S1_Fig.pdf
Principal Component Analysis (PCA) of secondary metabolites identified by UHPLC-QTOF-MS in Populus alba (in red), P. tremula (in green) and their hybrids, P. x canescens (in blue). Ellipses represent the 95% confidence intervals and colored dots represent averages for each group. For (A) and (B), symbols specific to each species and hybrid zone (Ticino: Italy, Ticino river hybrid zone; Danube: Austria, Danube river hybrid zone; Tisza: Hungary, Tisza river hybrid zone) represent individual trees. The first two principal components (PC’s) are plotted for (A) PCA of seven chlorogenic acids explaining 71.6% of the phenotypic variation; (B) PCA of the 13 salicinoids explaining 85.1% of the phenotypic variance within this subgroup; (C) PCA of all 38 phenylpropanoid in 133 individuals from the common garden explaining 58.9% of the phenotypic variance. For PCA for flavonoids see main paper.

10.1371/journal.pone.0128200.s001

S2_Fig.pdf
Normalized peak areas used as measures of relative abundances of compounds were summed within groups of aglycones and moieties for P. alba, P. tremula and their hybrids from three natural hybrid zones. For compound diversity see S3 Table.

10.1371/journal.pone.0128200.s002

S1_Materials.pdf
B) Molecular genetic analysis of common garden seedlings.

10.1371/journal.pone.0128200.s003

S1_Table.pdf
Hybrid individuals and plants from each parental species (P. alba and P. tremula) were characterized with 77 microsatellite DNA markers in a previous study [23]. The common garden plants were genotyped with 16 microsatellites for the present study (see text and S2 Table for details). Assignment to each taxon was based on Bayesian genomic admixture proportions Q as described in main text.

10.1371/journal.pone.0128200.s004

S2_Table.pdf
Information for the 16 microsatellite marker loci used to identify parental species and hybrids and to estimate the correlation of paternity (Cp) in the common garden. These 16 microsatellites are a subset of the genome-wide marker panel used for admixture mapping in natural hybrid zones and are fully described [23]. Localization on chromosomes, allele frequency differential (delta) between the parental reference populations of the Italian hybrid zone [23], number of alleles (N_A) and gene diversity (He) in the common garden trial are indicated.

10.1371/journal.pone.0128200.s005

S3_Table.xlsx

10.1371/journal.pone.0128200.s006

S4_Table.xlsx
Shown are means ± standard deviations of traits quantified for three natural hybrid zones (Italy, Austria, Hungary) and a common garden trial established from seeds from the Italian hybrid zone. Numbers of individuals (n) for each species in each hybrid zone are indicated.

10.1371/journal.pone.0128200.s007

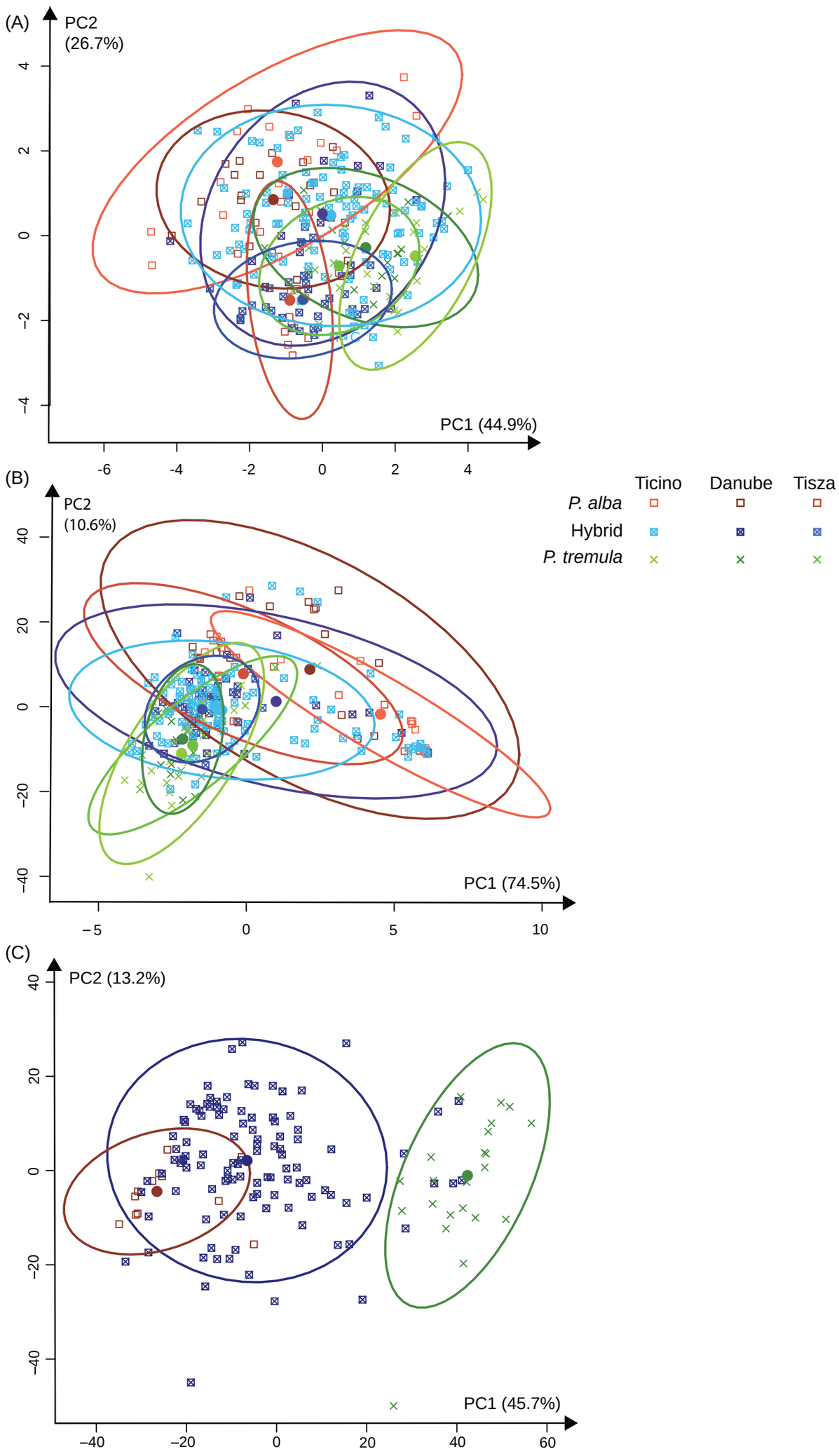
S5_Table.xlsx
10.1371/journal.pone.0128200.s008

S6_Table.pdf
Contingency tables presenting counts of the presence (yes) or absence (no) of an excess of interspecific heterozygote [23], for all 67 codominant genetic markers (S5 Table) studied in natural hybrid zones of P. alba and P. tremula, for markers representing putative QTL for salicinoids and flavonoids, and for all 34 markers representing putative phytochemical QTL in the present study.

10.1371/journal.pone.0128200.s009

S1 Figure. Principal component analysis of chlorogenic acids and salicinoids in natural populations and individuals of a common garden trial.

Principal Component Analysis (PCA) of secondary metabolites identified by UHPLC-QTOF-MS in *Populus alba* (in red), *P. tremula* (in green) and their hybrids, *P. x canescens* (in blue). Ellipses represent the 95% confidence intervals and colored dots represent averages for each group. For (A) and (B), symbols specific to each species and hybrid zone (Ticino: Italy, Ticino river hybrid zone; Danube: Austria, Danube river hybrid zone; Tisza: Hungary, Tisza river hybrid zone) represent individual trees. The first two principal components (PC's) are plotted for (A) PCA of seven chlorogenic acids explaining 71.6% of the phenotypic variation; (B) PCA of the 13 salicinoids explaining 85.1% of the phenotypic variance within this subgroup; (C) PCA of all 38 phenylpropanoid in 133 individuals from the common garden explaining 58.9% of the phenotypic variance. For PCA for flavonoids see main paper.


(A)
PC2
(26.7%)
PC1 (44.9%)
(B)
PC2
(10.6%)
PC1 (74.5%)
(C)
PC2 (13.2%)
PC1 (45.7%)
Ticino
Danube
Tisza
P. alba
Hybrid
P. tremula

S1 Materials

A) High-throughput quantification of phenylpropanoids in natural populations

Metabolic fingerprinting, the quantification of specific targeted phytochemical traits, was performed through Ultra-High-Pressure Liquid Chromatography coupled with Quadrupole-Time-Of-Flight Mass Spectrometry (UHPLC-QTOF-MS) on an Acquity UHPLC system (Waters corp., Milford, USA) coupled with an electrospray source Synapt G2 QTOF-MS (Waters corp.). The separation was achieved on a reversed-phase Acquity BEH C18 column (50x2.1 mm column, particle size 1.7 µm, Waters corp.). Solvents were A= water + 0.1% vol. formic acid; B = acetonitrile + 0.1% vol. formic acid. The gradient program at flow of 0.4 mL/min of solvents was: 2-40% B in 4 min, 40-100% B in 2 min, holding at 100% B for 1.5 min followed by re-equilibration at 2% B for 2 min. The column temperature was maintained constant at 30^{o}C and the injection volume of leaf extract was 5 µl. Mass over charge (m/z) data from the QTOF-MS were obtained in negative ion modes ([M-H]$^{-}$, S3 Table) over an *m/z* range of 85-1200 Da with the following parameters: capillary voltage at -2.5 kV, cone voltage at 25 V, source temperature at 120^{o}C, desolvation gas temperature at 330^{o}C, desolvation gas flow 800 L/hour. Argon was used as collision gas at a flow of 2.1mL/min. Internal calibration of the instrument was obtained by infusing a solution of leucinenkephaline at 600 ŋg/mL at a flow rate of 10 µL/min through the Lock Spray^{TM} probe. The abundance of targeted secondary metabolites was quantified in a relative manner on the basis of peak areas obtained from the QTOF-MS normalized over the total spectrum area. For each compound, the corresponding most abundant ion (e.g. ion of the molecular species, formate adduct or dimer) was considered for quantification. (S3 Table). The identification of targeted compounds by High-Resolution tandem Mass Spectrometry (HR-MS/MS) and comparison to pure standards is documented in [1].

B) Molecular genetic analysis of common garden seedlings

Leaf samples from each common garden seedling were dried with silicagel. Total genomic DNA was extracted with the DNeasy Plant Mini Kit (Qiagen) and quantified with a NanoDrop photometer (Thermo scientific). Three multiplex reaction mixes with six marker loci each were designed by selecting highly informative microsatellites in natural hybrid populations of *Populus alba* and *P. tremula* (S2 Table). The multiplexed microsatellites were amplified in 15 µl Polymerase Chain Reactions (PCR) including 10ng of DNA with Type-it Microsatellite PCR kit (Qiagen). Concentrations of primers including fluorescent dyes (FAM, VIC, NED and PET from Applied Biosystems) were adjusted in the

primer mix to adjust for differing signal intensities among markers. PCR conditions were: denaturation for 5 min at 95^{o}C followed by 28 cycles consisting of a denaturation step of 30 sec at 95 ^{o}C, an annealing step of 90 sec at 57 ^{o}C, and an extension step of 30 sec at 72 ^{o}C, followed by a final extension step of 30 min at 60 ^{o}C. Amplified fragments were analyzed on an Applied Biosystem 3130 Genetic Analyzer with LIZ (Applied Biosystem) as internal size standard. Two marker loci were dropped from downstream analyses because missing data frequencies were too high. For the remaining 16 markers, numbers of alleles (Table S2) were calculated with Microsatellite Analyzer [2] and gene diversity was estimated with the FSTAT software [3]. The genomic composition of the common garden seedlings was estimated through genomic admixture proportions (Q) based on 16 multiplexed microsatellite markers (Table S2) with the Bayesian approach implemented in the STRUCTURE software [4-6] using an admixture model and parental species samples (42 *P. alba*, 68 *P. tremula*) from the Ticino river hybrid zone as references. A burn-in of 50 000, followed by 100 000 iterations, a prior mean of F_k of 0.7 and k=2 populations were specified in STRUCTURE runs. Individual common garden plants (S1 Table) were characterized as *P. alba* (Q>0.95), *P. x canescens* hybrids (0.05<Q<0.95) and *P. tremula* (Q<0.05) according to their admixture proportions. The correlation of paternity (Cp) was calculated following [7] using the MLTR software to estimate percentages of full-siblings (=seedlings sharing the same father) in the open pollinated families. The microsatellite genotype data were also used to confirm the absence of seed contaminations (unrelated genotypes) within the families based on codominant inheritance of the markers, following Lexer et al. [8]. Inspection of the maternal genotypes (=seed parents of the open pollinated families) revealed two cases of exact genotype matches between spatially separated mother trees, raising the possibility that these mothers form part of ancient clones. Thus, all common garden analyses in this paper were carried out for 15 families (as expected from the seed harvest) and 13 families (progeny of matching mother pairs merged). As the results were highly similar, only results from the 15 family dataset are discussed throughout the paper. This was regarded more appropriate, since seeds from each maternal tree had been exposed to that tree's particular microenvironment during development, and the maternal genotype matches do not exclude the existence of genetic differences at other (untyped) loci or somaclonal mutations in the genomes of ancient clones.

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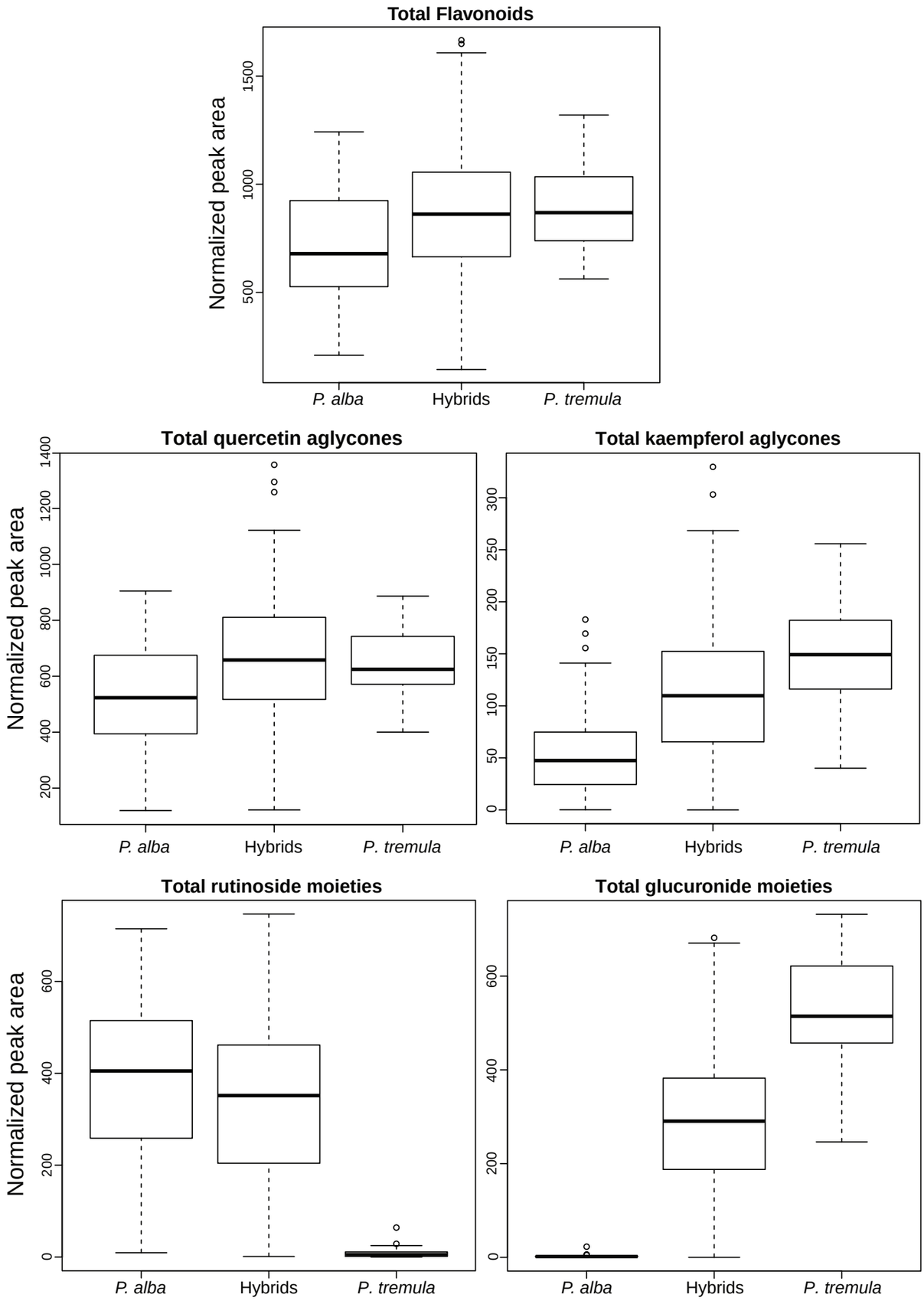
S1 Table. Number of individuals studied in three natural hybrid zones.
Hybrid individuals and plants from each parental species (*P. alba* and *P. tremula*) were characterized with 77 microsatellite DNA markers in a previous study [9]. The common garden plants were genotyped with 16 microsatellites for the present study (see text and S2 Table for details). Assignment to each taxon was based on Bayesian genomic admixture proportions Q as described in main text.

	Hybrids	*P. alba*	*P. tremula*
Ticino river hybrid zone	109	15	15
Danube hybrid zone	20	21	16
Tisza river hybrid zone	34	15	15
Common garden trial	104	9	20

S2 Figure. Interspecific variation for total flavonoids, quercetin and kaempferol aglycones, rutinoside and glucuronide moieties.

Normalized peak areas used as measures of relative abundances of compounds were summed within groups of aglycones and moieties for *P. alba*, *P. tremula* and their hybrids from three natural hybrid zones. For compound diversity see S3 Table.



S2 Table. Molecular marker used to characterize common garden seedlings

Information for the 16 microsatellite marker loci used to identify parental species and hybrids and to estimate the correlation of paternity (Cp) in the common garden. These 16 microsatellites are a subset of the genome-wide marker panel used for admixture mapping in natural hybrid zones and are fully described in Lindtke et al. [9]. Localization on chromosomes, allele frequency differential (delta) between the parental reference populations of the Italian hybrid zone [9], number of alleles (N_A) and gene diversity (He) in the common garden trial are indicated.

Name	Chr.	Delta	N_A	He
G124	1	0.78	7	0.794
ASP376	1	0.94	6	0.721
G1158	2	1	6	0.584
G1255	5	1	4	0.546
0G1831	6	0.72	10	0.824
G1074	6	0.98	4	0.517
O26	6	0.71	7	0.700
O167	6	0.96	2	0.498
ASP933	6	1	7	0.747
O190	6	0.95	4	0.519
ASP322	6	1	15	0.857
G2020	10	0.77	17	0.867
G1574	10	0.98	6	0.634
G1812	14	0.85	7	0.787
G1894	15	0.97	7	0.704
O214	18	0.83	4	0.463

S3 Table. Details of thirty-eight phenylpropanoids identified following Caseys et al. [1] by
mass spectrometry (UHPLC-QTOF-MS) in *P*

Abbreviation	Name	Molecular Formula	ID	Method
	Chlorogenic acids			
3CfQA	3-Caffeoyl quinic acid	C16H18O9		2
3CmQA	3-Coumaroyl quinic acid	C16H18O8		2
5CfQA	5-Caffeoyl quinic acid	C16H18O9		1,2
3fQA	3-feruloyl quinic acid	C17H20O9		2
1CfQA	1-Caffeoyl quinic acid	C16H18O9		2
5CmQA	5-Coumaroyl quinic acid	C16H18O8		2
DiCfQA	(1,5) Dicaffeoyl quinic acid	C25H24O12		1,2
	Salicinoids			
SC	Salicin	C13H18O7		1,2
St2	Salicortin isomer 2	C20H24O10		2
St3	Salicortin isomer3	C20H24O10		2
St	Salicortin	C20H24O10		1,2
Ac-St	Acetyl-salicortin	C22H26O11		2
Ac-St1	Acetyl-salicortin isomer 1	C22H26O11		2
Ac-St2	Acetyl-salicortin isomer 2	C22H26O11		2
HCH-St	HCH-Salicortin	C27H30O13		2
Td	Tremuloidin	C20H22O8		1,2
Tc1	Tremulacin isomer	C27H28O11		2
Tc	Tremulacin	C27H28O11		1,2
HCH-Tc	HCH-tremulacin	C34H34O14		2
Ac-Tc	Acetyl-tremulacin	C29H30O12		2
	Flavonoids			
Cat	Catechin	C15H14O6		1,2
Q-rut-p	Quercetin-rutinoside-pentose	C32H38O20		2
Q-glu-p	Quercetin glucuronide-pentose	C26H26O17		2
Q-h-p	Quercetin-hexose-pentose	C26H28O16		2
K-rut-p	Kaempferol-rutinoside-pentose	C32H38O19		2
I-rut-p	Isorhamnetin-rutinoside-pentose	C33H40O20		2

Q-rut	Quercetin-3-O-rutinoside	C27H30O16	1,2
Q-glu	Quercetin-3-O-glucuronide	C21H18O13	1,2
Q-glo	Quercetin-3-O-glucoside	C21H20O12	1,2
K-rut	Kaempferol-3-O-rutinoside	C27H30O15	1,2
I-rut	Isorhamnetin-3-O-rutinoside	C28H32O16	1,2
Q-ara	Quercetin-3-O-arabinopyranoside	C20H18O11	1,2
K-glu	Kaempferol-glycuronide	C21H18O12	2
Q-rha	Quercetin-rhamnoside	C21H20O11	2
I-glo	Isorhamnetin-glycoside	C22H22O12	2
I-glu	Isorhamnetin-glycuronide	C22H20O13	2
I-ac-h	Isorhamnetin acetyl-hexose	C24H24O13	2
I-rha	Isorhamnetin-rhamnoside	C22H22O11	2

Note: Compounds were defined by their retention time (tR) and exact mass in negative
compounds was assessed by analytical standards (1) or by tandem high-resolution mass spe
presence of dimers. Phenotypic interspecific differentiation was assessed with the Tuke

Ultra-High Pressure Liquid Chromatography coupled with Quadrupole-Time Of Flight
. *alba* , *P. tremula* and their hybrids.

	Hybrid zones		Common Garden		
Deriv.	t_R (min)	$[M-H]^-$ (m/z)	t_R (min)	$[M-H]^-$ (m/z)	Intersp. Diff.
b	1.3	353.0874	1.29	353.0875	
b	1.56	337.0926	1.53	337.0927	
b	1.61	353.0872	1.58	353.0876	-
	1.7	367.103	1.66	367.1031	+
b	1.9	353.0873	1.85	353.0876	-
b	2.19	337.0925	2.14	337.0927	-
b	2.63	515.1187	2.62	515.1191	-
a, b	1.36	285.0978	1.33	285.098	+
a,b	2.13	423.129	2.18	423.1289	
a	2.38	423.1289	2.34	423.1288	
a,b	2.49	423.1289	2.44	423.1291	
b	2.9	465.1396	2.93	465.1394	
	2.7	465.1396	2.7	465.1397	
	3.6	465.1395	3.53	465.1399	
b	3.29	561.1603	3.22	561.1602	-
a,b	3.29	389.1236	3.23	389.1234	+
b	3.56	527.155	3.49	527.1551	+
a,b	3.99	527.155	3.96	527.1548	+
a	4.58	665.186	4.51	665.1859	-
a	4.6	569.1656	4.54	569.165	
b	1.64	289.0714	1.61	289.0717	
	2.08	741.1866	2.03	741.1863	
	2.18	609.1089	2.14	609.1087	+
b	2.21	595.1296	12.16	595.1295	+
	2.29	725.1917	2.23	725.1913	
	2.3	755.2022	2.3	755.2005	

	2.34	609.1452	2.29	609.1452	-
b	2.41	477.067	2.37	477.0669	+
b	2.43	463.0875	2.39	463.0877	
b	2.53	593.1503	2.5	593.1501	-
	2.57	623.1605	2.55	623.1605	-
	2.58	433.0769	2.55	433.0771	
b	2.61	461.0719	2.61	461.0721	
b	2.63	447.093	2.62	447.0927	
	2.66	477.1029	2.66	477.103	
b	2.68	491.0824	2.69	491.0826	
	2.84	519.1138	2.84	519.1138	
	2.82	461.1085	2.83	461.1085	

mode (M-H)- following methods described in Caseys et al. [1]. The identification of
ctrometry (2); derivatives were documented as (a) presence of formic acid adducts, (b)
y HSD method, adjusted P-val<0.05; + P. tremula phenotype ; - P. alba phenotype.

S4 Table. Intra- and inter-specific variation in 38 phytochemical traits identified by UHPLC-QT
Shown are means ± standard deviations of traits quantified for three natural hybrid zones (Italy
species in each hybrid zone are indicated.

	Ticino river hybrid zone (Italy)			Danube
	P. alba **(n=15)**	**Hybrids (n=109)**	***P. tremula*** **(n=15)**	***P. alba*** **(n=21)**
Chlorogenic acids				
3CfQA	952.88 ± 419.77	599.41 ± 285.49	467.08 ± 196.24	776.41 ± 221.18
3CmQA	119.30 ± 74.70	157.71 ± 82.02	164.44 ± 67.78	97.62 ± 55.17
5CfQA	503.31 ± 159.96	305.77 ± 166.62	85.84 ± 75.42	489.61 ± 187.76
3fQA	25.63 ± 16.82	31.12 ± 12.96	47.12 ± 20.33	15.02 ± 7.44
1CfQA	107.55 ± 56.67	70.95 ± 46.72	13.45 ± 18.44	97.50 ± 49.07
5CmQA	46.81 ± 37.71	37.41 ± 28.41	4.34 ± 6.34	38.52 ± 15.26
DiCfQA	70.95 ± 47.57	47.67 ± 53.77	3.67 ± 4.62	66.21 ± 37.83
Salicinoids				
SC	51.56 ± 83.25	133.12 ± 105.95	204.87 ± 126.06	67.73 ± 60.29
St2	215.07 ± 213.28	404.35 ± 160.78	452.96 ± 59.47	362.81 ± 220.89
St3	31.03 ± 51.87	73.30 ± 49.09	62.21 ± 22.69	67.94 ± 69.12
St	3.38 ± 8.18	28.98 ± 29.61	38.46 ± 21.90	6.12 ± 8.59
Ac-St	6.13 ± 10.50	23.08 ± 22.93	30.54 ± 17.69	15.54 ± 14.99
Ac-St1	11.08 ± 15.00	24.52 ± 17.63	30.75 ± 15.25	18.72 ± 16.88
Ac-St2	7.47 ± 11.48	42.81 ± 28.13	76.82 ± 29.32	15.75 ± 18.03
HCH-St	51.00 ± 47.68	35.89 ± 26.33	11.82 ± 13.02	56.83 ± 43.33
Td	3.48 ± 6.10	83.06 ± 73.6	219.87 ± 150.26	12.38 ± 21.81
Tc	136.33 ± 177.72	469.49 ± 198.29	624.09 ± 80.57	239.22 ± 210.61
Tc1	16.31 ± 37.15	99.19 ± 65.23	131.90 ± 44.80	37.76 ± 54.84
HCH-Tc	44.49 ± 67.91	83.44 ± 57.48	37.84 ± 32.30	53.70 ± 67.19
Ac-Tc	0.02 ± 0.02	4.54 ± 13.95	10.58 ± 24.73	0.43 ± 1.91
Flavonoids				
Cat	181.18 ± 104.68	111.29 ± 81.74	119.66 ± 123.47	112.39 ± 55.70
Q-rut-p	16.35 ± 26.25	70.78 ± 44.76	1.16 ± 1.43	28.73 ± 20.63
Q-glu-p	0.00 ± 0.00	16.35 ± 16.71	62.01 ± 17.56	0.01 ± 0.06
Q-h-p	3.93 ± 10.52	39.95 ± 43.77	153.23 ± 26.86	10.38 ± 11.66
K-rut-p	3.12 ± 6.68	12.68 ± 9.65	0.29 ± 0.59	6.16 ± 10.74
I-rut-p	7.80 ± 8.42	39.43 ± 27.3	6.30 ± 6.19	14.72 ± 13.3
Q-rut	204.88 ± 57.85	109.78 ± 73.05	1.03 ± 3.96	204.59 ± 46.98
Q-glu	1.26 ± 0.94	172.25 ± 91.40	325.87 ± 57.74	3.15 ± 3.51

Q-glo	66.66 ± 56.17	43.9 ± 35.16	21.05 ± 12.10	115.65 ± 66.31
K-rut	76.36 ± 37.43	45.62 ± 28.57	0.01 ± 0.01	61.97 ± 34.26
I-rut	190.64 ± 50.15	96.63 ± 53.48	2.16 ± 8.33	138.97 ± 35.59
Q-ara	5.12 ± 5.01	11.84 ± 12.78	31.98 ± 19.20	6.99 ± 9.94
K-glu	0.04 ± 0.14	78.51 ± 49.61	171.28 ± 36.67	0.26 ± 1.16
Q-rha	37.16 ± 31.86	23.05 ± 22.57	14.64 ± 7.13	30.81 ± 29.4
I-glo	23.49 ± 11.33	21.80 ± 13.78	7.27 ± 3.96	40.97 ± 20.67
I-glu	0.05 ± 0.09	44.17 ± 30.48	14.26 ± 7.42	0.03 ± 0.07
I-ac-h	4.65 ± 5.63	4.83 ± 5.4	3.70 ± 12.98	25.60 ± 28.77
I-rha	2.51 ± 6.00	11.14 ± **8.78**	19.85 ± 8.78	5.88 ± 7.81
CT	46.22 ± 22.75	33.64 ± 26.71	11.83 ± 14.93	34.53 ± 14.12

OF-MS and condensed tannins.

y, Austria, Hungary) and a common garden trial established from seeds from the Italian hybrid

river hybrid zone (Austria)		Tisza river hybrid zone (Hungary)		
Hybrids (n=20)	***P. tremula* (n=16)**	***P. alba* (n=15)**	**Hybrids (n=34)**	***P. tremula* (n=15)**
659.85 ± 315.86	538.79 ± 164.23	404.67 ± 277.80	298.25 ± 147.38	466.95 ± 167.07
170.34 ± 95.46	154.07 ± 56.31	45.54 ± 31.63	68.36 ± 45.08	87.72 ± 47.41
363.22 ± 163.43	226.16 ± 138.20	276.66 ± 115.46	231.04 ± 75.56	234.80 ± 107.21
19.75 ± 9.84	25.99 ± 11.30	5.78 ± 3.24	10.58 ± 7.66	22.91 ± 9.43
66.38 ± 39.60	37.59 ± 36.93	57.99 ± 38.84	51.52 ± 28.49	43.68 ± 25.97
30.72 ± 28.97	11.26 ± 11.76	49.62 ± 24.06	36.64 ± 23.9	7.79 ± 6.71
46.22 ± 35.66	28.05 ± 30.68	43.38 ± 28.37	68.79 ± 42.58	46.26 ± 32.28
108.69 ± 98.34	222.87 ± 78.75	117.05 ± 79.30	189.31 ± 78.97	180.59 ± 84.93
361.18 ± 202.94	466.91 ± 64.24	411.29 ± 182.62	468.42 ± 58.07	457.53 ± 52.64
58.11 ± 49.95	66.95 ± 22.03	72.79 ± 45.97	78.33 ± 20.59	72.41 ± 22.6
15.21 ± 20.34	38.21 ± 24.51	28.39 ± 32.95	45.82 ± 31.73	48.28 ± 30.41
15.56 ± 13.86	27.77 ± 13.40	33.24 ± 24.96	39.75 ± 22.58	41.47 ± 27.64
13.28 ± 11.89	25.48 ± 13.01	19.35 ± 11.25	22.16 ± 10.63	28.95 ± 14.02
26.64 ± 24.85	54.97 ± 32.22	39.39 ± 24.99	55.32 ± 28.36	51.41 ± 30.54
20.04 ± 20.76	8.64 ± 9.13	42.63 ± 32.61	36.30 ± 21.24	5.87 ± 5.05
53.30 ± 58.14	163.42 ± 51.75	26.63 ± 17.95	106.82 ± 50.11	180.18 ± 117.53
347.95 ± 226.35	614.54 ± 74.39	391.99 ± 183.71	542.22 ± 71.43	560.57 ± 132.26
59.01 ± 63.15	124.71 ± 35.77	66.21 ± 50.22	133.49 ± 48.26	147.36 ± 72.17
36.95 ± 40.43	18.84 ± 18.67	77.09 ± 73.96	85.19 ± 52.87	14.86 ± 13.4
4.81 ± 12.98	7.32 ± 14.84	0.03 ± 0.07	2.91 ± 7.43	31.30 ± 58.43
79.30 ± 61.9	66.86 ± 55.62	112.76 ± 61.40	67.01 ± 42.4	65.30 ± 52.62
68.76 ± 47.15	1.28 ± 0.95	9.48 ± 5.39	35.97 ± 30.47	1.10 ± 0.93
13.13 ± 12.91	52.07 ± 16.34	0.01 ± 0.03	9.68 ± 9.55	52.49 ± 11.95
54.58 ± 49.67	159.48 ± 32.38	17.40 ± 17.83	52.99 ± 35.87	138.58 ± 34.37
7.10 ± 5.58	0.08 ± 0.14	0.83 ± 0.57	4.13 ± 3.87	0.17 ± 0.23
35.92 ± 29.12	8.54 ± 9.29	3.36 ± 3.64	18.47 ± 16.81	2.88 ± 2.91
119.99 ± 84.46	0.00 ± 0.00	105.18 ± 61.35	44.19 ± 42.7	0.00 ± 0.00
167.25 ± 112.21	308.42 ± 65.54	1.89 ± 0.73	124.86 ± 58.66	268.44 ± 67.18

75.19 ± 62.62	31.77 ± 13.74	76.34 ± 48.14	39.88 ± 22.17	35.18 ± 23.31
36.85 ± 31.73	0.00 ± 0.00	15.90 ± 10.41	10.04 ± 9.24	0.01 ± 0.05
93.66 ± 57.64	0.01 ± 0.05	79.03 ± 37.06	30.60 ± 26.41	0.02 ± 0.07
11.75 ± 12.87	29.20 ± 16.22	1.86 ± 3.44	12.38 ± 11.09	32.93 ± 33.77
52.42 ± 44.24	172.41 ± 40.59	0.03 ± 0.08	31.78 ± 21.46	111.79 ± 40.64
26.32 ± 22.49	16.66 ± 6.84	20.58 ± 16.45	19.73 ± 20.52	23.97 ± 12.7
30.44 ± 19.78	11.23 ± 5.54	29.45 ± 8.21	27.52 ± 13.92	12.05 ± 5.87
31.42 ± 28.35	12.26 ± 6.2	0.00 ± 0.00	22.22 ± 14.74	9.24 ± 5.36
20.44 ± 23.60	1.61 ± 5.95	37.30 ± 20.51	29.95 ± 19.3	2.37 ± 8.65
9.34 ± 11.04	20.17 ± 10.23	6.67 ± 5.9	22.45 ± 19.11	24.17 ± 17.77
31.27 ± 23.90	25.42 ± 29.38	60.34 ± 39.41	31.02 ± 20.81	20.31 ± 18.38

zone. Numbers of individuals (n) for each

Common Garden		
P. alba **(n=9)**	**Hybrids (n=104)**	***P. tremula*** **(n=20)**
85.53 ± 25.16	100.62 ± 77.04	186.18 ± 62.24
13.46 ± 4.36	24.13 ± 24.76	55.55 ± 36.8
213.90 ± 30.56	189.89 ± 46.66	159.39 ± 53.36
2.57 ± 1.34	4.77 ± 4.38	10.69 ± 4.23
67.80 ± 19.05	63.25 ± 26.01	29.28 ± 15.32
59.37 ± 14.59	44.01 ± 20.4	9.91 ± 4.56
81.00 ± 33.95	68.60 ± 39.8	26.63 ± 18.06
175.52 ± 52.03	178.68 ± 54.55	241.09 ± 86.05
838.04 ± 167.99	840.18 ± 103.47	853.66 ± 110.70
15.25 ± 13.13	8.31 ± 7.67	9.94 ± 5.33
10.72 ± 7.43	12.29 ± 10.36	22.98 ± 13.19
16.73 ± 12.29	31.52 ± 28.47	22.76 ± 13.42
19.45 ± 9.44	16.33 ± 9.21	14.57 ± 10.97
32.80 ± 13.27	50.73 ± 24.97	77.50 ± 34.39
68.33 ± 16.92	39.53 ± 19.41	11.31 ± 4.25
45.24 ± 39.86	84.63 ± 40.15	156.93 ± 63.89
720.39 ± 112.58	838.60 ± 123.56	1019.44 ± 133.77
55.87 ± 26.64	83.79 ± 29.15	99.06 ± 31.5
123.71 ± 38.76	92.54 ± 49.57	27.96 ± 11.75
0.19 ± 0.37	3.74 ± 7.41	0.34 ± 0.95
117.44 ± 31.27	105.34 ± 48.03	97.39 ± 44.39
18.33 ± 13.79	61.81 ± 52.89	1.00 ± 0.94
0.02 ± 0.03	4.27 ± 9.02	32.47 ± 11.33
4.75 ± 3.13	45.46 ± 59.25	211.28 ± 36.49
1.84 ± 1.53	6.33 ± 7.41	0.12 ± 0.17
1.57 ± 3.46	1.67 ± 7.66	8.49 ± 7.05
201.92 ± 33.09	132.90 ± 72.68	24.45 ± 16.96
33.57 ± 62.91	131.00 ± 126.26	430.33 ± 61.50

69.99 ± 30.17	85.26 ± 47.66	70.90 ± 23
80.09 ± 38.92	46.77 ± 28.35	0.00 ± 0.01
178.23 ± 16.87	107.34 ± 60.52	0.02 ± 0.09
4.39 ± 3.87	5.06 ± 8.45	13.34 ± 14.15
6.16 ± 14.84	35.71 ± 38.4	187.44 ± 41.96
24.76 ± 17.85	27.97 ± 22.12	29.29 ± 7.81
27.14 ± 9.76	38.73 ± 24.23	14.35 ± 5.24
10.96 ± 22.34	25.93 ± 30.3	6.05 ± 2.01
15.08 ± 22.32	21.75 ± 28.93	0.06 ± 0.11
4.09 ± 2.1	6.36 ± 7.02	5.90 ± 4.77
23.61 ± 11.30	19.51 ± 13.12	10.41 ± 6.57

S5 Table. Genomic architecture of phytochemical traits inferred fro

Marker	Chr.	Position (Mb)	Marker Type	Neutrality dev.
G1719	1	35.488312	codom	
G1688	3	17.574314	codom	
G1074	6	3.989388	codom	H+++
O190	6	13.718036	codom	
O60	6	23.655126	codom	
G1295	7	11.243952	dom	
G1574	10	16.527923	codom	H++
ASP302	1	30.444558	codom	H+
G1376	2	23.223025	codom	H+-
G1869	3	16.805774	codom	H+++
O127	4	6.447171	codom	H+
W15	5	25.424594	codom	
O369	6	22.796697	codom	
O167	6	5.82104	codom	T++
O312	7	11.625195	codom	H+
O374	8	6.575467	codom	T+
O21	9	5.179553	codom	H-
G2020	10	8.741893	codom	H+
G1574	10	16.527	codom	H++
O149	10	16.58154	codom	H+ T+
G1037	11	5.503115	codom	H-
G1186	12	13.872383	codom	H+++
G1894	15	0.809326	codom	H+++

G124	1	9.131303	codom	H++
ASP302	1	30.444558	codom	
G1719	1	35.488312	codom	
G1274	1	45.729682	dom	
G1158	2	2.787112	codom	H++
G1376	2	23.223025	codom	H+-
G1869	3	16.805774	codom	H+++
G139	6	2.281003	codom	A++
O26	6	5.786927	codom	
O167	6	5.82104	codom	T++
ASP933	6	13.019647	codom	H++
G2034	6	22.219025	codom	H++
O369	6	22.796697	codom	
G1260	7	3.438279	codom	
G1295	7	11.243952	dom	
O312	7	11.625195	codom	H+
G1250	10	1.067244	dom	
G1574	10	16.527923	codom	H++
G1037	11	5.503115	codom	H-
G154	12	8.796449	dom	
G1186	12	13.872383	codom	H+++
G1353	13	0.817449	codom	H-A+
G1292	14	8.054896	dom	
G1454	15	0.913028	codom	H++A+
O28	18	11.99325	codom	H++
G162	18	14.881684	codom	H+

Note: Shown are the names of markers flanking putative QTL, positio
along chromosomes (chr.) in megabases (Mb) correspond to P. tricho
from neutrality (genomic clines; Lindtke et al. [9]) were documented
(A+) and P. tremula alleles (T+) in one (+) to three (+++) of the studie
value) from two-step model selection as described in main text, the p
[additive (add) or dominant (dom)]. QTL direction indicates whether t
underdominance in plants with intermediate genetic ancestries and v

m admixture mapping in natural hybrid zones.

Traits	-LOG(Pval)	PVE (%)	Signif. model	QTL direction
Chlorogenic acids				
5-Coumaroyl quinic acid	3.716	12.3	add	-
5-Caffeoyl quinic acid	3.357	9	add	+
5-Caffeoyl quinic acid	4.288	4.8	dom	-
1-Caffeoyl quinic acid	3.017	7.4	dom	-
(1,5) Dicaffeoyl quinic acid	4.386	10.7	add	-
3-feruloyl quinic acid	3.131	16.6	dom	+
3-feruloyl quinic acid	5.451	12.5	add	+
Salicinoids				
Salicortin	4.041	21	add	+
Tremulacin	3.05	25.5	add	+
Salicortin	3.067	16.9	add	+
Tremuloidin	3.138	31.7	add	+
Tremulacin Isomer	4.718	27.2	add	+
Acetyl-salicortin	3.995	15.7	add	+
Acetyl-salicortin isomer 1	8.904	10.7	add	+
Acetyl-salicortin isomer 2	7.94	16.3	add	+
Tremuloidin	9.211	28	add	+
Tremuloidin	4.557	34.3	dom	+
Tremulacin isomer	3.439	14.8	add	+
Salicortin Isomer 3	3.092	7.8	add	+
Tremuloidin	3.07	39.2	dom	+
Tremulacin Isomer	5.793	21.6	dom	+
HCH-tremulacin	3.275	6.1	dom	o/u
Acetyl-salicortin Isomer 1	3.511	17.2	add	+
Salicortin Isomer 2	5.957	14.2	dom	o/u
HCH-Salicortin	3.656	18.6	dom	-
Acetyl-tremulacin	8.386	15.1	add	-
Salicin	4.231	7.9	dom	o/u
Acetyl-salicortin Isomer 1	3.113	27	dom	+
Salicin	3.341	14.1	add	+
Tremulacin	3.89	29.5	add	+
Acetyl-salicortin Isomer 1	3.736	21.3	dom	+
Acetyl-salicortin Isomer 2	3.561	20.8	dom	+
HCH-Salicortin	3.107	10.5	add	-

Flavonoids				
Isorhamnetin-3-O-rutinoside	3.55	49	dom	-
Kaempferol-glycuronide	4.182	46	dom	+
Condensed tannins	3.461	35.3	add	-
Quercetin-3-O-arabinopyranoside	3.225	17	dom	+
Quercetin-3-O-glucuronide	3.762	15.1	add	+
Catechin	4.44	3.8	add	+
Quercetin glucuronide-pentose	5.168	42.8	dom	+
Quercetin-3-O-glucoside	3.005	28.5	add	-
Kaempferol-glycuronide	3.681	40.2	add	+
Quercetin-rhamnoside	5.396	18.8	add	+
Isorhamnetin-3-O-rutinoside	3.012	55.5	add	-
Quercetin-3-O-arabinopyranoside	3.146	21	add	+
Kaempferol-3-O-rutinoside	7.04	29.1	add	-
Quercetin-hexose-pentose	5.054	41.5	add	+
Isorhamnetin-glycuronide	4.818	33.6	dom	o/u
Quercetin-rhamnoside	4.889	25.3	dom	-
Quercetin glucuronide-pentose	6.215	35.5	dom	+
Catechin	3.292	9.7	add	-
Quercetin-3-O-glucoside	3.136	19.6	add	-
Isorhamnetin-glycuronide	3.225	38.5	dom	o/u
Quercetin-3-O-rutinoside	8.075	34.6	add	-
Isorhamnetin-3-O-rutinoside	6.326	40.5	add	-
Quercetin-rhamnoside	6.951	8.8	add	-
Quercetin-hexose-pentose	4.432	50.4	add	+
Isorhamnetin-rhamnoside	4.135	18.4	dom	o/u
Isorhamnetin acetyl-hexose	3.297	13.9	add	-
Quercetin-3-O-glucoside	4.32	24.8	dom	-
Kaempferol-3-O-rutinoside	4.471	35.4	dom	-
Condensed tannins	4.634	30.3	add	-
Condensed tannins	6.423	27.1	dom	-
Quercetin-3-O-glucuronide	4.564	8.6	add	+
Isorhamnetin-rhamnoside	6.512	13.3	add	+

ns on chromosomes and marker type [codominant (codom) or dominant (dom); [9]]. Positions
carpa genome assembly V2 available at www.phytozome.net. Deviations of genetic markers
as heterozygosity excess (H+), heterozygosity deficit (H-), homozygosity excess of P. alba alleles
d hybrid zones. Genetic mapping results for phytochemical traits are presented as –log10 (p-
ercentage of variance explained (PVE) of each putative QTL, and type of significant model
the P. tremula allele increases (+) or decreases (-) trait values. †o/u indicates QTL with over- or
without significant difference in allelic effects between parental species

S6 Table. **Excess of interspecific heterozygosity linked with phytochemical QTL**.

Contingency tables presenting counts of the presence (yes) or absence (no) of an excess of interspecific heterozygotes [9], for all 67 codominant genetic markers (S5 Table) studied in natural hybrid zones of *P. alba* and *P. tremula*, for markers representing putative QTL for salicinoids and flavonoids, and for all 34 markers representing putative phytochemical QTL in the present study.

Ticino hybrid zone				
Heterozygote excess	All markers	Salicinoids	Flavonoids	All 34 QTL
Yes	30	**8**	**11**	**21**
No	37	**8**	**10**	**22**

≥ 2 hybrid zones				
Heterozygote excess	All markers	Salicinoids	Flavonoids	All 34 QTL
Yes	22	4	**9**	**15**
No	45	12	**12**	**28**

Note: Chlorogenic acids were not tested individually, because the number of detected QTL was low (S5 Table). Patterns were examined for the Italian hybrid zone (the population of origin of our common garden trial) and across hybrid zones. Bold type identifies comparisons with heterozygote excess. For these comparisons, Fisher's exact test could not reject the independence of count distributions for QTL-linked markers (salicinoids, flavonoids, all 34 QTL) *versus* all codominant markers, i.e. there was an elevated number of markers with heterozygosity excess among the phytochemical QTL.