This is an electronic appendix to the paper by Weir 2004 Ice sheets promote speciation in boreal birds. *Proc. R. Soc. Lond.* B **271**, 1881-1887. (DOI 10.1098/rspb.2004.2803.)

Electronic Appendix A: Supporting Online Materials and Methods

DNA Sequencing

Total genomic DNA was extracted from muscle or liver tissue using a Qiagen tissue extraction kit. A fragment of the mitochondrial cytochrome b was amplified using the primers L14990 (5' - CCATCCAACATCTCMGCATGATGAAA) and H16065 (5' - GGAGTCTTCAGTCTYTGGTTTACAAGAC). For the PCR reaction we used 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 200 pM of each primer, 0.02 U/µl Taq polymerase and 20 - 100ng/µl of DNA. PCR conditions were as follows: denaturation at 93°C for 3 minutes, annealing between 50 and 55°C for 1 minute, extension at 72°C for 10 minutes. Amplified products where purified on Qiagen spin columns and sequenced using the BigDye cycle sequencing kit with the primer L14990. We sequenced one or more samples from each superspecies complex in the reverse direction using the primer H16065. Sequenced products where run on a ABI sequencer and aligned in Clustal X. Between 826 to 1006 bp of the sequenced product were used in each complex.

Sequences generated for this study have been deposited in Genbank (Table 1).

Sequences Used

Sequences (and GenBank accession numbers) used for the analysis of the boreal avifauna in Table 2 are as follows: *Sphyrapicus* AF123532, U83296; *Empidonax* AF447597, AF447598, AY143200, AY143201, AY309248, AY309249; *Vireo* AF81963, AF81964, AF81967, AF81970, AF81978, AF81981, AF81984, AF81987, AF81991, AF81993, AF82000, AF82001; *Poecile* AF347948-AF347950, AY309247; *Catharus* AY049490, AY049495, AY049496, AY049502, AY049503; *Vermivora* AF256510, AY309245, AY309246, AY640935; *Oporornis* AF383029, AY309242-AY309244; *Passerella* U40162-U40166, U40168-U40171, AY138920, AY138922-AY138924, AY138927-AY138929, AY138933, AY138934. Additional *Sphyrapicus* sequences where obtained from Cicero and Johnson (1995) and *Dendroica* sequences (two sequences for each of three species) were provided by Irby Lovette.

Sequences used as outgroup taxa for phylogenetic analysis of superspecies complexes in Figure 1 are as follows: *Sphyrapicus* - *S. thyroideus* (*1*); *Empidonax* - *E. flavescens* (AY143191, AF447599, AF447600); *Vireo* - *V. flavifrons* (AF081961, AF081962); *Poecile* - *P. sclateri* (AF347947), *P. cincta* (AF347950), *P. gambeli* (AF347938), and *P. atricapillus* (AF347937); *Vermivora* - *V. celata* (AF489902, AY030123); *Oporornis* - *O. formosus* (AF383017) and *Geothlypis trichas* (AF383003); *Passerella* - *Junco hyemalis* (U26199, AY138936), *Zonotrichia capensis* (U40174, AF383139), *Spizella arborea* (U26190, AY138925) and *Pipilo chlorurus* (U26201, AY138935); *Dendroica* - *D. adelaidae* (AF256504).

Sequences used for the Sub-boreal avifauna (Figure 3, Table 3) are as follows: *Aphelocoma* U77335, AY030116; *Callipepla* AF028759, AF028762, AF028765, AF028768, AF028771; *Chaetura* AF168105, CVU50029, CVU50030; *Contopus* AF447607-AF447610; *Icterus spurius* group AY211197-AY211215; *Icterus galbula* group AF099290, AF099309; *Megascops* AF115864, AF294997, AJ004016; *Polioptila* AF027840-AF027843; *Parula* AY030122, AF256502, AF256503, AF256509; *Picoides* AF389322, AF389323, AF389326, AF389327; *Pipilo* AF092887, AF284075; *Piranga* AF290146, AF011759, AF011760, AF011773, AF011774; *Quiscalus* AF089055, AF089056, AF290171; *Sturnella* AF089063, AF089064, AF290164; *Toxostoma* AF130235, AF130237, AF130238, AF130240, AF287541, AF130242, AF287548, AF287560; *Tympanuchus* AF230179, AF230180; *Vireo* AF081960, AY030111.

Sequences used for the Neotropical avifauna (Figure 3, Table 4) are as follows: *Agelaius* AF089005, AF089013, AF290174; *Cacicus* AY117700-AY117704; *Columbina* AF182683, AF182684, AF483347; *Cranioleuca* AF053780-AF053782; *Icterus icterus* AF099296, AF099297, AF089031; *Megascops* AJ3904, AJ4039, AJ4040, AF295003, AF295004; *Petrochelidon* AF182380 AF182381 AF182388-AF182391; *Psarcolius* AF089053, AF472377-AF472381; *Ramphocelus* AF310048, RCU15723, U15717, U15719, U15720, U15722, U15724; *Selenidera* AF100552, AF123517, AF123518; *Tachycineta* AY052445-AY052448; *Xiphorhynchus* AY089791-AY089794, AY089799-AY089801, AY089803, AY089805, AY089807, AY089808, AY089812, AY089814-AY089816, AY089820, AY089824, AY089826, AY089827, AY089828, AY089830-AY089832; *Zenaida* AF182699, AF251533, AF251534.

Literature Cited

1. Cicero, C. & Johnson, N. K. 1995 Speciation in sapsuckers (*Sphyrapicus*): III. Mitochondrial-DNA sequence divergence at the cytochrome-b locus. *Auk* **112,** 546-563.
2. Baker, M. B., Lopez-Medrano, E., Navarro-Siguenza, A. G., Rojas-Soto, O. R., & Omland, K. E. 2003 Recent speciation in the Orchard Oriole group: Divergence of *Icterus spurius spurius* and *Icterus spurius fuertesi*. *Auk* **120**, 848-859.
3. Aleixo, A. 2002 Molecular systematics and the role of the "várzea"-"terra firme" ecotone in the diversification of *Xiphorhynchus* woodcreepers (Aves: Dendrocolaptidae). *Auk* **119**, 621-640.

Table 1 Tissue source, collecting locality and GenBank accession numbers for specimens sequenced in this study.

Taxon	**Institution***	**Tissue Number**	**Collection Locality**	**GenBank accession numbers**
Empidonax occidentalis	MVZ	167081	Custer Co., Colorado, USA	AY309249
Empidonax occidentalis	MVZ	167099	Apache Co., Arizona, USA	AY309248
Poecile rufescens	CVM	15432	Vancouver, British Columbia, Canada	AY309247
Vermivora (r.) ruficapilla	LSUMNS	B-27333	Baton Rouge, Louisiana, USA	AY640935
Vermivora virginiae	UWBM	56395	Canon City, Colorado, USA	AY309246
Vermivora (r.) ridgewayi	MVZ	173509	Plumas Co., California, USA	AY309245
Oporornis philadelphia	KUNHM	2404	Topeka, Kansas, USA	AY309244
Oporornis tolmiei	FMNH	393953	[to be included]	AY309242
Oporornis tolmiei	FMNH	393954	[to be included]	AY309243

*CVM = Cowan Vertebrate Museum, Vancouver; FMNH = Field Museum of Natural History, Chicago; LSUMNS = Louisiana State University Museum of Natural Science, Baton Rouge; KUNHM = Kansas University Natural History Museum; MVZ = Museum of Vertebrate Zoology, Berkley; UWBM = University of Washington Burke Museum, Seattle.

Table 2 GTR-gamma divergence and dates of coalescence between members of species complexes restricted to the boreal forest of North America. (Cyt B, Cytochrome B.)

Taxa Comparison	GTR-gamma Divergence (Standard Deviation)	Estimated Age of Coalescence	Data
Sphyrapicus varius versus *S. nuchalis* / *S. ruber* (Yellow-bellied, Red-naped and Red-breasted Sapsucker)	2.40% (± 0.11%)	1,090,000	711 bp Cyt B
Sphyrapicus nuchalis versus *S. ruber* (Red-naped, and Red-breasted Sapsucker)	0.71 % (± 0.00%)	320,000	711 bp Cyt B
Empidonax occidentalis versus *E. difficilis* (Cordilleran and Pacific-slope Flycatcher)	0.87% (± 0.14%)	390,000	1006 bp Cyt B
Vireo plumbeus versus *V. solitarius* / *V. cassinii* (Plumbeous, Blue-headed and Cassin's Vireo)	3.23% (± 0.31%)	1,470,000	1143 bp Cyt B
Vireo solitarius versus *V. cassinii* (Blue-headed and Cassin's Vireo)	2.96% (± 0.17%)	1,350,000	1143 bp Cyt B
Poecile hudsonica versus *P. rufescens* (Boreal and Chestnut-backed Chickadee)	3.60% (± 0.05%)	1,640,000	1001 bp Cyt B
Catharus minimus versus *C. bicknelli* (Gray-cheeked and Bicknell's Thrush)	1.12% (± 0.22%)	510,000	1066 bp Cyt B
Vermivora (r.) ruficapilla versus *V. virginiae* / *V. (r.) ridgwayi* (Nashville, Virginia's and Calaveras Warbler)	2.14% (± 0.53%)	970,000	961 bp Cyt B
Vermivora virginiae versus *V. (ruficapilla) ridgwayi* (Virginia's and Calaveras Warbler)	2.01% (± 0.19%)	910,000	961 bp Cyt B
Dendroica virens versus *D. townsendi* / *D. occidentalis* (Black-throated Green, Townsend's and Hermit Warbler)	2.37% (± 0.16%)	1,080,000	1143 bp Cyt B
Dendroica townsendi versus *D. occidentalis* (Townsend's versus Hermit Warbler)	0.95% (± 0.12%)	430,000	1143 bp Cyt B
Oporornis philadelphia versus *O. tolmiei* (Mourning and MacGillivray's Warbler)	2.49% (± 0.07%)	1,130,000	946 bp Cyt B
Passerella (i.) iliaca versus *P. (i.) schistacea*, *P. (i.) megarhyncha* and *P. (i.) unalaschensis* (Eastern, Slate-colored, Thick-billed and Sooty Fox Sparrows)	1.69% (± 0.22%)	770,000	433 bp Cyt B, 1030 bp ND2

Passerella (i.) schistacea versus *P. (i.) megarhyncha* and *P. (i.) unalaschensis* (Slate-colored, Thick-billed and Sooty Fox Sparrows)	1.57% (± 0.21%)	720,000	433 bp Cyt B, 1030 bp ND2
Passerella (i.) megarhyncha versus *P. (i.) unalaschensis* (Thick-billed and Sooty Fox Sparrows)	1.59% (± 0.22%)	720,000	433 bp Cyt B, 1030 bp ND2
Mean (Standard Deviation of means)	**1.98% (± 0.88%)**	**900,000 (± 400,000)**	

Table 3 GTR-gamma divergence and estimated dates of coalescence between members of species complexes restricted to sub-boreal habitats of North America. Cyt B, Cytochrome B.

Taxa Comparison	GTR-gamma Divergence (Standard Deviation)	Estimated Age	Data
Tympanuchus pallidicinctus versus *T. cupido* (Lesser and Greater Prairie-Chicken)	0.50%	250,000	609 bp Cyt B
Callipepla californica versus *C. gambelii* (California and Gambel's Quail)	2.46% (± 0.17%)	1,230,000	699 bp Cyt B
Megascops asio versus *M. kennicottii* (Eastern and Western Screech-Owl)	9.37% (± 2.12%)	4,690,000	395 bp Cyt B
Picoides nuttallii versus *P. scalaris* (Nuttall's and Ladder-backed Woodpecker)	1.05% (± 0.13%)	530,000	1028 bp Cyt B
Contopus sordidulus versus *V. virens* (Western and Eastern Wood-Pewee)	1.97% (± 0.14%)	990,000	1143 bp Cyt B
Aphelocoma californica versus *A. coerulescens* (Western and Florida Scrub-Jay)	5.68%	2,840,000	1143 bp Cyt B
Vireo gilvus versus *V. leucophrys* (Warbling and Brown-throated Vireo)	6.66%	3,330,000	1143 bp Cyt B
Toxostoma cinereum versus *T. bendirei* (Gray and Bendire's Thrasher)	1.46%	730,000	433 bp Cyt B
Toxostoma curvirostre versus *T. ocellatum* (Curve-billed and Ocellated Thrasher)	6.59% (± 0.50%)	3,300,000	433 bp Cyt B
Parula americana versus *P. pitiayumi* (Northern and Tropical Parula)	4.68% (± 0.00%)	2,340,000	1143 bp Cyt B
Piranga ludoviciana versus *P. bidentata* (Western and Flame-colored Tanager)	5.38% (± 0.33%)	2,690,000	1073 bp Cyt B

Pipilo maculatus versus *P. erythrophthalmus* (Spotted and Eastern Towhee)	1.20%	600,000	433 bp Cyt B
Quiscalus major versus *Q. mexicanus* (Boat-tailed and Great-tailed Grackle)	3.06% (± 0.00%)	1,530,000	881 bp Cyt B
Icterus galbula versus *I. abeillei* (Baltimore and Black-backed Oriole)	0.81%	410,000	876 bp Cyt B
Icterus spurius versus *I. fuertesi* (Orchard and Ochre Oriole)	0.19% (± 0.10%) *	100,000	925 bp Cyt B
Sturnella neglecta versus *S. magna* (Western and Eastern Meadowlark)	4.82% (± 0.00%)	2,410,000	893 bp Cyt B
Mean (Standard Deviation of means)		**1,750,000 (± 1,360,000)**	

* *Icterus spurius* and *I. fuertesi* share some haplotypes and are not reciprocally monophyletic as is expected for recently evolved species (Baker *et al.* 2003).

Table 4 GTR-gamma divergence and estimated dates of coalescence between members of species complexes restricted to tropical habitats of the Neotropical lowlands. Cyt B, Cytochrome B.

Taxa Comparison	GTR-gamma Divergence (Standard Deviation)	Estimate Age	Data
Zenaida asiatica versus *Z. meloda* (White-winged and Pacific Dove)	5.14% (± 0.05%)	2,570,000	1045 bp Cyt B
Columbina squammata versus *C. inca* (Scaled and Inca Dove)	3.16% (± 0.22%)	1,580,000	1039 bp Cyt B
Selenidera maculirostris versus *S. gouldii* (Spot-billed and Golden-collared Toucanet)	2.00%	1,000,000	925 bp Cyt B
Megascops usta versus *M. watsonii* (Southern and Northern Tawny-bellied Screech-owl)	1.73% (± 0.63%)	865,000	393 bp Cyt B

Xiphorhynchus spixii versus *X. elegans* * (Spix's and Elegant Woodcreeper)	5.34% (± 0.17%)	2,670,000	1003 bp Cyt B
Xiphorhynchus chunchotambo versus *X. padalotus / X. ocellatus* (Tschudi's, Chestnut-rumped and Ocellated Woodcreeper)	5.63% (± 0.62%)	2,820,000	964 bp Cyt B
Xiphorhynchus pardalotus versus *X. ocellatus* (Chestnut-rumped and Ocellated Woodcreeper)	4.03% (± 0.38%)	2,020,000	964 bp Cyt B
Xiphorhynchus erythropygius versus *X. triangularis* (Spotted and Olive-backed Woodcreeper)	5.81% (± 0.68%)	2,910,000	965 bp Cyt B
Xiphorhynchus guttatoides versus *X. guttatus / susurrans* (Lafresnaye's, Buff-throated and Cocoa Woodcreeper)	7.25% (± 0.83%)	3,630,000	1006 bp Cyt B
Xiphorhynchus guttatus versus *X. susurrans* (Buff-throated and Cocoa Woodcreeper)	4.39% (± 0.06%)	2,200,000	1022 bp Cyt B
Xiphorhynchus lachrymosus versus *X. flavigaster* (Black-striped and Ivory-billed Woodcreeper)	4.74% (± 0.57%)	2,370,000	968 bp Cyt B
Cranioleuca pyrrhophia versus *C. obsoleta* (Stripe-crowned and Olive Spinetail)	2.06% (± 1.26%)	1,030,000	209 bp Cyt B
Tachycineta albiventer versus *T. albilinea* (White-winged and Mangrove Swallow)	6.97%	3,490,000	936 bp Cyt B
Tachycineta meyeni versus *T. leucorrhoa* (Chilean and White-rumped Swallow)	4.12%	2,060,000	913 bp Cyt B
Petrochelidon fulva versus *P. rufocolaris* (Cave and Chestnut-collared Swallow)	1.97% (± 0.13%)	990,000	921 bp Cyt B
Ramphocelus carbo versus *R. bresilius* (Silver-beaked and Brazilian tanager)	4.35% (± 0.49%)	2,180,000	1045 bp Cyt B
Ramphocelus flammigerus versus *R. passerinii / R. costaricensis* (Flame-rumped, Scarlet-rumped and Cherrie's Tanager)	5.69% (± 0.26%)	2,850,000	1045 bp Cyt B
Ramphocelus passerinii versus *R. costaricensis* (Scarlet-rumped and Cherrie's Tanager)	1.67% (± 0.08%)	840,000	1045 bp Cyt B
Agelaius cyanopus versus *A. xanthophthalmus* (Unicolored and Pale-eyed Blackbird)	0.57% (± 0.00%)	290,000	879 bp Cyt B
Icterus icterus versus *I. jamacaii / I. croconotus* (Venezuelan, Campo and Orange-backed Troupial)	4.72% (± 0.60%)	2,360,000	890 bp Cyt B
Icterus jamacaii versus *I. Croconotus* (Campo and Orange-backed Troupial)	1.96%	980,000	890 bp Cyt B

Cacicus cela versus *C. vitellinus* (Yellow-rumped and Saffron-rumped Cacique)	4.86% (± 0.13%)	2,430,000	906 bp Cyt B
Psarocolius montezuma versus *P. bifasciatus / P. yuracares* (Montezuma, Para and Olive Oropendola)	1.76% (± 0.16%)	880,000	920 bp Cyt B
Psarocolius bifasciatus versus *P. yuracares* (Para and Olive Oropendola)	0.87% (± 0.04%)	440,000	920 bp Cyt B
Mean (Standard Deviation)		**1,890,000 (± 970,000)**	

* For current taxonomic treatment of *Xiphorhynchus* see Aleixo (2002).

Figure 4 (*Overleaf.***)** Approximate geographic distribution of boreal (black), sub-boreal (dotted) and lowland Neotropical (horizontal) avifaunas compared in this study. The Tropic of Cancer, Equator, and Tropic of Capricorn are indicated by the three dotted lines from north to south respectively.

Figure 4