (RIESEBERG *et al.* 2003), and the transfer of adaptive traits across “porous” species boundaries (KIM and RIESEBERG 1999; BARTON 2001; MARTIN *et al.* 2006; WHITNEY *et al.* 2006). The first two of these (genetic swamping and massive introgression of genes from the local species) appear unlikely here: *P. alba* is the species with the smaller effective population size N_e (LEXER *et al.* 2005) and phylogeographic data indicate that it recolonized Central Europe after *P. tremula* (FUSSI *et al.* 2010); yet integrity of its gene pool appears to be protected by surprisingly strong reproductive barriers (Figure 1; Figure 3; Figure 4) and possibly by sufficient gene flow at the within-species level ($N_e m > 3.0$; LEXER *et al.* 2005); intraspecific gene flow in the later colonizer is expected to prevent or slow down introgression and “surfing” of genes originating from the local species (EXCOFFIER *et al.* 2009b).

The third trajectory, hybrid speciation, appears already more likely, since our data suggest the build-up of partial RI between intermediate hybrids and their parents (Figure 1; Figure 3; LEXER *et al.* 2009). The great ecological divergence between the hybridizing species (flood plain *vs.* upland habitats) is also in line with expectations for conditions prevailing at the onset of hybrid speciation (BUERKLE *et al.* 2000; NOLTE and TAUTZ 2010). Nevertheless, opportunities for interspecific gene flow still exist (Figures 1 and 3), and interspecific heterozygosities in hybrids are higher than expected, based on models of recombinational speciation (BUERKLE *et al.* 2000); note that drift in isolated hybrid lineages tends to reduce interspecific heterozygosity. Hybrid speciation in European *Populus* spp. would require range shifts leading to stronger spatial separation of *P. × canescens* hybrids from both parental species or the presence of multiple genes with strong positive effects on hybrid fitness (“overdominance”; BUERKLE *et al.* 2000). The latter pattern was found for 11 out of 68 loci (16%) in our regression-based genomic cline analysis of the Danube population, compared to 19% of loci with indications of underdominance (Table 2; underrepresentation of heterozygotes). On the basis of the data, *P. × canescens* may be on a trajectory to hybrid speciation, but genetic data on the depth of coalescences of gene sequences are required to test this further.

We regard the fourth scenario, adaptive introgression, as the most likely evolutionary consequence of

hybridization between these species: our Structure-based ancestry analysis (Figure 1), genome scan of divergence between parental species (Figure 2), and two-dimensional analysis of heterozygosity *vs.* hybrid genomic composition (Figure 3) all indicate low but persistent (or recurrent) introgression into both parental species, consistent with the notion of the “porous genome” in speciation genetics (WU 2001; SCOTT-SAINTAGNE *et al.* 2004; MINDER *et al.* 2007). This suggests that the transfer of traits across porous species boundaries (KIM and RIESEBERG 1999, MARTIN *et al.* 2006; WHITNEY *et al.* 2006) is possible in these ecologically important forest trees, and this is corroborated by the results of recent morphometric analyses of many phenotypic characters in these species and hybrids (LEXER *et al.* 2009). A combination of common garden experiments and genetic mapping are currently underway to test whether introgressed morphological and chemical traits are adaptive in the recipient species.

Admixture mapping in highly divergent populations:

Admixture mapping in hybrid zones has been suggested as a means of mapping loci involved in reproductive isolation and adaptive differentiation in the wild (RIESEBERG and BUERKLE 2002; BUERKLE and LEXER 2008). The methodological approach is analogous to the mapping of disease gene loci in admixed human populations (REICH *et al.* 2005; ZHU *et al.* 2005). Our data allow us to comment on the prospects for genetic mapping in hybrid zones stemming from admixture between highly divergent lineages.

First, it is clear from Figure 3 that coarse-scale mapping of isolating factors in highly divergent populations with strong reproductive barriers (as often encountered in evolutionary genetics) will require the screening of multiple localities for recombinant, early-generation hybrids and subsequent pooling of these individuals to form a QTL mapping population of sufficient size. The same principle was used in *Homo sapiens* (REICH *et al.* 2005; ZHU *et al.* 2005), so this should not be a major obstacle in organisms with weak or absent population structure such as highly outcrossing, wind-pollinated forest trees. The linear models used for gene mapping in admixed populations are readily extended to accommodate the use of multiple populations and environments (BUERKLE and LEXER 2008). Sample sizes of several hundred individuals will be required for admixture mapping in early recombinant hybrid generations, as in QTL mapping experiments with early generation intercrosses.

Perhaps of even greater relevance is the potential created by ultra-high throughput genotyping and sequencing: the unstoppable movement of neutral or advantageous alleles across porous genomes (this study; BARTON 2001; MARTIN *et al.* 2006; YATABE *et al.* 2007; FAURE *et al.* 2008) indicates a great potential for the use of advanced introgressed populations (rather than hybrid zones with recent admixture) for high-resolution

mapping using next generation sequencing approaches (HOHENLOHE *et al.* 2010; TURNER *et al.* 2010). Divergent, introgressed alleles from related species should readily be identified in highly advanced introgressants using the genomic coverage afforded by these methods, and appropriate neutrality tests (NIELSEN 2005) facilitate the detection of fitness-related genes among those loci that do move across the barrier. This should allow the identification of functionally important heterospecific alleles in the process of spread through positive selection or neutral introgressed genes in the process of surfing on the colonization wave of rapidly expanding populations (EXCOFFIER *et al.* 2009b). The marker coverage required at this step will depend on how much time (in terms of generations) has passed since the onset of admixture, and population size requirements will depend primarily on the desired threshold frequency for the detection of introgressed variants.

Clearly, admixture mapping projects in wild species are confronted with challenges not seen in admixed human populations used for gene mapping, but they also bring novel opportunities to unravel the genetics of reproductive isolation and to identify fitness-related genes that function well in divergent genomic backgrounds. Much remains to be learned about the evolutionary consequences of the origin and breakdown of reproductive barriers from genomic studies of admixture in wild species of animals and plants.

We thank Hans Herz, Wilfried Nebenführ, Stefano Gomasasca, István Asztalos, and other colleagues for help during field work; Robyn Cowan and Ludwika Sygnarski for help in the lab; Zach Gompert for help with data analysis; and Loren Rieseberg, Roger Butlin, and Sergey Gavrilov for helpful discussions. C.L.’s research on the evolutionary genomics of species barriers in *Populus* is supported by grant no. NE/E016731/1 of the U.K. Natural Environment Research Council and grant no. 31003A_127059 of the Swiss National Science Foundation.

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Communicating editor: M. KIRST

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Genomic Admixture Analysis in European *Populus* spp. Reveals Unexpected Patterns of Reproductive Isolation and Mating

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DOI: 10.1534/genetics.110.118828

FILES S1-S3

Files S1-S3 are available for download as Excel files at <http://www.genetics.org/cgi/content/full/genetics.110.118828/DC1>.

File S1: Genotype data used for admixture analysis of the Danube hybrid zone of *Populus alba* and *P. tremula*, following structure 2.2 input format. Individual ID's starting with alb refer to *P. alba*, ID's starting with trem refer to *P. tremula*, ID's starting with can refer to *P. x canescens* hybrids. See text for marker details and structure 2.2 manual for specifics of the input file format.

File S2: Genotype data used for admixture analysis of the Ticino hybrid zone of *Populus alba* and *P. tremula*, following structure 2.2 input format. Individual ID's starting with alb refer to *P. alba*, ID's starting with trem refer to *P. tremula*, ID's starting with can refer to *P. x canescens* hybrids. See text for marker details and structure 2.2 manual for specifics of the input file format.

File S3: Genotype data used for admixture analysis of the Tisza hybrid zone of *Populus alba* and *P. tremula*, following structure 2.2 input format. Individual ID's starting with alb refer to *P. alba*, ID's starting with trem refer to *P. tremula*, ID's starting with can refer to *P. x canescens* hybrids. See text for marker details and structure 2.2 manual for specifics of the input file format.

TABLE S1

Essential information for all 93 genetic marker loci typed in this study, including locus name, assignment to chromosomes, marker type, marker source, information about marker scoring, gene diversity (H_E) in *P. alba* and *P. tremula*, interspecific F_{ST} and G'_{ST} and probability for greater-than-neutral F_{ST} .

Locus ^a	Chrom	Type ^b	Source ^c	Scoring ^d	H_E alba	H_E trem	F_{ST}	G'_{ST}	P
G1274	1	mic	genomic	dom	0.402	0.032	0.052	0.041	0.463
G1719	1	mic	genomic	codom	0.867	0.628	0.182	0.694	0.701
A302	1	mic	EST	codom	0.852	0.778	0.070	0.358	0.338
P2852	1	mic	genomic	codom	0.807	0.868	0.042	0.245	0.082
O30_2	1	mic	genomic	codom	0.937	0.801	0.085	0.636	0.353
G1568	1	mic	genomic	codom	0.932	0.969	0.033	0.660	0.012
O137	1	mic	genomic	codom	0.512	0.489	0.488	0.968	0.994*
G124	1	mic	genomic	codom	0.560	0.708	0.350	0.947	0.905
G1376	2	mic	genomic	codom	0.024	0.619	0.669	0.979	1.000*
G1158	2	mic	genomic	codom	0.042	0.165	0.897	1.001	1.000*
G1133	3	mic	genomic	codom	0.646	0.697	0.306	0.920	0.866
A376	3	mic	EST	codom	0.477	0.788	0.366	0.994	0.916
G1629	3	mic	genomic	codom	0.820	0.826	0.124	0.681	0.545
O30_1	3	mic	genomic	codom	0.082	0.449	0.189	0.180	0.816
G1887	3	mic	genomic	dom	0.175	0.000	0.143	0.092	1.000*
G1416	3	mic	genomic	codom	0.452	0.379	0.000	0.000	0.192
AQ	3	seq	EST	codom	0.000	0.079	0.957	0.993	1.000*
O203	3	mic	genomic	codom	0.202	0.616	0.173	0.225	0.780
G1869	3	mic	genomic	codom	0.816	0.836	0.174	1.000	0.703
G1688	3	mic	genomic	codom	0.726	0.296	0.394	0.758	0.979
O220	4	mic	genomic	codom	0.000	0.515	0.740	0.996	1.000*

GAD	4	indel	EST	dom	0.361	0.000	0.184	0.146	0.989
O127	4	mic	genomic	codom	0.064	0.695	0.375	0.513	0.994*
G1809	4	mic	genomic	codom	0.028	0.839	0.431	0.695	0.992*
DE	4	seq	EST	codom	0.103	0.191	0.571	0.537	1.000*
G1255	5	mic	genomic	codom	0.021	0.000	0.990	1.000	0.500
G1192	5	mic	genomic	codom	0.658	0.361	0.146	0.243	0.754
PER1	5	indel	EST	dom	0.000	0.411	0.671	0.766	1.000*
G1838	5	mic	genomic	codom	0.694	0.792	0.208	0.788	0.748
G20	5	mic	genomic	codom	0.565	0.000	0.558	0.691	1.000*
W15	5	mic	genomic	codom	0.799	0.656	0.141	0.479	0.619
G1065	6	mic	genomic	codom	0.000	0.749	0.628	1.006	1.000*
O369	6	mic	genomic	codom	0.446	0.545	0.026	0.039	0.463
G2034	6	mic	genomic	codom	0.922	0.307	0.388	1.007	0.913
A322	6	mic	EST	codom	0.779	0.805	0.188	0.892	0.719
O60	6	mic	genomic	codom	0.759	0.265	0.397	0.767	0.979
W12	6	mic	genomic	codom	0.767	0.823	0.163	0.775	0.659
O190	6	mic	genomic	codom	0.266	0.374	0.638	0.908	1.000*
A933	6	mic	EST	codom	0.387	0.534	0.538	0.995	1.000*
G1485	6	mic	genomic	codom	0.000	0.139	0.061	0.036	0.882
AS1	6	seq	EST	codom	0.000	0.229	0.829	0.891	1.000*
AS1MIC	6	mic	EST	codom	0.372	0.289	0.046	0.047	0.419
O26	6	mic	genomic	codom	0.863	0.000	0.548	0.950	1.000*
O167	6	mic	genomic	codom	0.061	0.021	0.959	1.000	1.000*
G1074	6	mic	genomic	codom	0.044	0.000	0.978	0.999	1.000*
G1831	6	mic	genomic	codom	0.696	0.737	0.247	0.853	0.811
G139	6	mic	genomic	codom	0.000	0.778	0.624	1.033	1.000*
G1260	7	mic	genomic	codom	0.614	0.494	0.208	0.404	0.727
W17	7	mic	genomic	codom	0.616	0.628	0.194	0.461	0.726
G1295	7	mic	genomic	dom	0.492	0.000	0.000	0.000	0.151

O312	7	mic	genomic	codom	0.782	0.756	0.159	0.663	0.670
G2062	8	mic	genomic	codom	0.862	0.847	0.077	0.511	0.331
PHYB	8	seq	EST	codom	0.113	0.352	0.234	0.213	1.000*
O374	8	mic	genomic	codom	0.778	0.889	0.160	0.961	0.663
O202	8	mic	genomic	codom	0.525	0.573	0.283	0.567	0.881
O268	8	mic	genomic	codom	0.650	0.401	0.254	0.468	0.906
THAU	9	mic	EST	codom	0.797	0.536	0.331	0.991	0.887
G1949	9	mic	genomic	codom	0.568	0.805	0.096	0.271	0.512
O23	9	mic	genomic	codom	0.858	0.724	0.209	1.001	0.762
O21	9	mic	genomic	codom	0.198	0.000	0.090	0.058	1.000*
G2020	10	mic	genomic	codom	0.857	0.666	0.205	0.844	0.770
O149	10	mic	genomic	codom	0.369	0.533	0.548	0.996	1.000*
O344	10	mic	genomic	codom	0.041	0.538	0.721	1.022	1.000*
G1574	10	mic	genomic	codom	0.250	0.781	0.458	0.930	0.991*
G114	10	mic	genomic	codom	0.087	0.745	0.572	0.970	1.000*
G1250	11	mic	genomic	dom	0.000	0.000	0.945	0.895	0.500
G1037	11	mic	genomic	codom	0.410	0.396	0.000	0.000	0.187
G154	12	mic	genomic	dom	0.000	0.000	0.495	0.329	0.500
W5	12	mic	genomic	codom	0.689	0.787	0.244	0.923	0.802
G1186	12	mic	genomic	codom	0.781	0.502	0.353	0.980	0.893
G1353	13	mic	genomic	codom	0.497	0.646	0.076	0.145	0.452
O16	13	mic	genomic	dom	0.408	0.082	0.009	0.008	0.210
PHYA	13	seq	EST	codom	0.029	0.110	0.895	0.931	1.000*
G162	13	mic	genomic	codom	0.821	0.854	0.148	0.902	0.664
ENDO	14	seq	EST	codom	0.045	0.172	0.798	0.825	1.000*
G1292	14	mic	genomic	dom	0.000	0.000	0.946	0.897	0.500
G1812	14	mic	genomic	codom	0.831	0.671	0.176	0.679	0.712
G2014	14	mic	genomic	dom	0.447	0.000	0.114	0.095	0.644
G1306	14	mic	genomic	dom	0.243	0.000	0.079	0.052	0.670

G1894	15	mic	genomic	codom	0.837	0.084	0.516	0.941	1.000*
G1454	15	mic	genomic	codom	0.820	0.781	0.131	0.632	0.569
G1608	15	mic	genomic	codom	0.669	0.215	0.558	1.000	1.000*
O430	15	mic	genomic	dom	0.000	0.000	0.946	0.897	0.500
O14	16	mic	genomic	codom	0.000	0.100	0.041	0.023	0.864
G1381	17	mic	genomic	dom	0.000	0.000	0.512	0.345	0.500
SOS2	18	mic	EST	codom	0.485	0.083	0.280	0.292	0.972
O214	18	mic	genomic	codom	0.194	0.142	0.819	0.974	1.000*
G1577	18	mic	genomic	codom	0.831	0.042	0.564	1.000	1.000*
O28	18	mic	genomic	codom	0.468	0.233	0.161	0.183	0.725
O276	19	mic	genomic	dom	0.000	0.254	0.346	0.270	1.000*
O206	19	mic	genomic	codom	0.062	0.553	0.517	0.659	1.000*
AP2	scaffold 28	seq	EST	codom	0.214	0.096	0.726	0.779	1.000*
PAL	scaffold 28	seq	EST	codom	0.259	0.816	0.201	0.371	0.765

^aMarkers starting with G refer to GCPM, markers starting with O refer to ORPM, markers with W refer to WPMS, markers with P refer to PMGC microsatellite loci available at <http://www.ornl.gov/sci/ipgc>. For all other markers see JOSEPH and LEXER (2008) and DE CARVALHO *et al.* (2010). See text for details.

^bMarker type: mic, microsatellite; seq: sequence polymorphism, indel: insertion-deletion polymorphism.

^cMarker source: genomic, genomic library or shotgun genome sequence; EST, expressed sequence tag.

^dMarker scoring: codom, codominant; dom, dominant (presence/absence information). Loci with greater-than-neutral F_{ST} at the 99% level are indicated by an asterisk.

TABLE S2

Eighty (80) microsatellite loci used for Bayesian admixture analysis with a linkage model, including marker name, chromosome assignment, and assumed chromosomal distance in centimorgan (cM).

Locus	Chrom	cM ^a	Locus	Chrom	cM ^b
G1274	1	-1	G139	6	10.41
G1719	1	42.5	G1260	7	-1
ASP302	1	6.76	W17	7	0
P2852	1	96.44	G1295	7	12.5
O30_2	1	26.9	O312	7	2.5
G1568	1	9.3	G2062	8	-1
O137	1	31.23	O374	8	5.4
G124	1	1.77	O202	8	73
G1376	2	-1	O268	8	0
G1158	2	144.86	G1949	9	-1
G1133	3	-1	O23	9	10.22
ASP376	3	21.92	O21	9	13.2
G1629	3	9.48	G2020	10	-1
O30_1	3	9.48	O149	10	54.2
G1887	3	9.48	O344	10	9
G1416	3	6.7	G1574	10	14.68
O203	3	1.7	G114	10	25.2
G1869	3	43.34	G1250	11	-1
G1688	3	7.7	G1037	11	31.56
O220	4	-1	G154	12	-1
O127	4	55.52	W05	12	12.22
G1809	4	18.7	G1186	12	46.48
G1255	5	-1	G1353	13	-1
G1192	5	22.2	O16	13	13.8
G1838	5	35.26	G162	13	178
G20	5	61.56	G1292	14	-1
W15	5	29.92	G1812	14	17.19
G1065	6	-1	G2014	14	25.73
O369	6	11	G1306	14	2.08
G2034	6	12.6	G1894	15	-1
ASP322	6	1.96	G1454	15	0.59
O60	6	4.06	G1608	15	36.33
W12	6	12.88	G430	15	36
O190	6	11.3	O14	16	-1
ASP933	6	2.11	G1381	17	-1
G1485	6	26.49	O214	18	-1
O26	6	14.9	G1577	18	5.2
O167	6	10.02	O28	18	18.9

G1074	6	3.48	O276	19	-1
G1831	6	2.2	O206	19	21.5

^aThe first marker sampled on each chromosome is indicated by -1. ^bApproximate chromosomal distances were obtained from *P. trichocarpa* genome assembly v.1 and genetic maps; for markers that mapped to more than one chromosome in *P. trichocarpa*, map positions were clarified by using segregation data from a controlled cross between *P. alba* and *P. tremula* (Macaya-Sanz, Gonzalez-Martinez, Alba, unpublished data).

TABLE S3

Genetic diversity statistics and results of genomic clines for mapped codominant microsatellites studied in three hybrid zones of *P. alba* and *P. tremula*, including number of alleles (A), expected (H_E) and observed (H_O) heterozygosity in each parental population, allele frequency differential (delta) and the following genomic cline parameters: ln likelihood, probability for departure from neutrality, and indications for significant over- or underrepresentation (+ or -) of individual genotypic classes. A: Danube (Austria), 68 loci; B: Ticino (Italy), 18 loci; C: Tisza (Hungary), 18 loci.

A) Danube/Austria

Locus	Ch	A alba	He alba	Ho alba	A trem	H_E trem	H_O trem	delta	lnL ratio	P	Selection ^a		
G1719	1	12	0.867	0.844	7	0.628	0.568	0.625	7.49	0.004	TT:+	TA:	AA:-
A302	1	9	0.852	0.841	9	0.778	0.773	0.448	1.24	0.648	TT:	TA:	AA:
P2852	1	15	0.807	0.563	19	0.868	0.813	0.490	2.11	0.341	TT:	TA:	AA:
O30_2	1	19	0.937	0.911	8	0.801	0.915	0.677	26.60	0.000	TT:+	TA:	AA:-
G1568	1	25	0.932	0.514	36	0.969	0.644	0.740	46.82	0.000	TT:+	TA:	AA:-
O137	1	3	0.512	0.313	7	0.489	0.229	0.969	20.55	0.000	TT:	TA:-	AA:+
G124	1	5	0.560	0.617	6	0.708	0.721	0.885	6.28	0.015	TT:	TA:	AA:
G1376	2	2	0.024	0.024	12	0.619	0.341	0.906	4.49	0.049	TT:+	TA:-	AA:
A376	2	3	0.477	0.362	12	0.788	0.475	0.948	6.70	0.008	TT:	TA:	AA:
G1158	2	3	0.042	0.043	4	0.165	0.174	0.979	10.20	0.000	TT:	TA:	AA:
G1133	3	10	0.646	0.196	9	0.697	0.109	0.802	49.75	0.000	TT:+	TA:-	AA:
G1629	3	18	0.820	0.705	17	0.826	0.457	0.729	7.10	0.007	TT:+	TA:	AA:
O30_1	3	2	0.082	0.043	3	0.449	0.340	0.292	3.76	0.060	TT:	TA:	AA:
G1416	3	5	0.452	0.283	2	0.379	0.125	0.083	15.48	0.000	TT:	TA:	AA:+
O203	3	4	0.202	0.087	6	0.616	0.533	0.406	6.81	0.009	TT:	TA:	AA:
G1869	3	10	0.816	0.614	13	0.836	0.745	0.979	5.36	0.031	TT:	TA:	AA:
G1688	3	11	0.726	0.565	6	0.296	0.222	0.698	5.86	0.013	TT:+	TA:	AA:-

O220	4	1	0.000	0.000	4	0.515	0.583	1.000	8.68	0.001	TT:-	TA:+	AA:
O127	4	3	0.064	0.065	6	0.695	0.325	0.594	18.49	0.000	TT:+	TA:-	AA:
G1809	4	2	0.028	0.028	7	0.839	0.714	0.677	13.22	0.000	TT:+	TA:	AA:
G1255	5	2	0.021	0.021	1	0.000	0.000	1.000	11.09	0.001	TT:	TA:+	AA:
G1192	5	5	0.658	0.660	9	0.361	0.152	0.458	5.42	0.018	TT:	TA:	AA:
G1838	5	9	0.694	0.600	8	0.792	0.696	0.781	12.46	0.000	TT:-	TA:+	AA:-
G20	5	8	0.565	0.444	1	0.000	0.000	0.615	8.60	0.001	TT:+	TA:	AA:-
W15	5	7	0.799	0.688	6	0.656	0.646	0.542	5.94	0.010	TT:-	TA:	AA:
G1065	6	1	0.000	0.000	9	0.749	0.636	0.938	31.03	0.000	TT:+	TA:-	AA:
O369	6	5	0.446	0.417	6	0.545	0.521	0.219	9.43	0.000	TT:	TA:+	AA:-
G2034	6	16	0.922	0.756	4	0.307	0.277	0.958	6.72	0.012	TT:-	TA:	AA:
A322	6	11	0.779	0.787	8	0.805	0.783	0.813	10.78	0.000	TT:	TA:	AA:+
O60	6	9	0.759	0.646	6	0.265	0.167	0.813	59.90	0.000	TT:-	TA:	AA:+
W12	6	13	0.767	0.444	14	0.823	0.386	0.719	16.14	0.000	TT:+	TA:-	AA:+
O190	6	2	0.266	0.311	2	0.374	0.400	0.792	4.72	0.025	TT:-	TA:	AA:+
A933	6	6	0.387	0.364	8	0.534	0.511	0.969	8.35	0.001	TT:-	TA:	AA:
G1485	6	1	0.000	0.000	2	0.139	0.149	0.083	27.08	0.000	TT:+	TA:-	AA:
O26	6	9	0.863	0.909	1	0.000	0.000	0.875	13.14	0.000	TT:+	TA:-	AA:
O167	6	2	0.061	0.063	2	0.021	0.021	0.990	13.54	0.002	TT:	TA:+	AA:-
G1831	6	8	0.696	0.732	7	0.737	0.732	0.667	1.52	0.462	TT:	TA:	AA:
G1074	6	2	0.044	0.044	1	0.000	0.000	0.938	7.47	0.004	TT:-	TA:	AA:
G139	6	1	0.000	0.000	7	0.778	0.372	1.000	0.25	0.940	TT:	TA:	AA:
G1260	7	5	0.614	0.542	2	0.494	0.468	0.469	9.75	0.000	TT:+	TA:	AA:-
W17	7	9	0.616	0.489	8	0.628	0.556	0.521	15.86	0.000	TT:+	TA:	AA:-
O312	7	7	0.782	0.804	7	0.756	0.708	0.677	2.66	0.231	TT:	TA:	AA:
G2062	8	15	0.862	0.809	11	0.847	0.851	0.573	7.92	0.002	TT:-	TA:+	AA:
O374	8	15	0.778	0.575	17	0.889	0.625	0.760	24.97	0.000	TT:+	TA:	AA:-
O202	8	3	0.525	0.447	4	0.573	0.565	0.594	1.52	0.487	TT:	TA:	AA:
O268	8	4	0.650	0.591	5	0.401	0.214	0.531	3.75	0.092	TT:	TA:	AA:

G1949	9	7	0.568	0.351	9	0.805	0.457	0.479	7.08	0.010	TT:	TA:	AA:
O23	9	20	0.858	0.660	12	0.724	0.396	1.000	12.46	0.000	TT:-	TA:	AA:+
O21	9	3	0.198	0.196	1	0.000	0.000	0.125	8.39	0.001	TT:	TA:	AA:
G2020	10	14	0.857	0.850	13	0.666	0.667	0.740	6.38	0.014	TT:	TA:+	AA:
O149	10	3	0.369	0.333	4	0.533	0.543	0.958	11.45	0.000	TT:+	TA:	AA:-
O344	10	2	0.041	0.042	3	0.538	0.442	1.000	5.12	0.039	TT:	TA:+	AA:-
G1574	10	2	0.250	0.289	11	0.781	0.659	0.792	26.17	0.000	TT:+	TA:-	AA:
G114	10	3	0.087	0.089	8	0.745	0.341	0.885	2.52	0.290	TT:	TA:	AA:
G1037	11	5	0.410	0.478	3	0.396	0.395	0.073	10.92	0.000	TT:	TA:+	AA:-
W05	12	9	0.689	0.708	12	0.787	0.489	0.917	6.37	0.015	TT:	TA:	AA:
G1186	12	7	0.781	0.636	3	0.502	0.500	0.865	3.50	0.132	TT:	TA:-	AA:+
G1353	13	2	0.497	0.522	6	0.646	0.435	0.271	9.18	0.001	TT:	TA:	AA:
G162	13	9	0.821	0.786	14	0.854	0.773	0.823	0.63	0.741	TT:	TA:	AA:
G1812	14	11	0.831	0.432	6	0.671	0.700	0.656	5.97	0.010	TT:	TA:-	AA:+
G1894	15	11	0.837	0.830	4	0.084	0.085	0.906	3.77	0.101	TT:	TA:	AA:
G1454	15	11	0.820	0.667	12	0.781	0.667	0.708	7.27	0.008	TT:	TA:-	AA:+
G1608	15	10	0.669	0.488	3	0.215	0.116	0.896	46.01	0.000	TT:	TA:-	AA:+
O14	16	1	0.000	0.000	2	0.100	0.104	0.052	6.24	0.001	TT:+	TA:+	AA:-
O214	18	4	0.194	0.167	2	0.142	0.152	0.927	13.74	0.000	TT:	TA:+	AA:-
G1577	18	10	0.831	0.766	2	0.042	0.043	0.979	4.12	0.086	TT:	TA:	AA:
O28	18	3	0.468	0.542	4	0.233	0.255	0.333	3.38	0.119	TT:	TA:	AA:
O206	19	4	0.062	0.063	5	0.553	0.422	0.635	2.03	0.341	TT:	TA:	AA:

B) Ticino/Italy

Locus	Ch	A alba	He alba	Ho alba	A trem	H_E trem	H_O trem	lnL ratio	P	delta	Selection ¹		
P2852	1	10	0.577	0.350	15	0.870	0.590	8.90	0.001	0.825	TT:-	TA:	AA:
O30_2	1	20	0.933	0.909	8	0.686	0.750	10.01	0.001	0.900	TT:-	TA:+	AA:
O137	1	3	0.235	0.184	6	0.256	0.139	11.95	0.000	0.938	TT:-	TA:+	AA:

O30_1	3	2	0.075	0.077	2	0.119	0.125	23.91	0.000	0.063	TT:+	TA:-	AA:+
O220	4	1	0.000	0.000	6	0.632	0.600	7.66	0.001	0.500	TT:-	TA:	AA:+
O127	4	1	0.000	0.000	6	0.702	0.282	9.19	0.000	0.875	TT:	TA:	AA:
W15	5	8	0.835	0.872	7	0.771	0.632	3.66	0.095	0.563	TT:	TA:	AA:
O60	6	7	0.818	0.763	5	0.659	0.256	23.58	0.000	0.738	TT:+	TA:-	AA:+
O167	6	2	0.099	0.103	3	0.120	0.125	44.91	0.000	0.950	TT:+	TA:-	AA:-
O312	7	5	0.714	0.825	4	0.619	0.538	14.35	0.000	0.913	TT:	TA:+	AA:-
O202	8	3	0.607	0.550	2	0.501	0.450	8.69	0.001	0.550	TT:-	TA:	AA:+
O21	9	2	0.315	0.179	1	0.000	0.000	30.78	0.000	0.213	TT:	TA:-	AA:+
O149	10	3	0.515	0.150	3	0.229	0.200	39.54	0.000	1.000	TT:+	TA:-	AA:-
O344	10	2	0.025	0.025	7	0.820	0.526	15.75	0.000	0.950	TT:-	TA:	AA:+
W05	12	4	0.649	0.487	8	0.723	0.300	8.99	0.001	1.000	TT:-	TA:+	AA:
O214	18	3	0.164	0.125	2	0.073	0.075	32.13	0.000	0.950	TT:-	TA:+	AA:-
O28	18	3	0.560	0.400	3	0.228	0.250	5.92	0.021	0.488	TT:	TA:	AA:-
O206	19	6	0.580	0.385	5	0.618	0.487	17.86	0.000	0.163	TT:	TA:-	AA:+

C) Tisza/Hungary

Locus	Ch	A alba	He alba	Ho alba	A trem	H_E trem	H_O trem	lnL ratio	P	Delta	Selection ¹		
P2852	1	9	0.768	0.725	14	0.836	0.850	2.77	0.208	0.625	TT:	TA:	AA:
O30_2	1	20	0.880	0.850	9	0.830	0.750	7.30	0.006	0.625	TT:	TA:+	AA:-
O137	1	5	0.534	0.475	7	0.690	0.258	7.93	0.003	0.975	TT:	TA:	AA:-
O30_1	3	2	0.141	0.150	4	0.397	0.300	2.41	0.266	0.238	TT:	TA:	AA:
O220	4	3	0.249	0.026	3	0.563	0.667	29.94	0.000	0.838	TT:+	TA:	AA:-
O127	4	1	0.000	0.000	7	0.737	0.343	5.39	0.040	0.663	TT:	TA:	AA:
W15	5	4	0.715	0.667	7	0.786	0.769	5.26	0.026	0.588	TT:	TA:	AA:-
O60	6	8	0.771	0.675	4	0.291	0.200	12.30	0.000	0.825	TT:	TA:	AA:
O167	6	4	0.310	0.282	1	0.000	0.000	18.20	0.000	1.000	TT:+	TA:-	AA:
O312	7	7	0.761	0.775	7	0.753	0.625	5.65	0.013	0.575	TT:+	TA:	AA:

O202	8	4	0.567	0.538	3	0.574	0.650	11.27	0.000	0.775	TT:+	TA:	AA:-
O21	9	2	0.240	0.075	1	0.000	0.000	10.94	0.000	0.138	TT:	TA:	AA:+
O149	10	5	0.658	0.368	4	0.636	0.425	7.31	0.007	0.888	TT:+	TA:	AA:
O344	10	3	0.143	0.150	5	0.687	0.575	4.03	0.078	0.975	TT:-	TA:	AA:
W05	12	12	0.834	0.769	15	0.876	0.575	14.88	0.000	0.800	TT:	TA:-	AA:+
O214	18	3	0.227	0.200	3	0.297	0.300	9.02	0.000	0.838	TT:	TA:	AA:
O28	18	3	0.492	0.475	4	0.317	0.256	4.99	0.020	0.375	TT:+	TA:	AA:
O206	19	2	0.025	0.025	6	0.581	0.550	2.30	0.273	0.675	TT:-	TA:	AA:

^aAA, interclass homozygotes for *P.alba*; AT, interclass heterozygotes for *P. alba* and *P. tremula*; TT, interclass homozygotes for *P. tremula*.