Blow to the northeast? Intraspecific differentiation of populus davidiana suggests a northeastward skew of a phylogeographic break boundary in East Asia

RESEARCH PAPER

Blow to the Northeast? Intraspecific differentiation of *Populus davidiana* suggests a northeastward skew of a phylogeographic break boundary in East Asia

Running title: Phylogeography of *Populus davidiana*

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Data Accessibility
All newly obtained DNA sequences (MH768816-MH768887, MH768600-MH768671, MH768672-768743) were uploaded to GenBank. The microsatellite data generated in this study has been deposited in the Dryad database (doi:10.5061/dryad.bzkh1896r).

Conflict of Interest Statement
The authors declared that they have no conflicts of interest to this work.

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ABSTRACT

Aim: There is increasing interest in the role that biological traits, and historical and biogeographic processes, play in the formation of phylogeographic patterns. An arid belt that once existed in northern China might have affected many plants, but this has yet to be untested in an arid-tolerant, wind-dispersed species. Here we tested how intrinsic and extrinsic factors have affected the phylogeography of *Populus davidiana*.

Location: East Asia

Methods: Genetic variation was surveyed across 40 populations (555 individuals) covering the Chinese range of *P. davidiana*, using 16 nuclear microsatellite loci (nSSRs) and four chloroplast fragments (cpDNA). Demographic and migration hypotheses were tested using coalescent-based approaches, and the present and past potential distributions were predicted using species distribution modelling.

Results: Molecular data divides *P. davidiana* into two lineages, northeastern China (NECR), and central and northern China (CNCR); however, the dividing line is around 118ºE for nSSRs, but 122ºE for cpDNA. The range and habitat of the two lineages barely overlap at present, and their ecological separation may have initiated around the Pliocene-Quaternary boundary, when major intraspecific cpDNA clades diverged. NECR and CNCR experienced postglacial northeastward and northward range shifts, respectively. Bi-directional historical gene flow was detected between NECR and CNCR for both bi-parentally inherited nSSRs and maternally inherited cpDNA. Demographic inferences suggest a severe bottleneck for CNCR and especially NECR, around the latest Pleistocene.

Main conclusions: The phylogeographic break within *P. davidiana* reflects the impacts of biogeographic history, climate and biological traits. Its plumed, wind-dispersed seeds might be especially significant, because prevailing southwestern spring winds may have moved the NECR-CNCR boundary further east than similar phylogenetic breaks in other species, and also moved the cpDNA boundary relative to that for nuclear markers. Biological traits, therefore, should also be considered when examining the genetic and ecological differentiation between closely related taxa.

KEYWORDS

phylogeographic break, arid belt, demographic history, species distribution modelling, biological traits, aspen

1 | INTRODUCTION

Biogeographic patterns within and between species are determined by both extrinsic (e.g. Avise, 2009; Hewitt, 2000; Hickerson et al., 2010; Gavin et al., 2015), and intrinsic factors (e.g. Lê, Josse, & Husson, 2008; Papadopoulou & Knowles, 2016). Extrinsic factors include historical and biogeographic processes, such as climate shifts and their knock-on effects (e.g. Avise, 1992; Matlack, 1987; Ye et al., 2017a), the uplift of mountains (e.g. Du et al., 2017), and the formation of rivers (e.g. Yue et al., 2012). Intrinsic factors, such as biological traits, have received considerably less attention (e.g. Papadopoulou & Knowles, 2016; Paz et al., 2015; Sukumaran & Knowles, 2018), but can affect phylogeography by influencing gene flow, effective population size (N_e), ecological adaptation and establishment in new habitats (Freeland, 2011). For similarly distributed but unrelated lineages, extrinsic factors generate concordant patterns (Avise, 1992; Chen et al., 1989; Joseph et al., 1995; Milne & Abbott, 2002; Minami & Azuma, 2003; Wang et al., 2016; Qiu, et al., 2011; Ye et al., 2017a), whereas intrinsic factors usually underlie taxon-specific patterns (Papadopoulou & Knowles, 2016). Traits that affect ecological adaptation are difficult to measure directly, but can be examined indirectly via range and niche reconstruction through species distribution modelling (Catullo et al., 2015; Elith & Leathwick, 2009).

The mesic vegetation of China was divided by an arid belt lying between ~35°N and ~45°N, which varied in width and intensity especially during the late Tertiary period (Guo et al., 2008). Though its intensity is now reduced, this arid belt divides the East Asiatic floristic kingdom, and may account for clear phylogenetic divides between these regions in many Tertiary relict groups (Donoghue & Li, 2001; Milne & Abbott, 2002). The biogeographic effect of this belt on any lineage would depend on its tolerance of aridity, and many lineages exhibit varying degrees of gene flow between the two sides of the arid belt (Guo et al., 2014; Liu & Ko, 2014; Zong et al., 2015; Ye et al., 2017b; Bai et al., 2016).

Populus davidiana Dode is an arid- and cold-tolerant, wind-pollinated and wind-dispersed deciduous tree species distributed across northern China, with extensions into the Korean Peninsula, easternmost Mongolia and the Russian Far East (Zheng et al., 2017; Hou et al., 2018; Fang, Zhao, & Skvortsov, 1999), and hence on both sides of the arid belt. Zheng et al. (2017) studied species delimitation and lineage divergence history of the *Populus davidiana* complex (which includes *P. davidiana* and *P. rotundifolia* Griffith), and detected intraspecific genetic differentiation within this species, across where the arid belt lay, suggests a barrier to intraspecific gene flow in the past. Yet, only 165 individuals from 33 populations of the species were sampled (Zheng et al., 2017).

In this study, we sampled 555 individuals from 40 populations of *P. davidiana* across

northern and northeastern China, and examined intraspecific variation using 16 nuclear microsatellite loci (nSSRs) and four chloroplast fragments (cpDNA). Historical rates of gene flow and demographic histories were inferred, and species distribution modelling (SDM) was used to predict distributions for nine time periods. We aimed to (1) determine whether the phylogeographic divide identified in *P. davidiana* matches that of other plant lineages, and (2) to figure out the roles that external and intrinsic factors have played in shaping the phylogeographic pattern of this species. Throughout this paper, "*P. davidiana*" refers to *P. davidiana sensu stricto*, i.e. excluding *P. rotundifolia* as per Zheng et al. (2017).

2 | MATERIALS AND METHODS

2.1 | Population sampling and genotyping

We sampled 555 individuals from 40 populations throughout the main range of *Populus davidiana*, in northern China (Fig. 1, Table S1 in Appendix S3), including the 170 individuals from 33 populations sampled by Zheng et al. (2017). Between three and 27 trees were sampled from each population, with all sampled individuals at least 100 m apart from one another. A total of 541 individuals were successfully genotyped using a set of 16 nuclear microsatellite loci (Appendix S3, Table S2), and four chloroplast DNA (cpDNA) regions (*mat*K, *trn*G-*psb*K, *ps*bK-*psb*I, and *ndh*C-*trn*V) (Appendix S3, Table S2) were sequenced for 370 individuals across all sampled populations, plus one *P. adenopoda* individual as outgroup. For leaf collection, DNA extraction, PCR and sequencing methods see Appendix S1, Text 1.1. Allele sizes for each nSSR locus were analyzed with GeneMarker version 2.2.0 (Softgenetics, Pennsylvania, USA).

2.2 | Statistical Analysis of cpDNA data

CpDNA sequences were edited, aligned, manually checked, and concatenated with Clustal W in MEGA 5.0 (Tamura et al., 2011). All sequences generated in this study were then deposited in NCBI GenBank (Accession Numbers: MH768816-MH768887, MH768600-MH768671, MH768672-768743). Insertions/deletions (indels, excluding mononucleotide repeats) were encoded by software Gapcoder (Young & Healy, 2003), and then the 0/1 characters (except '–' gaps after coding) were replaced manually by A/T to use indel information (e.g. Havrdová et al., 2015). The haplotype variant sites were detected using DNASP v.5.10 (Librado & Rozas, 2009). NETWORK v.4.6 was adopted to infer network relationships between cpDNA haplotypes based on sequence variation (Bandelt, Forster, & Rohl, 1999). After that, to compare the genetic diversity of each population, haplotype diversity (H_d) and nucleotide diversity (π) were calculated at the population level using DNASP v5 (Librado & Rozas, 2009). In addition, a test of

phylogeographic structure was conducted in PERMUT (available at: http://www.pierroton.inra.fr/genetics/labo/Software/Permut) using 1,000 permutations (Appendix S1, Text 1.2). Finally, to perceive the distribution pattern of genetic variation within the cpDNA dataset, analyses of molecular variance (AMOVA) were carried out in ARLEQUIN version 3.0 (Excoffier, Laval, & Schneider, 2005), with significance tests based on 1,000 permutations. Genetic variation was hierarchically partitioned into three levels: among groups, among populations within group, and within populations.

The genetic structure and the potential genetic barriers between populations were analysed using SAMOVA version 1.0 (Dupanloup, Schneider, & Excoffier, 2002) based on a simulated annealing procedure (Appendix S1, Text 1.3). We calculated the F_{CT} value for each group number from 2 to 8, and set the number of simulated annealing processes to 100.

To calculate divergence times, we adopted a two-step approach in BEAST v.1.7.5 (Drummond & Rambaut, 2007), taking one to two haplotypes from each haplotype lineage, and adopting node ages from Zhang et al. (2018). Three additional outgroups, *P. laurifolia*, *P. tremula*, *P. lasiocarpa*, were included, using sequence data from Zhang et al. (2018).

2.3 | Statistical Analysis of Microsatellite data

Microsatellite data were read by GeneMarker (Softgenetics, Pennsylvania, USA) and then corrected by FlexiBin Excel macro (Amos et al., 2007). Allele sizes at each locus were scored and checked for possible genotyping errors like stuttering, large allele dropouts and null alleles in CERVUS v3.0 (Kalinowski et al., 2010). One locus (GCPM_126) at which high frequency null alleles (F [Null] > 0.4) were detected was eliminated, whereas the remaining 15 nSSR loci (Appendix S3, Table S2) were employed to estimate genetic diversity indices in GenAlEx version 6.5 (Peakall & Smouse, 2012) (Appendix S1, Text 1.4). Subsequently, BayeScan v.2.1 (Foll & Gaggiotti, 2008) was employed to detect nonneutral evolutive forces that have acted on microsatellite loci, such as diversifying and purifying selection (Appendix S1, Text 1.4), and 10 neutral loci were retained to conduct the following population genetic analyses if not stated otherwise.

To investigate population subdivision within *P. davidiana*, a Bayesian clustering method was used, as implemented in STRUCTURE v.2.3.4 based on microsatellite data (Pritchard et al., 2000) (Appendix S1, Text 1.5). STRUCTURE results were summarized and visualized using Structure Harvester (Earl & vonHoldt, 2012). To cross-validate the results of STRUCTURE, we also conducted a Principal Coordinates Analysis (PCoA) based on the nSSR data using GenAlEx version 6.5 (Peakall & Smouse, 2012).

For microsatellite data, analyses of molecular variance (AMOVA) were also carried out in ARLEQUIN version 3.0 (Excoffier et al., 2005), with significance tests based on 1,000 permutations.

2.4 | Isolation by distance, gene flow and demographic history

In order to test the influence of geographical distance on the genetic structure, we first used the software GenAlEx v 6.5 (Peakall & Smouse, 2012) to obtain the matrix of geographic distances between each pair of populations, and then imported the matrix of F_{ST} values calculated by the software ARLEQUIN v 3.5 into EXCEL. Subsequently, a Mantel test (between geographic distance and genetic differentiation, i.e. isolation-by-distance test) was performed using the software GenAlEx v 6.5, with 1,000 random permutations. With similar parameter setting, a stratified Mantel test was conducted for core populations of each putative intraspecific groups considering the results from STRUCTURE based on nSSRs and SAMOVA based on cpDNA.

To estimate the amount and direction of gene flow between groups of *P. davidiana* based on neutral nSSR data, the software MIGRATE v 3.3.1 was employed (Beerli & Felsenstein, 1999; Beerli and Palczewski, 2010). The parameters M (migration rate divided by the mutation rate) and the effective number of migrants ($2N_em$, where N_e is effective population size and m is the migration rate) were calculated using the Brownian motion model (Appendix S1, Text 1.6).

Given that MIGRATE analyses assume a constant effective population size for each lineage, which might be violated in our case according to the STRUCTURE results, we also analyzed our data using DIYABC, which allowed us to test explicitly the hypotheses of demographic history that each lineage may have experienced. To do so, we have simulated and compared seven scenarios concerning the history of effective population size changes for each presumed evolutionary lineage, using the approximate Bayesian computation procedure (Beaumont, Zhang & Balding, 2002) as implemented in DIYABC v.1.0.4.46 (Cornuet et al., 2008) based on nSSR data. The seven possible scenarios allowed for ancient (100,000 to 450,000 generations ago) expansion, contraction, or neither, followed by recent (one to 20,000 generations ago) expansion, contraction, or neither (Appendix S2, Fig. S1; full details were given in Appendix S3, Table S3 and Appendix S1, Text 1.7). Using a direct approach and logistic regression analyses, the posterior probability of all scenarios was calculated and compared. Following Macaya-Sanz et al. (2012) we set the generation time of *P. davidiana* as 40 years, with a span of 20 to 60 years. To alleviate the impact of inter-lineage gene flow on the testing of demographic history scenarios, when $K = 2$ according to STRUCTURE results, all individuals that were potential hybrids

(identified as such from Q values > 0.125 for each of the two assumed genetic groups), were eliminated.

2.5 | Species distribution modellings (SDMs)

SDMs were conducted using the maximum entropy method implemented in MAXENT v.3.2.1 (Phillips et al., 2006) to predict the distribution of potentially suitable habitat for *P. davidiana* in four time periods: the present, the Last Glacial Maximum (LGM; c. 21 thousands years ago (kya)), the Last Interglacial (LIG; c. 130 kya), and the Mid-Holocene (MH; c. 6 kya). Nineteen bioclimatic variables at 2.5-arc-minute resolution were downloaded from the WorldClim database (Hijmans et al., 2005). Strong co-linearity between bioclimatic variables may affect the accuracy of the model. Therefore, a Pearson correlation test was performed on these bioclimatic variables, across the 40 populations. Eight bioclimatic variables between which all pairwise Pearson correlation coefficients r were ≤ 0.70 (Appendix S3, Table S4) were retained and used for subsequent SDM analysis. For full details of SDM analysis see Appendix S1, Text 1.8. DIVA-GIS v.7.5 (Hijmans et al., 2001) was used to map the distribution of habitat suitability. We also conducted a Principal Component Analysis (PCA) of the eight bioclimatic variables that were used for SDMs, based on two R packages: FactoMineR (Lê, Josse & Husson, 2008) and FactoExtra (Kassambara & Mundt, 2017).

The SDM analysis was repeated in the same way for five further time slices, except that in these cases only 14 bioclimatic factors were available, and only six were retained following Pearson correlation tests (Appendix S3, Table S5). These time slices were the Younger Dryas Stadial (12.9-11.7 Ka), the Bølling-Allerød period (14.7-12.9 Ka), the Marine Isotope Stage 19 (MIS19) in the Pleistocene (~787 ka), the mid-Pliocene Warm Period (~3.264–3.025 million years ago [Ma]), and MIS M2 in the Late Pliocene (~3.3 Ma).

All SDM analyses were conducted on the full set of 40 populations, and also on each of the two subsets CNCR and NECR defined by nSSR data (see Results; Figs 2a, d).

3 | RESULTS

3.1 | Variations of nuclear microsatellite data and population subdivision

Across all 541 individuals and 40 populations genotyped, a total of 150 alleles were scored from 15 microsatellite loci, and the number of alleles per locus varied from 4 to 19 alleles, with an average of 10 (Appendix S3, Table S6). Among populations, the mean values for descriptive variables were: number of alleles (A_a) was 35.73, the number of effective alleles (A_e) was 2.43, the Shannon index (I) was 0.86, the observed heterozygosity (H_o) was 0.41, the expected heterozygosity (H_e) was 0.46, and the allelic richness based on

5 samples (Ar_{5}) was 2.23 (Appendix S3, Table S7). The genetic differentiation index (F_{ST}) and standardized F_{ST} (F_{ST}') averaged across all loci was 0.18 and 0.45 (Appendix S3, Table S6), indicating a pronounced level of genetic differentiation among populations.

For the ten neutral nSSR loci (40 populations, n = 541), STRUCTURE yielded the highest likelihood when K = 2 (Appendix S2, Fig. S2a, b), suggesting the existence of two genetic clusters, and subsequently two population groups (Fig. 2a). We also conducted STRUCTURE for the five non-neutral loci, as well as all 15 loci, both suggested similar population grouping schemes when K = 2 (Appendix S3 Fig. S2c, d). All populations of the CNCR group (populations 1-19; central and northern China region) except p15 (44%) had between 56% and 98% of genetic ancestry assigned to cluster I, whereas all populations in the NECR group (populations 20-40; Northeastern China Region) had between 73% and 96% of genetic ancestry assigned to cluster II (Fig. 2a; Table S8 in Appendix S3). The PCoA based on genetic distance showed a similar population genetic structure (Fig. 2c). Hence, all subsequent analyses that considered information from nuclear DNA, were conducted considering this subdivision of sampled populations into CNCR and NECR.

3.2 | Variations and distribution pattern of cpDNA sequences

The total length of the aligned matrix that concatenated the four cpDNA fragments was 2,296 bp, among which 27 substitutions and 28 indels were detected (Tables S9, S10 in Appendix S3). A total of 72 haplotypes (Tables S9, S10 in Appendix S3) were differentiated based on these, but 37 were excluded because they were singletons that could have resulted from PCR errors (Tables S9, S10 in Appendix S3); therefore 35 haplotypes were retained (Fig. 3b). Nevertheless, for reference, we have presented basic analyses based on all 72 haplotypes in the supplemental information (Appendix S2, Fig. S3).

The phylogenetic network of these 35 haplotypes revealed five distinct haplotype lineages (HLs) with adjacent geographical ranges yet little overlap (Fig. 3b). Haplotype lineages A, B, C, D, and E comprised haplotypes H8-H15, H16-17, H18-26, H29-35, and H1-H7, respectively (Fig. 3b), and molecular dating suggested that HL-E diverged from the others (0.90-) 2.50 (-3.43) Ma (i.e. 2.50 Ma with 95% HPD: 3.43-0.90 Ma; Appendix S2, Fig. S4). In general, HL-E occurred mainly in NECR, whereas the other four mostly (HL-A and HL-D) or only (HL-B and HL-C) occurred in CNCR (Fig. 3a). Nevertheless, the admixture of different haplotypes and haplogroups was found to be prevalent, with 17 CNCR and 10 NECR populations containing ≥2 haplogroups (Fig. 3) (see more details in Appendix S1, Text 1.9). Because HL-E corresponds mostly but not precisely with NECR, we use the terms cpNECR and cpCNCR to describe the two biogeographic groups defined by cpDNA data (Figs 2d, 3a).

3.3 | Population genetic structure

Our AMOVA analysis based on nSSR data revealed that 79.93% of the overall variation was within populations, with 13.38% among populations within groups, and just 6.69% between groups (Table 1). Genetic differentiation among and within populations accounted for 14.86% and 85.14% respectively for CNCR (Appendix S3, Table S11), and 7.67% and 92.33% respectively for NECR (Appendix S3, Table S12). The isolation-by-distance test revealed that pairwise geographical distance was weakly but significantly correlated with genetic differentiation in all populations ($R^2 = 0.1124$, $P = 0.0003$; Appendix S2, Fig. S5a) and core NECR populations (p24-38; $R^2 = 0.2606$, $P = 0.0002$; Appendix S2, Fig. S5c), yet no significant correlation was found in core CNCR populations (p1, p3-10, p12-19; $R^2 = 0.0031$, $P = 0.67353$; Appendix S2, Fig. S5b). The core NECR and CNCR populations correspond to cpDNA SAMOVA groups IV and II, respectively (see below).

AMOVA based on cpDNA found that 45.69% of variation was distributed within populations, 39.01% was portioned among populations within groups, and 15.30% between groups (Table 1). For CNCR, 52.35% of variation was among populations and 47.65% within; for NECR these values were 39.52% and 60.48%, respectively (Tables S11, S12 in Appendix S3). The permutation test (based on cpDNA) indicated that N_{ST} was significantly higher than G_{ST} across all populations, and also when either CNCR or NECR were considered separately ($P < 0.05$ in each case; Table S13 in Appendix S3), pointing to a strong phylogeographical structure for *P. davidiana*.

SAMOVA based on cpDNA showed the highest F_{CT} value for a model with six population groups (Appendix S3, Table S14; Fig. 3a). However, four of these groups contained only one population each (Fig. 3a). Of the two large groups, IV comprised fifteen NECR populations (p24-p38), but the three NECR populations furthest west (p21-p23) were placed in Group II, together with most CNCR populations (p1, p3-p10, p12-p20) (Fig. 3a). Group IV individuals mostly possess HL-E haplotypes, whereas other haplogroups occurred mainly in group II.

3.4 | Demographic history of NECR and CNCR, and gene flow between them

In the DIYABC analyses, scenario 7 was consistently the best supported based on direct estimate, logistic regression and PCA plots (Figs S6, S7, S8, S9 in Appendix S2) and also the least error-prone (Tables S15, S16 in Appendix S3) for each of CNCR and NECR (excluding p20-23). In this scenario, *P. davidiana* in each region independently experienced an ancient expansion and a recent bottleneck (i.e. contraction then expansion). Expansion in CNCR and NECR groups occurred around 226.8 Ka and 214.8 Ka, respectively, and a strong bottleneck occurred between 20.0 and 10.7 Ka for CNCR, and between 23.7 and

13.2 Ka for NECR (Fig. 4; Tables S17, S18 in Appendix S3). The bottleneck event in NECR (N2/N3≈1/382) was much stronger than that in CNCR (N2/N3≈1/37).

The MIGRATE analyses based on the ten neutral nSSR loci under both the variable theta and the same theta models output results where the posterior distribution of all parameters exhibited a normal distribution and the effective sampling size of all parameters exceed 200, indicating that both models reached convergence (Figs S10, S11). The MIGRATE analyses based on the variable theta model suggested that the mean estimated gene flow ($2N_em$) from NECR to CNCR (excluding p20-23) is 22.57, which is smaller than that in the opposite direction (36.88). However, based on the same theta model, gene flow in each direction was similar, i.e. 16.24 from NECR to CNCR, vs 15.27 the other way (Table 2, Fig. 2b). Meanwhile, the estimated N_e of CNCR was slightly larger under the variable theta model (173.91 vs. 147.55) but smaller under the same theta model (100.02 vs 116.35; Table 2, Fig. 2b).

3.5 | Species distribution modelling

The AUC values of SDMs varied from 0.931 to 0.985 (mean 0.960) in CNCR and from 0.973 to 0.981 (mean 0.978) in NECR, which indicated that all models performed better than random expectation. Variable jackknife analyses suggested that Mean Temperature of Driest Quarter (35.2%), was the environmental variable that contributed most to potential distribution modelling for CNCR, whereas Annual Precipitation (34.6%) and Mean Temperature of Driest Quarter (32.9%) were the two environmental variables that contributed most to potential distribution modeling for NECR (Appendix S3, Table S19). The predicted current distribution range of both NECR and CNCR of *P. davidiana* was highly consistent with the actual occurrence of our sampled populations (Figs 5a-b), and also very similar to that during the mid-Holocene, especially for CNCR (Figs 5a, c). However, when only the potential distribution with high habitat suitability (>0.6) is considered, then compared to the current predicted range (Fig. 5a), CNCR occupied a similar range yet with a slightly larger and continuous distribution in its eastern range during the MH (Fig. 5c) and its central range during the LGM (Figs 5e), whereas it migrated southward and westward and occupied a fragmented range during the LIG (Fig. 5g). The SDMs also suggested that NECR migrated southwards during the LIG, and could potentially have disjunctly occupied the Qinghai-Tibetan Plateau (Fig. 5h), which is ~2000 km from the rest of its predicted range, and not suitable for it at present (Fig. 5b). As with CNCR, a larger continuous distribution was predicted for NECR during the LGM (Fig. 5f), yet unlike CNCR, the range of NECR was shifted some way southwestwards at this time, expanding southward as far as ca. N34° while its northern fringe retreated. At the same time, the potential distribution of NECR was similar, by and large, between the present

(Fig. 5b) and the MH (Fig. 5d), although its MH distribution extended further southward, by ca. 5° to N35°.

According to the niche identity test undertaken using ENMTools, the observed values of Schoener's *D* (0.354) and Hellinger's *I* (0.639) were significantly different from the null distributions, indicating that CNCR and NECR were ecologically distinct lineages (Appendix S2, Fig. S12). The results of PCA analysis for climate variables were consistent with SDMs (Appendix S2, Fig. S13).

Concerning the connection between the predicted distribution of CNCR and NECR, SDMs suggested that they were connected by areas of no more than intermediate suitability during LGM, the Holocene and the present (Figs 5a-f). During the LIG, the suitable area for NECR included the Qinghai-Tibet Plateau, as noted above, and overlapped with that of CNCR (Figs 5g-h), although that part of NECR's potential range might not have been occupied. For time periods with fewer bioclimatic variables available, the potential distribution for NECR was larger during each of the Younger Dryas Stadial (12.9-11.7 Ka), the Bølling-Allerød period (14.7-12.9 Ka) and the MIS19 in the Pleistocene (~787 ka), yet smaller and fragmented for CNCR in all these periods (Appendix S2, Figs S14a-h), generating a few areas of overlapping suitability between CNCR and NECR, mostly around central China. Furthermore, during two Pliocene time slices (~3.264–3.025 Ma and ~3.3 Ma, Figs S14i-l in Appendix S2), the potential distribution areas were narrower for CNCR and slightly larger but less continuous (compared to the present) for NECR, but with less potential overlap between them.

4 | DISCUSSION

Both cpDNA (Fig. 3) and nSSR markers (under both STRUCTURE and PCoA; Figs 2, S15) clearly divided *Populus davidiana* into two intraspecific groups of populations in the northeastern China region (NECR), and the central and northern China region (CNCR). However, the boundary between regions is different between markers, lying around 118ºE for nSSRs (CNCR vs NECR), and 122ºE for cpDNA (cpCNCR vs cpNECR) (Figs 2, 3). Moreover, historical bi-directional gene flow between the NECR and CNCR regions was indicated for both SSRs and cpDNA data (Figs 2b, 3a). An identity test of ecological niches reveals clear habitat separation between the two (Fig. 6), and SDM analysis also indicates strong but incomplete separation between these groupings, with each having only medium habitat suitability to the main range of the other (Figs 5a-b).

4.1 | The separation of NECR and CNCR lineages reflects the impact of different factors

Network analysis resolved two major cpDNA haplotype clades. Of these, HL-E was the dominant haplotype within cpNECR (91% of individuals), whereas HL-A+B+C+D accounted for 93% of individuals in cpCNCR. Hence it seems likely that this biogeographic subdivision originated with divergence between these two clades, but bidirectional haplotype flow between them followed. The two clades diverged (0.90-) 2.50 (-3.43) Ma (Appendix S2, Fig. S4), i.e. around the Pliocene/Quaternary boundary. This is somewhat younger than the estimated origin of similar central-northern China vs. northeastern China divides within *Acer mono* ((4.13-) 6.98 (-9.84) Ma; Guo et al., 2014), and *Lindera obtusiloba* ((1.13-) 4.40 (-8.75) Ma; Ye et al., 2017b), and between *Juglans mandshurica* and *J. cathayensis* ((5.97-) 10.93 (-17.21) Ma; (Bai et al., 2016), but older than that between *Quercus liaotungensis* and *Q. mongolica* ((0.20-) 0.92-2.15 (-9.23) Ma; Yang et al., 2016), and subdivision within *Bupleurum longiradiatum s.s.* (<1.54 Ma; Zhao et al., 2013). Therefore, *P. davidiana* may be unique among these, in that intraspecific divergence might have been caused by the onset of the Quaternary, implying a likely role of climatic changes at that time.

Populus davidiana diverged from *P. tremoloides*, then *P. tremula* and finally *P. rotundifolia* during the Pliocene and Quaternary (Du et al., 2015; Zheng et al., 2017; Hou et al., 2020; Li et al., 2020), in the process probably moving from higher to lower latitudes (Du et al., 2015), and undergoing a long history of range shifts. Around the Pliocene-Quaternary boundary (2.5 Ma), the two major cpDNA lineages diverged. SDM analysis for the closest available time periods (~3.3 Ma and ~3.264–3.025 Ma; Figs S14i-j, S14k-l in Appendix S2), indicate a more restricted range for *P. davidiana* than more recent periods, which might have initiated geographical and ecological separation between CNCR and NECR. However, differences between SDMs for those two periods indicate ranges fluctuating with the climate, so there could have been periods of contact later on. Based on late Quaternary (LIG and LGM; Figs 5e-h) SDMs, the ranges of CNCR and NECR might have remained largely separated for most of the Quaternary, though again varying climates might have allowed overlap during some interglacials. The degree of predicted overlap increased slightly after the LGM during the Younger Dryas Stadial, the Bølling-Allerød period, the Holocene and the present (Figs 5a-d) following the step-wise northward retreat of the southern edge of NECR, accounting perhaps for the evidence of gene flow between regions (Figs 2a-b, 3a, and see below). However, even if a substantial gap existed between NECR and CNCR during the Quaternary, this might not have prevented gene flow at the time, because *Eleutherococcus senticosus* (Wang et al., 2016) and *Bupleurum longiradiatum* (Zhao et al., 2013), though not *Lindera obtusiloba* (Ye et al., 2017b), show evidence of gene flow and shared haplotypes across a range gap of 400 km or more in this

region (Figs 2a, 3a). Our MIGRATE analyses based on nSSRs (Table 2) as well as *fastsimcoal2* analyses based on population genomic data (Hou et al., 2020) both suggested that bi-directional historical gene flow may have occurred between NECR and CNCR lineages after their divergence**.** Nevertheless, our SDMs support that intrinsic factors, i.e. differentiated ecological niches between CNCR and NECR through different time periods, may have been more important for driving their separation than extrinsic factors, such as the arid belt in northern China.

In addition to the above, the divergence between the two lineages may have been promoted by genetic drift, exacerbated in each lineage by a recent bottleneck event. Our DIYABC inferences revealed severe bottleneck events, which started around the LGM and ended around the beginning of the postglacial period (i.e. the Holocene), for both for NECR (ca. 23.7 to 13.2 Ka) and CNCR (ca. 20.0 to 10.7 Ka) (Fig. 4; Tables S17, S18 in Appendix S3); MSMC analyses based on population genomic data, from a sample of fewer populations and individuals of *P. davidiana*, also suggested a bottleneck event for both lineages (Hou et al., 2020). In addition, a similar demographic scenario was also favored for a congener, *P. adenopoda*, that occurs in subtropical China (Fan et al., 2018). Perhaps because the NECR populations occupy higher latitudes than the CNCR population, NECR experienced not only an earlier (see above) but also stronger bottleneck event, i.e. a ratio of 1/382 between bottleneck and pre-bottleneck N_e, compared to a 1/37 ratio for CNCR. This might be due to climate shifts, since both lineages might have experienced southward retreat during the Bølling-Allerød period, 14.7- 12.9 Ka, (Appendix S2, Figs S14h-i) and the Younger Dryas Stadial, 12.9-11.7 Ka (Appendix S2, Figs S14e-f). During these periods, the area of high habitat suitability (>0.6) excludes ~half or more of current occurrence sites. However, while DIYABC and SDM results appear to be consistent with one another, many sources of uncertainty apply for ABC inferences, concerning the choice of models, assumptions about generation times, overlapping of generations, confidence interval of estimated parameters, and especially natural selection and gene flow (Kuhner, 2009; Tsuda et al., 2015, 2017). Specifically, we were not able to consider gene flow between CNCR and NECR in our ABC model, and this may have affected the accuracy of our model testing. Instead, we sought to minimise this issue by eliminating non-neutral loci as well as individuals that are potential F1 and BC hybrids in both lineages.

Considering the substantial difference between the ecological niches of CNCR and NECR lineages (Appendix S2, Fig. S12), it is likely that natural selection may have played a role during lineage divergence. The BayeScan analysis revealed that five of 15 microsatellite loci examined may have experienced natural selection, and based on Bayesian clustering analyses, these five non-neutral loci produce population subdivisions

similar to those for all loci, as do the ten neutral loci (Appendix S2, Fig. S2c, d). Such a congruence between non-neutral and neutral markers, as well as slightly higher average F_{ST}' value of the former (0.53 vs. 0.45; Appendix S3, Table S6), may be explained by a model wherein natural selection acted on non-neutral loci, lead to divergence of non-neutral loci, after which divergence hitchhiking and genomic hitchhiking caused a divergence of neutral loci (e.g. Feder et al., 2012). This has been partly confirmed by a recent population genomic survey (Hou et al., 2020). It is also possible that natural selection act upon non-neutral loci after allopatric divergence of the two lineages. Unfortunately, the evidence collected here is not sufficient to test all of the above hypotheses thoroughly. We propose that increased genome coverage, coupled with the dense population sampling employed here, will enable a better testing on the relative roles of ecological barriers, demographic history, genetic drift and natural selection in driving the lineage divergence of *P. davidiana* (e.g. Wang et al., 2016, 2020; Ma et al., 2018; Hou et al., 2020).

4.2 | A southwesterly prevailing wind in the spring skewed the intraspecific phylogeographic break within *P. davidiana*

One interesting intraspecific divergence pattern in *P. davidiana* is that the boundary between NECR and CNCR regions is different between markers. SAMOVA separated two regional groupings, each comprising one large group and two single population groups, here termed the cpCNCR (p1-23) and cpNECR (p24-40; Fig. 3a), with the boundary around 122ºE. Conversely, nSSR data separates CNCR (p1-19) from NECR (p20-40) at around 118ºE. Therefore, there is consensus between nSSR and cpDNA markers regarding populations to the west of 118ºE (p1-19, sCNCR) and those to the east of 122ºE (p24-40, sNECR); however, populations 20-23 (termed as introgressed populations in Fig. 2d) inhabit a region of overlap and discordance between 118ºE and 122ºE, because they have northeastern nDNA but southwestern cpDNA. These occupy an area that SDMs suggested to be a contact zone of the two groups (Figs 5a-b). Among similarly distributed taxa that have been biogeographically examined, all show a northeast-southwest subdivision around this region. Indeed, the overlap area (p20-23) corresponds closely with the region of overlap between two *Quercus* species that share many haplotypes (Yang et al., 2016), a gap in the distribution of *Eleutherococcus senticosus* (Wang et al., 2016), and a west to east dividing line within *Corylus mandshurica* (Zong et al., 2015) (Fig. 2a). However, comparable dividing lines within *Acer mono* and for *Juglans mandshurica* vs *J. cathayensis* both lie further southwest (Bai et al., 2016; Guo et al., 2014; Liu & Ko, 2014), as do the large range gaps within *Lindera obtusifolia* (Ye et al., 2017b) and *Bupleurum longiradiatum s.s*. (Zhao et al., 2013) (Fig. 2a). Curiously, the width of the gap does not correlate with the degree of detectable gene flow across these examples, because there is

no haplotype overlap within *A. mono* (Guo et al., 2014), and only very limited overlap (one population) in *Juglans* (Bai et al., 2016); conversely, ample gene flow across the gaps is detectable in *Eleutherococcus* (Wang et al., 2016), though not *Lindera* (Ye et al., 2017b) or *Bupleurum* (Zhao et al., 2013). This might be due to past range shifts in these species. Notably, all of these dividing lines lie to the west of that between the temperate coniferous broadleaved mixed forest vegetation zone and the warm temperate deciduous broadleaved forest zone, which lies around 124ºE and between p25 and p26 (Figs 1, 2a).

In *Populus*, cpDNA is maternally inherited and hence dispersed by seeds only, whereas nDNA is bi-parentally inherited and dispersed by both seeds and pollen. However, the prevailing wind direction during the flowering (March-April) and fruiting (April-May) seasons of *P. davidiana* is SW to NE (Fig. 6). This might facilitate the migration of insects into Northeastern China (Chen et al., 1989), and explain why asymmetric nuclear gene flow is mainly SW to NE between *Quercus liaotangensis* and *Q. mongolica* (Yang et al., 2016). It might also explain why the CNCR and NECR boundary, as reflected by nSSRs, lies further northeast than those in other species discussed above. *Quercus* seeds are unaffected by wind, but *Populus* seed is light and has plumes for wind dispersal (Fang, Zhao, & Skvortsov, 1999). Based on a calculated falling velocity of only 0.23 m/s for *P. sieboldii* (Minami & Azuma, 2003), and a calculation for the slightly faster falling plumed seeds of *Asclepias syriaca* (Matlack, 1987), *Populus* seeds might typically travel 18 km in 10 kph winds. At the same time, pollen typically travels 0.05-10 Km but may also travel more than 100 Km (Ashley, 2010). Hence, the prevailing wind from SW to NE might have facilitated both seed flow and pollen flow from SW to NE for *P. davidiana*, and therefore skewed the nSSRs boundary between southwestern and northeastern populations, moving it eastward.

Meanwhile, the cpDNA dividing line of *P. davidiana* lies further east than the nSSR dividing line, as well as dividing lines in most of the other species mentioned (Fig. 2a). A hypothesis to explain this pattern is a series of hybridization events that have led to the introgression of CNCR cpDNA into NECR populations. SDM analysis suggests that CNCR and NECR occupy different niches: the CNCR lineage is mainly affected by Mean Temperature of the Driest Quarter (bio_9), whereas both Mean Temperature of the Driest Quarter (bio_9) and Annual Precipitation (bio_12) may have affected NECR (Appendix S3, Table S19). SDM analysis suggests that CNCR material might have invaded populations p20-23 while NECR was retreating northward following the middle Holocene (Figs 5c-d). When a few CNCR immigrants, probably via seed propelled by the prevailing southwesterly wind, arrived among populations p20-23 and encountered native trees, they may have formed inter-lineage hybrids. Assuming the existence of pollen competition

mechanisms that favour material of the same lineage, immigrant individuals would have tended to be the female parent to hybrid seed due to a scarcity of pollen from the same lineage. This would gradually lead over time to introgressed individuals tending to possess SW cpDNA and mostly NE nSSRs. If introgressed individuals had a selective advantage over standing NECR stock at that time, perhaps because the local climate was becoming more suitable to SW material, then introgressed individuals might have gradually replaced most native trees in this area. This process, therefore, may have moved the cpDNA division line to the east between p23 and p24, while the nSSRs division line remained static between p19 and p20.

Other species exhibiting phylogeographic breaks in this area are either not wind-dispersed (*Quercus, Juglans, Lindera, Eleutherococcus*, and *Corylus*) or have winged seeds that are either larger (*Acer mono*) or borne closer to the ground (*Bupleurum*) (Guo et al., 2014; Zhao et al., 2013). Hence none of these may respond to the prevailing wind as strongly as *P. davidiana,* and this could explain why the *Populus* cpDNA dividing line is further east than for most of the other species mentioned (Fig. 2a). Curiously, *Juglans*, with similar seed and pollen dispersal to *Quercus*, exhibits very little between species gene flow (Bai et al., 2016), implying that other factors like historical range shifts may also be important in these cases. Bird-dispersal of edible fruits could explain the ample gene flow in *Eleutherococcus* (Wang et al., 2016).

In conclusion, we have presented evidence for a phylogeographic break within *P. davidiana*, where the predominant southwesterly wind in the spring may have skewed the boundary between NECR and CNCR, moving it northeastward relative to other species, and separating the boundaries indicated by cpDNA and nuclear data. The ecological separation between NECR and CNCR may have formed since the divergence of major cpDNA haplotype groups around the Pliocene-Pleistocene boundary. Our study highlights that biological traits, climate and biogeographic history should all be considered when examining the genetic and ecological differentiation between closely related taxa.

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BIOSKETCH

Xinya Song is a researcher on phylogeography at the College of Life Science, Sichuan University. The research interests of the team led by **Kangshan Mao** rested on the biogeographic and evolutionary history of plants, especially trees, in mountainous regions.

Author contributions: K.S.M. conceived the research; K.S.M., L.Z., J.M.C. collected samples; X.Y.S., X.Y.F., S.Y.X., H.L.Z, L.Q.F, J.M.C. T.M., X.T.X., J.W. collected and analysed the data; X.Y.S., R.I.M., K.S.M. wrote the manuscript; K.S.M., X.Y.F., S.Y.X., M.G.C., J.W. revised the manuscript.

TABLE 1 Analysis of molecular variance (AMOVA) for the two population groups (two putative lineages, *Populus davidiana* in CNCR and *P. davidiana* in NECR) based on nSSR and cpDNA data.

Source of variation	*df*	*SS*	*VC*	*V*%	*F*-statistic
SSR markers					
Among groups	1	110.18	0.20	6.69	F_{CT} = 0.07**
Among populations within groups	39	543.17	0.40	13.38	F_{ST} = 0.13**
Within populations	1041	2562.10	2.39	79.93	F_{SC} = 0.11**
Total	1081	3215.46	2.99		F_{ST}' = 0.45[a]
CpDNA					
Among groups	1	127.26	0.59	15.30	F_{CT} = 0.15**
Among populations within groups	38	605.80	1.51	39.01	F_{ST} = 0.54**
Within populations	336	594.01	1.77	45.69	F_{SC} = 0.46**
Total	375	1327.06	3.87		F_{ST}' = 0.77

Abbreviations: *df*, degrees of freedom; *SS*, sum of squares; *VC*, variance components; *V%*, percent variation; F_{ST}, the proportion of differentiation among populations; F_{SC}, the proportion of differentiation among populations within species; F_{CT}, the proportion of differentiation among species; **, $P < 0.01$, 1,000 permutations. [a] the mean F_{ST}' value, average over ten microsatellite loci (for F_{ST}' of each locus, see Table S6).

TABLE 2 Effective population sizes (N_e) and the effective number of immigrants per generation ($4N_em$) between the consensus CNCR (p1-19) and NCER (p24-40) of *Populus davidiana* based on the ten neutral nSSR loci.

			4 N_em	
		N_e	4 N_em CNCR→	4 N_em NECR→
Variable Theta	sCNCR	173.91		22.57
		(126.67-218.33)		(0.00-61.13)
	sNECR	147.55	36.88	
		(100.00-195.00)	(5.33-80.60)	
Same Theta	sCNCR	100.02		16.24
		(56.67-141.67)		(1.13-41.93)
	sNECR	116.35	15.27	
		(35.00-168.33)	(0.00-43.77)	

Abbreviations: N_e, effective population size; μ, mutation rate (μ= 10^{-3} per gamete per generation); 4 N_em CNCR→, the effective number of migrants from group CNCR to groups NECR; 4 N_em NECR→, the effective number of migrants from group NECR to groups CNCR; the mean value of θ was adopted to calculate the effective number of immigrants per generation.

FIGURE CAPTIONS

FIGURE 1 A map showing the sampling locations of 40 populations of *Populus davidiana* (black discs) and vegetation zones within China (Editorial Committee for Vegetation Atlas of China, 2001). Abbreviations: A, Cold temperate deciduous coniferous forest; B, Temperate coniferous broad-leaved mixed forest; C, Warm temperate deciduous broad-leaved forest; D, Subtropical evergreen broad-leaved forest; E, Tropical forests; F, Temperate grassland; G, Temperate deserts; H, Alpine vegetation of the Qinghai-Tibet Plateau.

FIGURE 2 A brief summary of the geographic distribution, genetic clustering and gene flow between groupings of *Populus davidiana* based on ten neutral nuclear microsatellite (nSSR) loci. (a) Geographic origin of the 40 populations of *P. davidiana* and their genetic components in regard to genetic clusters at the most likely K = 2. West to east dividing lines found in other seven species are inserted. (b) Illustrative representation of the effective population size (N_e) and effective number of migrants ($4N_em$) among the two population groups under two different hypotheses: (1) variable theta and (2) same theta. (c) Principal Coordinates Analysis (PCoA) of the 40 populations of *P. davidiana*, see Fig. S15 for more details. (d) Histogram of the STRUCTURE assignment test for 40 populations of *P. davidiana* based on ten neutral nSSR loci.

FIGURE 3 A brief summary of the geographic distribution, SAMOVA groupings and phylogenetic relationships of cpDNA haplotypes of *Populus davidiana*. (a) Geological distribution of haplotype lineages (colors in each pie) and SAMOVA groupings (dashed lines) and (b) maximum-parsimony network of 35 non-singleton haplotypes. Population codes are identified in Table S1. In (a), each section in any pie represent a distinct haplotype in that population. In (b), the unlabelled small black dots represent missing haplotypes, and circle sizes are proportional to the number of samples per haplotype.

FIGURE 4 Schematic representation for the estimates of effective population size (N_e) and the N_e-transition time of the best DIYABC scenarios (Sc7) for CNCR and NECR, respectively. Black vertical bars and white horizontal bars represent 95% confidence interval for the estimates of N_e and N_e-transition time, respectively. Note that the y-axis of N_e value is in Log_{10} format.

FIGURE 5 Habitat suitability of the two groups of *Populus davidiana*, CNCR (left) and NECR (right), from the late Quaternary to the present based on ecological niche modelling using MAXENT. Predicted distributions are shown for: the present day (a) CNCR and (b) NECR; the middle Holocene (MH) (c) CNCR and (d) NECR; the last glacial maximum (LGM) (e) CNCR and (f) NECR; and the last interglacial (LIG) for (g) CNCR and (h) NECR. The bioclimatic variables adopted for species distribution modelling for all time slices were downloaded from the WorldClim database (Hijmans et al., 2005).

FIGURE 6 Prevalent wind direction in the regions around Beijing, Hebei and Liaoning, where cpDNA and nSSR division line between groups are inconsistent, in March (black arrow), April (yellow arrow) and May (red arrow) averaged over 30 years from 1981 to 2010. Note that west to east dividing lines found in Juglans spp. (white dash line) and Acer mono (purple dash line) are inserted.