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Comparative phylogeography of Nearctic and Palearctic fishes

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Abstract

Combining phylogeographic data from mitochondrial DNA (mtDNA) of Nearctic and Palearctic freshwater and anadromous fishes, we used a comparative approach to assess the influence of historical events on evolutionary patterns and processes in regional fish faunas. Specifically, we (i) determined whether regional faunas differentially affected by Pleistocene glaciations show predictable differences in phylogeographic patterns; (ii) evaluated how processes of divergence and speciation have been influenced by such differential responses; and (iii) assessed the general contribution of phylogeographic studies to conservation issues. Comparisons among case studies revealed fundamental differences in phylogeographic patterns among regional faunas. Tree topologies were typically deeper for species from nonglaciated regions compared to northern species, whereas species with partially glaciated ranges were intermediate in their characteristics. Phylogeographic patterns were strikingly similar among southern species, whereas species in glaciated areas showed reduced concordance. The extent and locations of secondary contact among mtDNA lineages varied greatly among northern species, resulting in reduced intraspecific concordance of genetic markers for some northern species. Regression analysis of phylogeographic data for 42 species revealed significant latitudinal shifts in intraspecific genetic diversity. Both relative nucleotide diversity and estimates of evolutionary effective population size showed significant breakpoints matching the median latitude for the southern limit of the Pleistocene glaciations. Similarly, analysis of clade depth of phylogenetically distinct lineages vs. area occupied showed that evolutionary dispersal rates of species from glaciated and nonglaciated regions differed by two orders of magnitude. A negative relationship was also found between sequence divergence among sister species as a function of their median distributional latitude, indicating that recent bursts of speciation events have occurred in deglaciated habitats. Phylogeographic evidence for parallel evolution of sympatric northern species pairs in postglacial times suggested that differentiation of cospecific morphotypes may be driven by ecological release. Altogether, these results demonstrate that comparative phylogeography can be used to evaluate not only phylogeographic patterns but also evolutionary processes. As well as having significant implications for conservation programs, this approach enables new avenues of research for examining the regional, historical, and ecological factors involved in shaping intraspecific genetic diversity.

Keywords: conservation genetics, fish, glaciation, mtDNA, phylogeography, speciation

Introduction

The fusion of phylogenetics and population genetics with biogeography has had widespread effects on many areas of evolutionary biology and ecology. By making it possible to assess the impact of historical events on the genetic composition and structure of modern populations, phylogeography has revolutionized historical ecology and population genetics (Avise *et al*. 1987). Although its most common usage has been in delineating patterns of evolutionary distinct population segments within species, the phylogeographic approach has also been used to infer historical demographic processes such as gene flow, effective population sizes

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and evolutionary trajectories (Wilson *et al*. 1985; Avise *et al*. 1984, 1988).

Among the vertebrates, fish have perhaps been the most intensely studied group for local and regional genetic structure, largely due to the sustained long-term interest in the geographical structure of intraspecific diversity for fisheries management. Despite the applied focus of much of this work, however, a sizeable body of phylogeographic data has also been obtained. In turn, phylogeographic studies have had a significant impact on modern fish biology. As well as confirming major intraspecific subdivisions hypothesized from other evidence, phylogeographic data have highlighted unexpected genetic structuring within species and in some cases have revealed discrepancies between genetic and phenotypic variation. Perhaps the most significant contribution, however, has been to provide a historical dimension for evolutionary, ecological, and applied studies alike. Phylogeographic studies have consistently revealed the dominant influence of historical biogeographic and demographic events in shaping existing patterns of mtDNA variation. By doing so, phylogeographic studies have enabled us to retrace the movements, events and histories that have helped shape the modern-day genetic and geographical structure of fish species.

Comparative phylogeography has recently emerged as a powerful method for assessing the roles of historical events and demographic processes in shaping genetic diversity. This multispecies approach enables broader conclusions to be drawn than those generated from species-specific studies, and has led to the recognition that phylogeographic data may be used to depict the effects of historical events on processes of population differentiation that may ultimately lead to speciation (Avise 1994; Hewitt 1996). Although most such studies have examined species with similar distributions (Avise 1992; Turner *et al*. 1996; Zink 1996), phylogeographic patterns of species from different regions can also be compared to infer differential effects of large-scale historical events.

In this study, we use a comparative phylogeographic approach to assess mtDNA data from Nearctic and Palearctic freshwater and anadromous fishes. Together, these represent one of the best-documented groups for mtDNA diversity to date. As genetic diversity of freshwater fishes is largely controlled by the island-like nature of their habitats (Ward *et al*. 1994), current phylogeographic patterns of mtDNA diversity can be more readily interpreted in terms of historical effects rather than contemporary dispersal. The large number of studies published on species from areas differentially affected by historical events (see below) enables us to assess phylogeographic congruence within regional faunas and draw contrasts between regions. In this context, we specifically attempted to (i) test the general hypothesis that regional fish faunas were differentially affected by major historical events, resulting in predictable differences in regional phylogeographic structure; (ii) determine how processes of population divergence and speciation have been influenced by such differential effects; and (iii) assess the general contribution of phylogeographic studies to conservation issues in fish.

Pleistocene glaciations

The Pleistocene glaciations were arguably the most significant historical events to have occurred during the evolutionary lifespan of most extant species. Large areas of the world's landmasses were repeatedly buried under vast sheets of ice, causing drastic alterations on continental scales. It is estimated that up to 20 glaciation events (consisting of glacial advances, stabilization and retreat) occurred during the Pleistocene (Martinson *et al*. 1987). Each glaciation spanned ≈ 100 000 years, with interglacial periods lasting 10 000–12 000 years (Dawson 1992). Glacial impacts were greatest in North America, Eurasia and Antarctica, with glacial ice covering major portions of their surfaces. The North American ice sheets were particularly large, exceeding the combined coverage of the European and Asian glaciers (Dawson 1992). The most recent ('Wisconsinan') North American glaciation event reached maximum ice coverage 23 000–18 000 years ago, with major deglaciation occurring between 15 000 and 8000 years ago (Dyke & Prest 1987).

Freshwater habitats were altered on an unprecedented scale, through both the destruction of old systems and the widespread creation of new lakes and rivers. Advancing glacial fronts caused widespread habitat destruction, displacement or extirpation of local populations, and biome compression at glacial margins (Pielou 1991). Aquatic species were particularly affected, as opportunities for dispersal were limited to direct water connections leading away from the advancing ice fronts. Fish species displaced by the glaciers would also have had to survive as best they could for periods lasting thousands of years in fringe habitats along the glacial margins.

In some regions, meltwater from retreating glaciers during deglaciation events formed large proglacial lakes which facilitated the dispersal of freshwater species. These lakes dwarfed the largest freshwater lakes existing today, and were both dammed and fed by the glaciers along their northern margins. As a result, their sizes and positions changed along with the shifting ice sheets, with their volumes largely determined by glacial melting rates (Dyke & Prest 1987). For aquatic species able to gain access to them, the proglacial lakes provided tremendous opportunities for dispersal over vast geographical ranges. The boundaries and extents of these lakes are well preserved in the geological record, through the remains of their past shorelines and spillways. These data aid zoogeographic reconstructions of postglacial dispersal, and make it possible to assign approximate time windows for species movements into and across deglaciated areas.

As well as direct impacts on ice-covered regions, glaciation events had indirect effects on global climates (Shackleton 1987). Global sea levels fluctuated as glaciers formed and melted, with sea levels decreased by as much as 100 m (relative to present levels) as recently as 18 000 years ago. Glacial maxima were accompanied by increased aridity which was felt around the world (Shackleton 1987; Dawson 1992). From the global magnitude of disturbances caused by the glaciers, it is reasonable to assume that glaciations had significant impacts on the genetic composition of freshwater and anadromous species.

There is little doubt that glaciated regions were more dramatically affected by glaciation events than nonglaciated ones. These were nevertheless affected indirectly both by climatic and sea-level changes. It is also important to keep in mind that although we emphasize the role of Pleistocene glaciations in this study, more ancient historical events that occurred during the Miocene through the Pliocene may have also played a role in shaping the pattern of genetic diversity observed in extant populations, particularly in southern latitudes (Bermingham & Avise 1986).

Phylogeographic predictions based on glaciation history

Several a priori predictions can be made regarding genetic diversity and phylogeographic structure of fishes from glaciated vs. nonglaciated regions. First, fish species which were displaced by glacial advances are likely to have lower levels of genetic diversity relative to species from nonglaciated areas. Initial loss of diversity would have accompanied the destruction or displacement of many northern populations by glacial advances. Subsequent reduction in habitat numbers and sizes due to species' restriction to glacial refugia, as well as reduced evolutionary effective population sizes over many millenia, would have caused further substantial losses of intraspecific diversity. Actual bottlenecks in refugia would not be necessary for decreases in genetic diversity to occur, as long-term reductions in population size would have a comparable effect (Avise *et al*. 1984).

Northern species should also show less intraspecific divergence and appear to be 'younger' in evolutionary terms than species from nonglaciated areas, despite their true evolutionary ages. The repeated widespread disturbances and founder-flush cycles caused by multiple glacial expansions and retreats make it probable that the majority of intraspecific mitochondrial diversity originally present would be lost (Avise *et al*. 1984). Given the large number of glacial events that occurred during the Pleistocene (Martinson *et al*. 1987; Dawson 1992), it is therefore conceivable that extant intraspecific mitochondrial lineages within northern species would trace back to a more recent common ancestor than is the case for species present in nonglaciated regions.

Avise *et al*. (1987) predicted that intraspecific phylogeographic structure should correspond with recognized zoogeographic provinces. This should also hold true for the majority of northern species dispersing from single glacial refugia, as in many cases the distributions of these species contributed to the delineation of zoogeographic regions (McPhail & Lindsey 1970; Crossman & McAllister 1986). Similar congruence is also probable for species which persisted in two or more refugia but have disjunct distributions (i.e. no secondary contact between refugial groups). However, the phylogeographic structure of continuously distributed multiple-refuge species may not fit zoogeographic predictions, as the same conditions that enabled secondary contact to occur may also have favoured subsequent dispersal.

It is generally assumed that allopatric groups will be reciprocally monophyletic and readily distinguishable. This assumption may not be justified for multiple-refuge northern species, however, as repeated glacial advances may have caused vicariant displacement of a single lineage or closely related groups into separate refuges. If insufficient evolutionary time elapsed during their period of allopatry, fish from separate refugia may show little or no detectable phylogenetic differences.

Many northern species also typically have larger range sizes than species from nonglaciated areas (McAllister *et al*. 1986). This is largely due to postglacial dispersal opportunities provided by proglacial lakes (Hocutt & Wiley 1986; Dyke & Prest 1987). As a result of these dispersal opportunities, species with similar ranges that are present in deglaciated regions may not show good phylogeographic concordance, either in number of phylogenetic clades/refugial groups, geographical ranges of clades from shared refugia, or location and extent of secondary contact among refugial groups. Ecological differences between species may also have resulted in marked differences in phylogeographic patterns. Habitat conditions in glacial refugia and proglacial lakes changed regularly, as their locations and boundaries altered with shifting glacial borders (Pielou 1991). As a result, species with dissimilar ecological characteristics and dispersal abilities may have responded to events differently, resulting in different phylogeographic patterns.

Case studies

Comparison of case studies exemplifies several contrasts between species from glaciated (northern) and nonglaciated (southern) regions. Perhaps the best known study of fish phylogeography is Bermingham & Avise (1986), which documents concordant phylogeographic patterns in four freshwater species, three species of sunfish (*Lepomis* sp.) and the bowfin, *Amia calva* (Fig. 1). The details of the study are not reiterated here, as the work has been extensively reviewed elsewhere (Avise *et al.* 1987; Bermingham 1990; Avise 1992, 1994, 1995).

Bermingham and Avise observed strikingly similar intraspecific patterns for numbers of phylogenetic lineages (clades), geographical distributions of lineages, and clade depth (Fig. 1, a and b). Intraspecific divergence in the bowfin was an exception for clade depth, but all sunfish species showed a minimum divergence of at least six percentage (Fig. 1a). Each species showed clear geographical separation of eastern and western lineages, with the Florida peninsula serving as a barrier to gene exchange. Secondary contact between lineages was virtually nonexistent, and was only observed in three *L. microlophus* populations and one coastal population of *L. gulosus* (Fig. 1b). The strong interspecific concordance in phylogeographic structure has been supported by studies of other freshwater

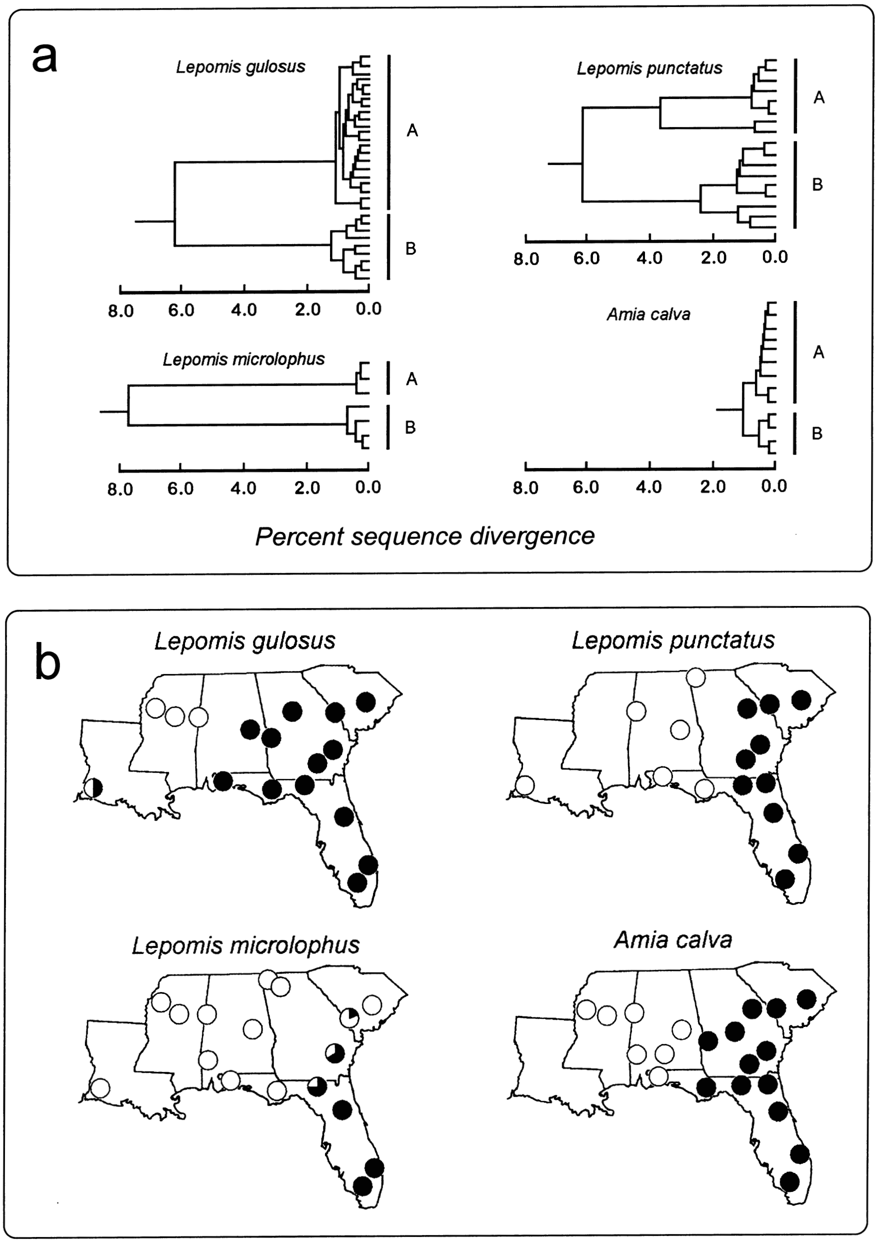

Fig. 1 Phylogeographic data from Bermingham & Avise (1986) for three species of sunfish (*Lepomis* sp.) and the bowfin *Amia calva*. (a) UPGMA dendrograms showing intraspecific mtDNA diversity for each species; (b) distributions of major intraspecific mtDNA lineages in the southeastern USA. Figures modified from Bermingham & Avise (1986).

fishes (Avise 1992; Scribner & Avise 1993) and more-distant taxonomic groups (Avise 1992, 1994). The shared pattern of interspecific congruence in geographical and genetic structure led Bermingham and Avise to conclude that genetic subdivision within these species was determined by geographical landforms as barriers to gene flow, and that habitat availability, dispersal opportunities, and long-term demographic processes were strongly influenced, although not exclusively, by Pleistocene climatic events, (Bermingham & Avise 1986; Avise 1992).

There have been relatively few studies examining the phylogeographic structure of species from formerly glaciated regions. Of those that have been studied, two species in particular have ranges that largely coincide with Pleistocene glacial limits. Lake whitefish (*Coregonus clupeaformis*) and lake trout (*Salvelinus namaycush*) both have distributions which closely match the areas covered by North American glaciers, excepting islands in the Canadian Arctic, and have been used as model organisms to examine postglacial dispersal (McPhail & Lindsey 1970; Crossman & McAllister 1986).

A survey of lake whitefish (*C. clupeaformis*) from 41 populations across the species range by Bernatchez & Dodson (1991) used RFLP analysis to screen for mtDNA variation. Three major clades were detected, with significant substructuring observed within the largest clade (Fig. 2a). In contrast to the sunfish divergence values observed by Bermingham and Avise (Fig. 1), divergences among the major groups ranged from 0.96% to 1.15%. Based on their geographical distributions, each of the four clades observed could be traced back to single glacial refuges (Fig. 2b). Groups A and B were only observed in Alaska and northwestern Canada, and were later resolved as having survived glaciation in Beringia and northern Eurasia, respectively (Bernatchez & Dodson 1994). The most abundant clade (Group C in Fig. 2b) dispersed from a Mississippian refuge to occupy most of the species' range, which corroborated Mississippian dispersal predictions based on multispecies distributions (McPhail & Lindsey 1970; Crossman & McAllister 1986). One Group C haplotype (C2 in Fig. 2b) was restricted to several populations in southern Quebec and northern Maine, and was interpreted as having persisted in an Atlantic refuge. Group D haplotypes occurred to the east of these Atlantic-refuge populations, and were attributed to a second Atlantic (Acadian) refuge. Dispersal of whitefish from their Beringian and eastern refugia appears to have been limited, whereas Mississippian-refuge fish dispersed widely across the species' range.

Secondary contact among refugial groups was quite limited (Fig. 2c). Groups A and B co-occurred in three of the seven sampled Beringian populations, and Atlantic and Acadian fish were found together in three eastern populations. Contact between Mississippian and Beringian whitefish was limited to a single population in the North-west Territories, and Mississippian contact with eastern-refuge fish was similarly limited to a single population in southeastern Quebec (Fig. 2b). As a result, intrapopulation nucleotide diversity was highest in areas occupied by the Beringian and eastern lineages (Fig. 2c). Intrapopulation diversity throughout the rest of the species range was due solely to local sympatry of two or more Mississippian haplotypes. As a result of the limited secondary contact among *C. clupeaformis* refugial groups, the zoogeographic aspects of Bernatchez and Dodson's (1994) data fit well with data from other morphological and allozyme-based studies of *C. clupeaformis*. This congruence among complementary data sets is similar to that observed in species from nonglaciated regions (Avise 1994), and enables robust conclusions to be drawn about species' history and evolution.

A similar study by Wilson & Hebert (1996), examining mtDNA diversity and phylogeographic structure in lake trout, *Salvelinus namaycush*, found 21 haplotypes among 61 geographically representative populations based on analysis with 30 restriction enzymes (Fig. 2d). Similar to data from *C. clupeaformis*, haplotypes showed little divergence and fell into three major clades with interclade divergences ranging between 0.45% and 0.86% (Fig. 2d). Some additional substructure was detected in one of the clades (Group B/D, Fig. 2d), although this was not supported by bootstrapping.

As was the case with lake whitefish, the geographical distributions of *S. namaycush* clades indicated their dispersal from multiple refugia (Fig. 2e). Groups A, B and D each appeared to have emanated from single refugia, group A being Mississippian and groups B and D dispersing from Atlantic and Beringian refugia, respectively. In contrast, the distributions of haplotypes from group C suggested their dispersal from three separate refugia. The most abundant C haplotype and its mutational derivatives (C in Fig. 2e) were found in Alaska, across the Canadian Arctic, and throughout western and central Canada, and appear to have dispersed from a Beringian refuge (Wilson & Hebert 1997). The distribution of the next most common group C haplotype (C2 in Fig. 2e) fits closely with that of the Mississippian group A fish. Finally, a third haplotype was only common in Montana and southern Alberta, although scattered individuals were observed in central Canada as well as the Laurentian Great Lakes region (Fig. 2e). In contrast to the data for lake whitefish, the phylogeographic evidence indicates that dispersal from non-Mississippian refugia was extensive, enabling Beringian fish to colonize close to two-thirds of the species' range, penetrating as far as central Quebec (Fig. 2e). Atlantic-refuge fish were also more widespread than was observed for lake whitefish, although their westward colonization did not reach

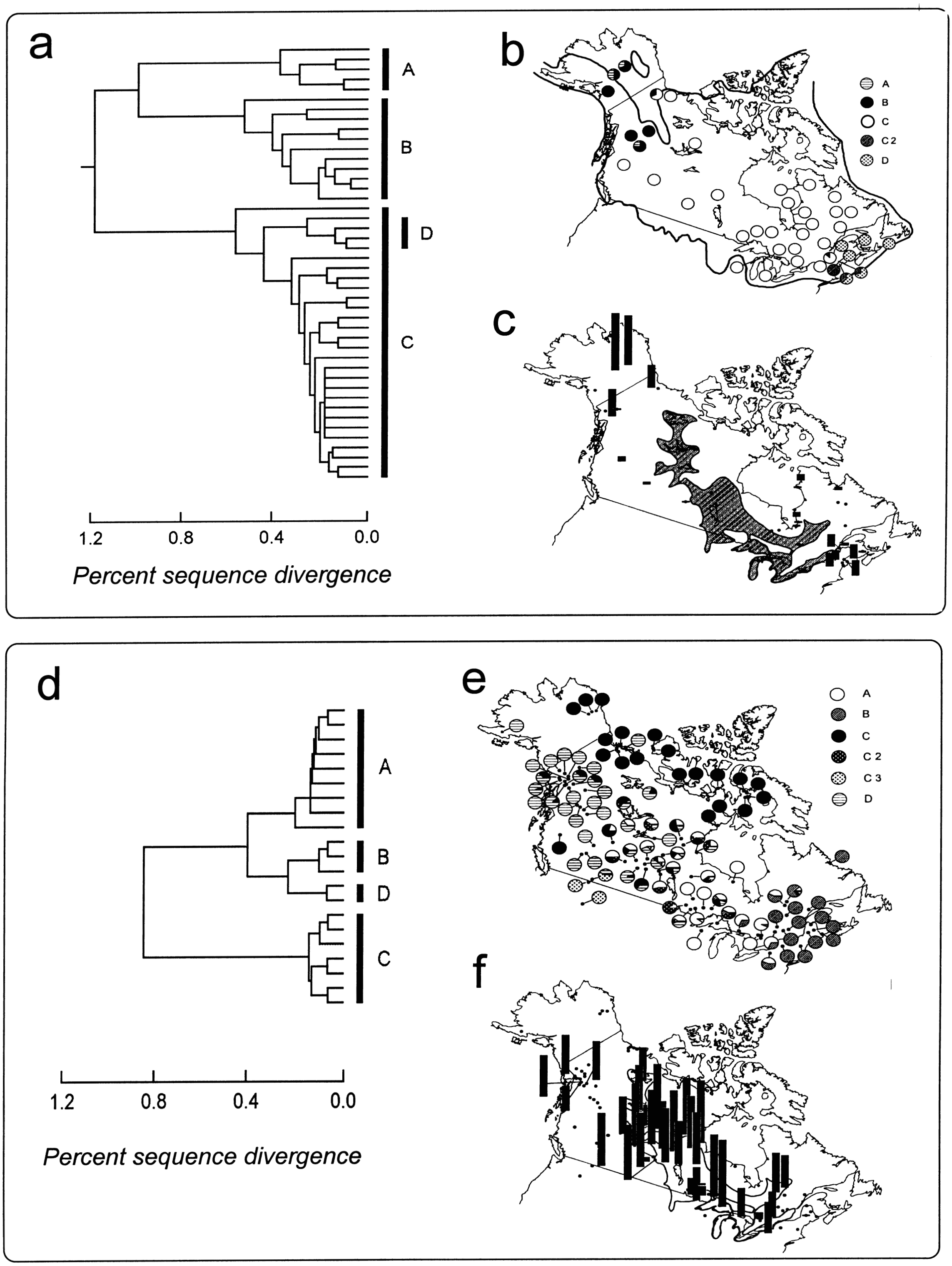

Fig. 2 Phylogeographic data for lake whitefish, *Coregonus clupeaformis*, (a–c) and lake trout, *Salvelinus namaycush* (d–f). (a) Intraspecific mtDNA diversity of *C. clupeaformis*, showing percentage sequence divergence of major phylogenetic lineages. (b) Geographical distribution of refugial groups, with respect to maximum extent of glacial coverage (black outline). (c) Intrapopulation nucleotide diversity among surveyed *C. clupeaformis* populations, with relation to areas formerly inundated by major proglacial lakes (shaded area). Dots represent populations with diversity values of zero. (d) Intraspecific diversity of *S. namaycush*, showing percentage sequence divergence of phylogenetic groups. (e) Geographical distributions of *S. namaycush* refugial groups. (f) Intrapopulation nucleotide diversity among surveyed *S. namaycush* populations, using the same scale as in (c). (a–c) modified from Bernatchez & Dodson (1991); (d–f) modified from Wilson & Hebert (1997).

beyond the Great Lakes region. Mississippian-refuge fish colonized much of central Canada, but mitochondrial evidence of their north-western emigration did not extend into arctic Canada (Fig. 2e).

Consequently, secondary intergradation among refugial groups was also extensive (Fig. 2e). Contact zones extend from Quebec to the Yukon Territory, with many populations containing representatives from three or more refugial groups. Contact was particularly extensive between the two Beringian lineages and Mississippian-refuge fish, with their secondary contact zone encompassing virtually all of central Canada (Fig. 2e). Contact between phylogenetic groups was also primarily responsible for observed levels of intrapopulation nucleotide diversity (Fig. 2f). The distribution of populations containing multiple refugial groups was strongly correlated with areas formerly covered by the major post-Wisconsinan proglacial lakes (Fig. 2f), indicating the extensive use of these proglacial habitats for *S. namaycush* dispersal from the different glacial refugia. As a result of this extensive secondary contact among refugial groups, there is little concordance in the geographical structure of data from complementary methods such as morphometric, allozyme and mtDNA analyses. This, in turn, has lead to considerable disagreement in zoogeographic interpretations of the postglacial history of *S. namaycush* among studies utilizing these various approaches (summarized in Wilson & Hebert 1997a,b).

These two examples highlight the similarities and contrasts evident in phylogeographic data obtained from fish species in formerly glaciated areas. Both species occupy large geographical ranges, which is typical of fishes in formerly glaciated regions. More significantly, both species also possess levels of intraspecific divergence that are sharply reduced in comparison with more southern species, and are comparable to divergence levels recorded from other northern fishes (Billington & Hebert 1991). These low levels of intraspecific divergence are all the more striking, considering the subdivision, displacement and subsequent isolation of these and other northern species into multiple refugia by glacial advances. As each glaciation event would have significantly altered the genetic structure of northern species, it appears that these vicariant events were not sufficiently long term for major sequence divergence to occur between allopatric isolates. It is also probable that the vast majority of historical intraspecific diversity was lost by species directly affected by glacial advances, as each glaciation event would have effectively reshuffled the species' overall genetic structure approximately every 100 000 years.

Despite these similarities, however, the contrasts in phylogeographic structure between these two species are striking. Both *C. clupeaformis* and *S. namaycush* appear to have utilized the giant proglacial meltwater lakes as avenues for dispersal, based on the distributions of refugial lineages in both species. However, their differing phylogeographic patterns indicate that the two species had markedly different colonization histories. In lake whitefish, Mississippian-refuge fish were apparently the only group able to access proglacial lake habitats for subsequent dispersal; in contrast, each of the lake trout refugial groups dispersed widely via proglacial lakes, particularly Beringian and Mississippian-refuge fish. These differences are highlighted not only by the geographical distributions of the different lineages (Fig. 2b,e), but by the stark contrasts in intrapopulation nucleotide diversity and admixture of refugial groups (Fig. 2c,f).

Additional studies of species from formerly glaciated areas also highlight the enormous variation in intraspecific phylogeographic structure among the species examined, with reduced interspecific congruence compared to species from nonglaciated regions. While many species show evidence of having survived in multiple refugia, the numbers, origins, and relative postglacial contributions of refugial groups varies considerably among the species examined to date (Baby *et al*. 1991; Taylor & Bentzen 1993a; Bernatchez 1997; Murdoch & Hebert 1997; Wilson *et al*. 1996; Lafontaine & Dodson 1997).

One feature shared by many northern species is their reduced intraspecific divergence relative to species from nonglaciated regions (Appendix 1). For example, mtDNA diversity among either anadromous or freshwater populations of northern salmonids such as Atlantic salmon (*Salmo salar*) and arctic charr (*Salvelinus alpinus*) is impoverished (Bermingham *et al*. 1991; Tessier *et al*. 1995; Wilson *et al*. 1996), whereas Pacific salmon (*Oncorhynchus* spp.) show considerable genetic heterogeneity (Wilson *et al*. 1985, 1987; Thomas *et al*. 1986). This was noted previously by Billington & Hebert (1991), who recorded that mean intraspecific percentage sequence divergence values averaged 0.9% for the 13 'glaciated' fish species they reviewed, vs. 6.6% for the seven 'nonglaciated' species. This contrast in intraspecific diversity was directly examined by Bernatchez *et al*. (1989) and Bernatchez & Dodson (1994), who compared levels of mtDNA diversity in Nearctic whitefish populations (taxonomically recognized as *C. clupeaformis*) with Palearctic populations (*C. lavaretus*). European populations of *C. lavaretus* contained much higher levels of diversity, which was attributed to the smaller extent and lesser impact of Eurasian ice sheets relative to those in North America.

Glacial impacts on intraspecific mtDNA diversity are even more apparent when the phylogeographic structure of species with ranges spanning both glaciated and nonglaciated regions is examined. Billington & Hebert (1988) detected three mtDNA lineages in Great Lakes populations of walleye, *Stizostedion vitreum*, which were subsequently attributed to Mississippian, Atlantic, and

Missourian refugial groups (Ward *et al*. 1989; Billington *et al*. 1992). Although more than half of the range of *S. vitreum* occurs within formerly glaciated portions of North America (Scott & Crossman 1973), only 10 haplotypes were detected within this area, and comprised one haplotype and mutational derivatives from each refugial group. In contrast, populations in the nonglaciated portion of its range had higher diversity, with 24 additional haplotypes detected (Billington *et al*. 1992). In addition, clade divergences were greater among nonglaciated haplotypes than among those occupying deglaciated habitats (N. Billington, unpublished data). Similarly, the brook charr (*Salvelinus fontinalis*) displays marked differences in phylogeographic structure between glaciated and nonglaciated portions of its range (Bernatchez & Danzmann 1993; R. G. Danzmann *et al*. unpublished). Although members of three clades are present in formerly glaciated regions, populations within this area are overwhelmingly dominated by a single haplotype. Conversely, the nonglaciated portion of the species range contains much richer phylogenetic diversity, and deeper clades with clear geographical structuring. Richardson & Gold (1995a) observed similar latitudinal differences in phylogeographic structuring of the shiner *Cyprinella lutrensis*, which they attributed to glacial effects. Interestingly, none of the area occupied by *C. lutrensis* was covered by glaciers, indicating that glacial impacts were felt beyond the ice margins. In Eurasia, brown trout (*Salmo trutta*) also display varying degrees of phylogeographic structure that correspond with glacial history (Bernatchez *et al*. 1992; Giuffra *et al*. 1994; Bernatchez & Osinov 1995). Of the five major *S. trutta* mtDNA clades observed, only one occupied previously glaciated regions of Northern Europe (Bernatchez 1995). In addition, most *S. trutta* populations were dominated by one or two haplotypes which were widely distributed throughout the Atlantic basin (Bernatchez *et al*. 1992; Hynes *et al*. 1996).

Global latitudinal patterns

General methodology

In order to provide a more empirical test of the relationship between genetic diversity and latitude, we quantified levels of intraspecific genetic diversity as a function of specific latitudinal distribution. To do so, we combined data from available studies dealing with intraspecific mtDNA diversity over broad geographical ranges, i.e. hundreds of kilometres or more. Thus, local studies of stock structure were not considered. Otherwise, there was no selection of data made a priori. We included anadromous species despite their potential for marine dispersal, as their overall intraspecific diversity was probably affected in a similar manner due to the availability of freshwater spawning habitats being a limiting factor for reproduction. Because most species were generally not selected a priori to initially test a general phylogeographic hypothesis, they represent a random sampling of phylogeographic diversity. The middle latitude (mean of extreme latitudes) was used as a measure of species distribution and determined from various distribution maps (Lelek 1987; Scott & Crossman 1973; Page & Burr 1991). As most studies differed substantially in the way that mtDNA genetic diversity was measured (RFLP analysis of the entire mtDNA molecule, or RFLP and/or sequencing of various mtDNA segments with different numbers of enzymes or nucleotides sequenced) or analysed (i.e. overall nucleotide diversity computed or not, different methods of tree reconstruction), strict standardization of the different data sets was not possible. On the other hand, retaining only those data sets that were strictly comparable would have resulted in a very reduced number of cases. Therefore, we accepted the potential variance in measurements imposed by the diversity of analyses under the rationale that the increased noise caused by nonuniform data sets should contribute more to blurr significant patterns rather than causing them. Consequently, evidence for latitudinal patterns should be very conservative in such circumstances.

In the absence of nucleotide diversity estimates for all species, we computed a common measure of genetic diversity by multiplying the deepest branch length of intraspecific trees by the overall haplotype diversity (h; Nei & Tajima 1981) for that species to generate a relative index of nucleotide diversity (Appendix 1). Although not absolute, this parameter approximated nucleotide diversity estimates in species for which it was available. We then statistically analysed the relationships between median latitude and nucleotide diversity using linear, negative exponential, and piecewise regression models. The piecewise regression algorithm generates a composite of multiple linear models (two in the present case) separated by breakpoint on the abciss coordinate in such a way as to optimize the percentage of explained variance. Similar procedures were also applied for interspecific comparisons (see below).

Intraspecific diversity

Figure 3 clearly illustrates the general trend of reduced nucleotide diversity with increasing latitude among the 42 species that could be compared. Both linear and negative exponential regression models indicated that this relationship was highly significant ($P < 0.0002$) and explained a substantial proportion (exponential = 29%, linear = 31%) of the total variance in nucleotide diversity. A piecewise linear regression model proved to be much more powerful, explaining 87% of the total

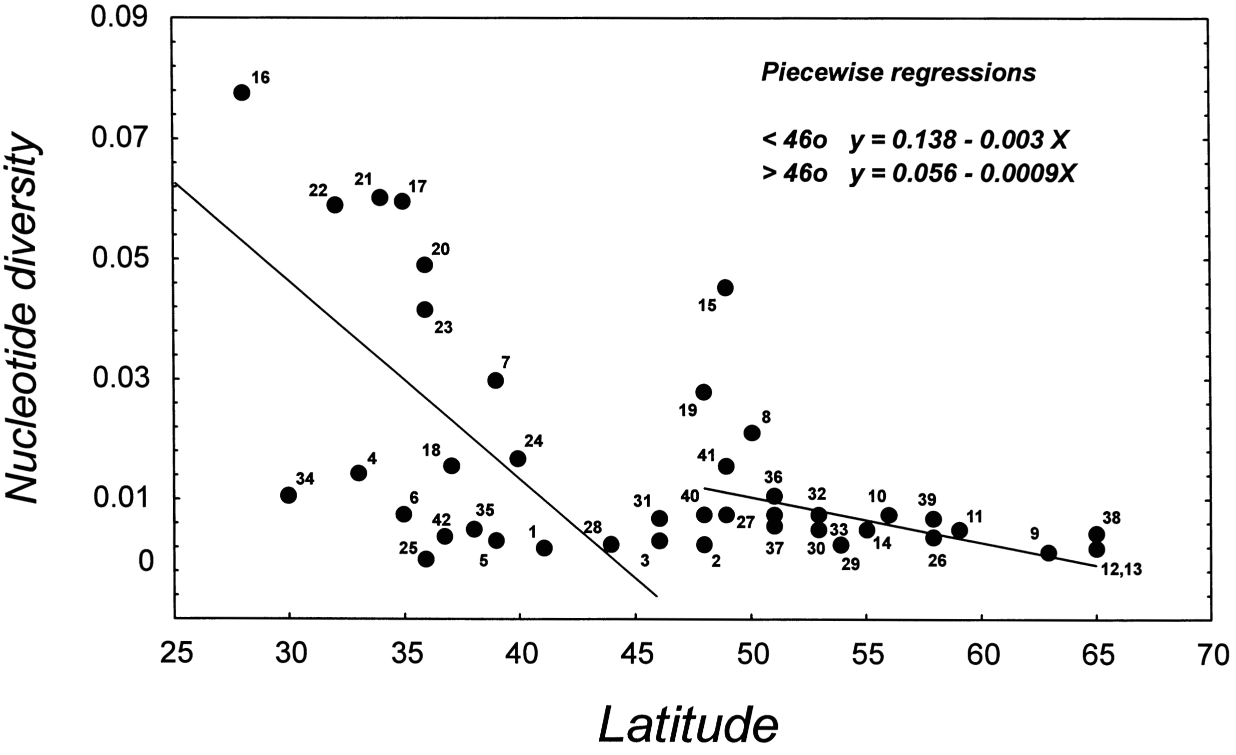

Fig. 3 Relationships between intraspecific nucleotide diversity index as a function of median latitudinal distribution of Nearctic and Palearctic freshwater and anadromous fish species. Numbers refer to species identification in Appendix 1. Piecewise regression models that best explained the total variance below and above breakpoint (46°) are given on the graph.

genetic variance. The breakpoint between the two linear relationships that composed the piecewise model was at 46° latitude, largely coincident with the median latitude of maximum Pleistocene glacial advance in North America (≈ 44°; Fulton & Andrews 1987). These results strongly indicate the dominant effects of glaciation events in shaping intraspecific genetic diversity, and that such effects can to some extent be quantified in a predictive manner. Interestingly, McAllister *et al.* (1986) found that glacial impacts on fish communities, as indicated by reduced species numbers and low incidence of endemic species, extended approximately 3° beyond glacial limits. It therefore appears that the latitudinal gradient of mtDNA diversity within species fits closely with species diversity patterns (Hocutt & Wiley 1986; Mayden 1992), and suggests that the same historical events had comparable effects within and above the species level.

Case-by-case comparisons of diversity are also suggestive of other general patterns that could be confirmed by studies of additional species. For instance, nucleotide diversities of the three species from the southwestern USA (nos 4, 34, and 42) were much lower than those generally observed in their southeastern counterparts. Although not included here because of the likely effects of human-induced perturbations on their genetic diversity, two other species (*Tiagora cobitis*, *Meda fulgida*) from the same area showed similarly reduced diversity values (Tibbets & Dowling 1996). Maximum divergence estimates between sister species from this region (nos 11, 12, and 16) also had comparable values (Fig. 4). These observations are suggestive of general differences in intraspecific diversity between southwestern and southeastern North American fishes, corroborating differential patterns of species diversity (Hocutt & Wiley 1986) which can be related to the more pronounced habitat and climatic disturbance in the southwest during Pleistocene times (Riddle 1996). Another trend illustrated by the diversity vs. latitude relationship was the globally low genetic diversity observed in holostean and chondrostean fishes (nos 1, 2, 3, 6, and 35), regardless of their median latitudinal distribution. This suggests that differences in either mtDNA mutation rates and/or evolutionary female effective population sizes may exist between teleost and nonteleost fishes (see below).

A basic prediction of the neutral theory is that levels of polymorphism within and divergence between species should be correlated, which implies that the ratio of intraspecific diversity of a given species to its estimated divergence from sister species should be more or less constant (Hudson 1990). Differences of this ratio among

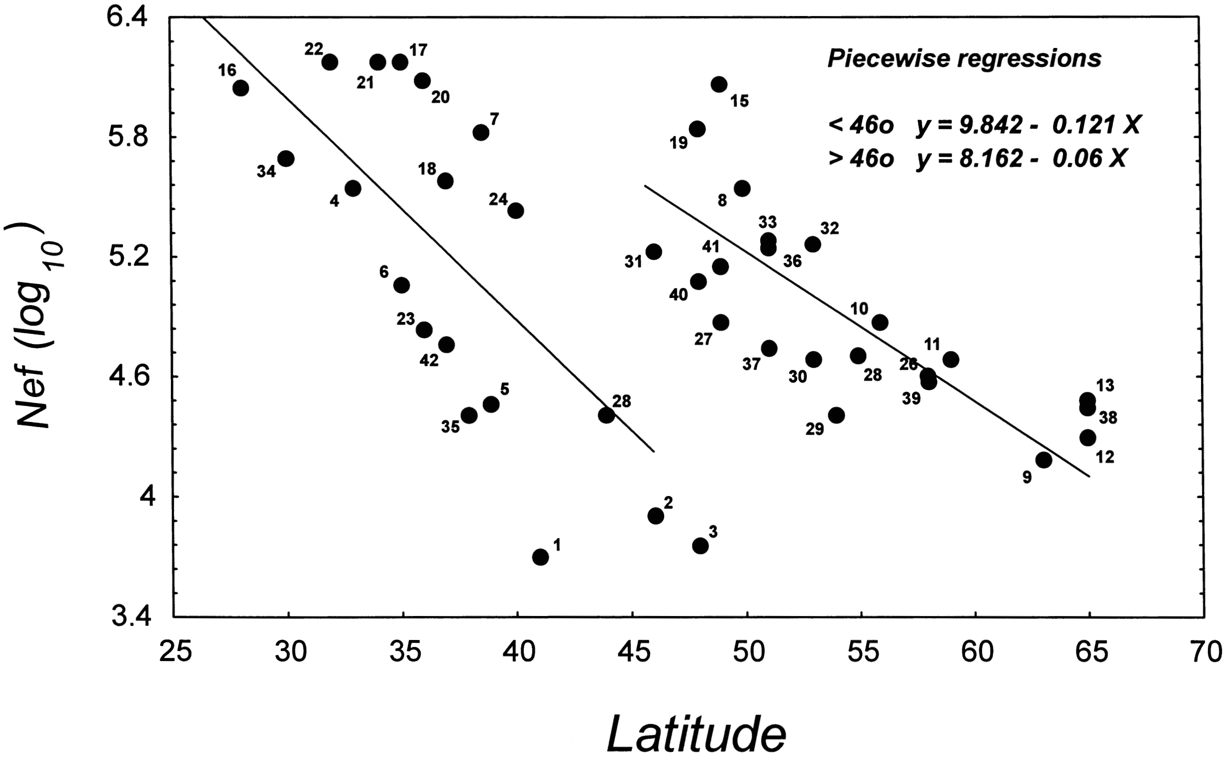

Fig. 4 Relationships between the logarithm of evolutionary female effective population size as a function of median latitudinal distribution of Nearctic and Palearctic freshwater and anadromous fish species. Numbers refer to species identification in Appendix 1. Piecewise regression models that best explained the total variance below and above breakpoint (46°) are given on the graph.

species may be indicative of differential selective and/or demographic effects and, therefore, be used to compare patterns of genetic diversity among regions. We computed the ratio of sister-species divergence to intraspecific nucleotide diversity for those species where both types of data were available (n = 28; Appendices 1 and 2). Cases with interspecific introgressive hybridization were excluded. While no significant latitudinal cline was observed as in our previous comparisons, there was a clear dichotomy in mean ratio values between species with median latitudinal distributions above and below the breakpoint (46°) depicted in the piecewise regression analyses. Divergence between sister species tended to be much lower among northern than southern taxa (see Fig. 5, and related section below). However, the greatly reduced levels of intraspecific divergence within northern species (Fig. 3) resulted in their mean ratio value being significantly higher (4.70 ± 2.21) than that for southern species (1.31 ± 0.35) (Mann–Whitney U-test = 7.5, P = 0.0014).

Evolutionary effective population size

Theoretical and simulation studies have revealed the relationship between the average number of generations (G) since two randomly chosen mtDNA variants within a sample shared a common ancestor (coalescence time) and the effective female population size (N_{ef}) (e.g. Avise *et al*. 1984; Hudson 1990). Assuming the validity of applying a given 'molecular clock', one can therefore estimate N_{ef} with knowledge of the mean pairwise divergence between mtDNA sequences and a species' average generation time, under the assumptions imposed by the inbreeding theory for neutral mutations (Avise *et al*. 1988). In the present case, such quantification could not strictly be made for all species as the mean sequence divergence was often not available, mutation rates are unknown, reproduction within species is obviously nonrandom and population size has probably not been constant. Thus, we computed a relative (rather than absolute) index of N_{ef} by using average specific generation times reported in the fish-related literature and the relative index of nucleotide diversity described above as a comparative measure of mean divergence estimate within species, and tentatively applying an evolutionary mutation rate of 2×10^{-8} mutations/site/year. Given the partial dependence of N_{ef} on the nucleotide diversity index described above, its relationship with median latitudinal species distri-

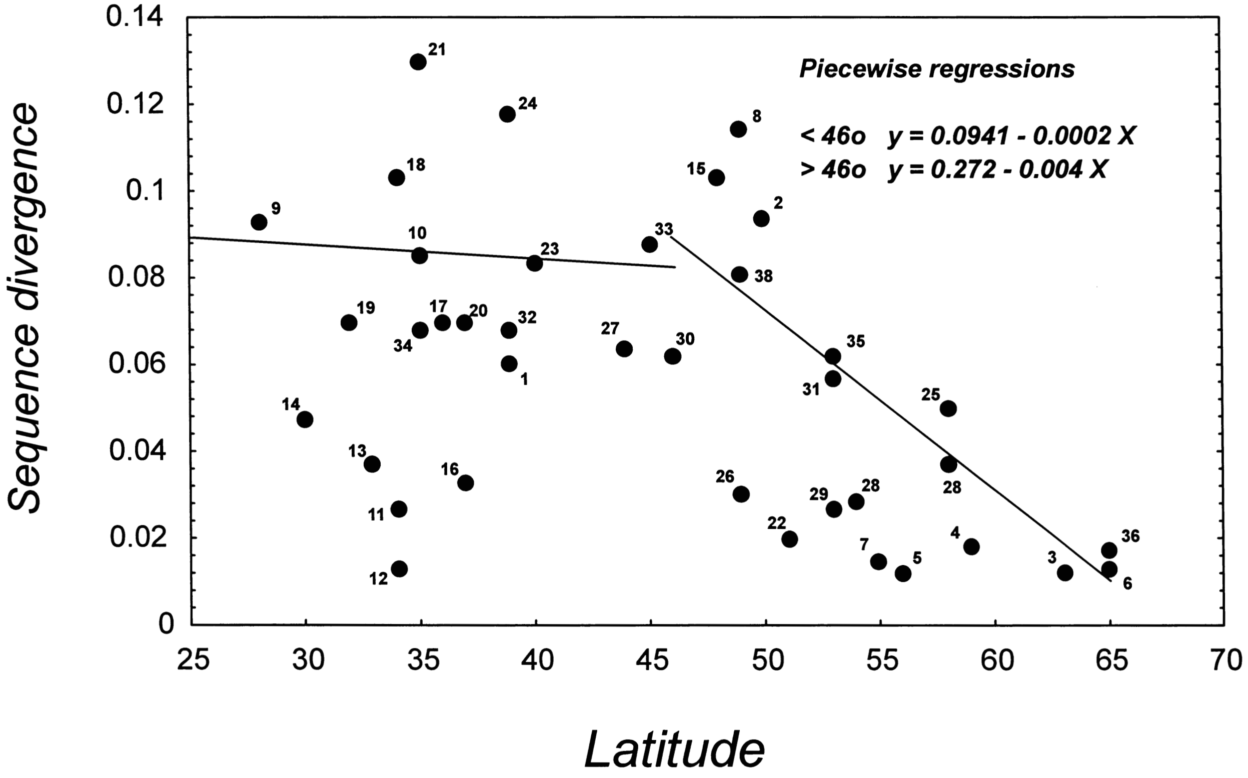

Fig. 5 Relationships between sequence divergence among pairwise comparisons of sister species as a function of median latitudinal distribution for Nearctic and Palearctic freshwater and anadromous fish species. Numbers refer to species identification in Appendix 2. Piecewise regressions that best explained the total variance below and above breakpoint (46°) are given on the graph.

butions was similarly negative and highly significant ($P < 0.001$) (Fig. 4). Linear and piecewise regression models, respectively, explained 26% and 75% of the variance. The breakpoint between the two linear relationships that comprised the piecewise model was at 46° latitude. This relationship is therefore indicative of either latitudinal variation in mtDNA mutation rates or, more likely, differential population reduction as a function of habitat loss/disturbance imposed by glacial movements. In accordance with similar previous studies (e.g. Avise 1992), all estimated N_{ef} values appeared to be much lower than contemporary species abundances. This discrepancy may be due to fluctuations in population sizes over evolutionary time, female reproductive contributions not conforming to Poisson distribution (Avise *et al.* 1988), and/or rates of mtDNA evolution variably much slower than the 2% sequence divergence/MY (e.g. Martin & Palumbi 1993; Bentzen *et al.* 1993; Smith 1992). N_{ef} estimates for nonteleost fishes also appear lower compared to teleosts (Fig. 3, Appendix 1). Thus, the proportion of explained variance by regression models substantially increased (linear, 26% to 47%; piecewise, 75% to 84%) when nonteleost species were omitted. This suggests uniquely shared demographic characteristics or average mtDNA mutation rates for these fishes as a group.

Rates of dispersal

As described previously, proglacial lakes provided geographically isolated races of northern species with tremendous dispersal opportunities (Fig. 2, b and e). As fish from nonglaciated areas had limited access to such dispersal routes, phylogenetic lineages of southern species have been isolated for much longer than their northern counterparts. One can therefore predict major differences in the scale and evolutionary rates of dispersal as a function of latitude. First, one would expect that clades of comparable sequence divergence (which assumes similar age) would be distributed over larger surface areas in formerly glaciated regions compared to nonglaciated ones. Second, one would also expect a poorer relationship between clade divergence and occupied surface areas in northern latitudes, as recent dispersal within formerly glaciated regions has only been possible for the last 15 000 years for all clades, regardless of their age.

We tested these predictions by quantifying surface areas occupied by phylogenetically distinct clades as a

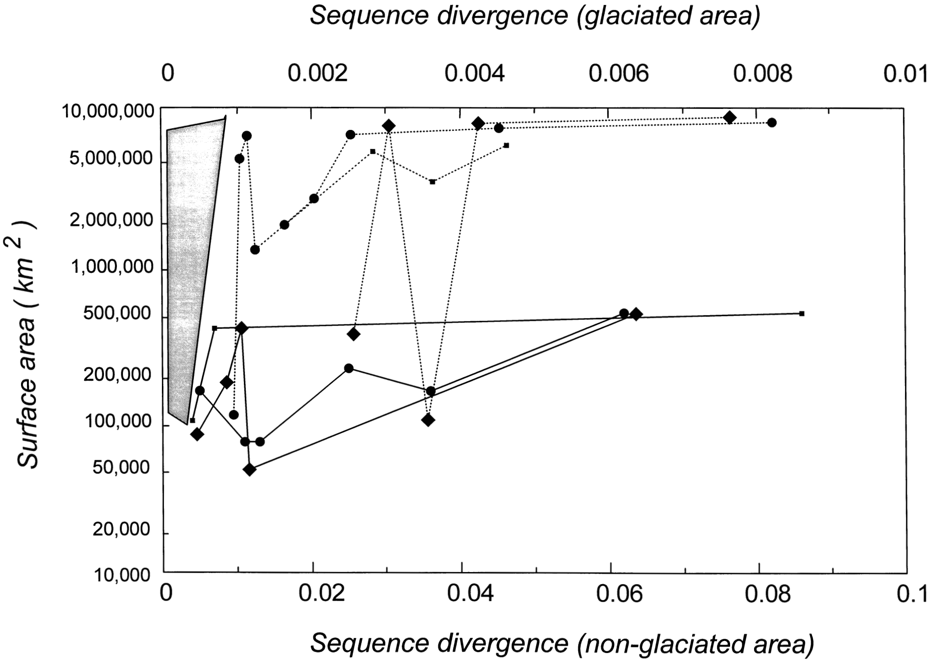

Fig. 6 Relationships between surface area occupied by mtDNA clades as a function of their depth in sequence divergence for three North American freshwater fish species from formerly glaciated and nonglaciated regions. The dotted lines represent species from glaciated areas: *Coregonus clupeaformis* (diamond), *Salvelinus namaycush* (circle), *Stizostedion vitreum* (square). Full lines represent species from nonglaciated areas: *Lepomis gulosus* (diamond), *Lepomis punctatus* (circle), *Lepomis microlaphus* (square). Note the difference of one order of magnitude between scales of sequence divergence for both fish groups. The shaded area represents the projection of relationships among species from glaciated areas on the scale of those from nonglaciated regions.

function of their estimated sequence divergence. Figure 6 illustrates such relationships for species found in formerly glaciated and nonglaciated areas. The discrepancy between species from the two regions is striking. Surface areas occupied by clades of southern species were approximately an order of magnitude smaller (50 000–500 000 km^2) than those occupied by their northern counterparts (500 000–5 000 000 km^2), despite the 10-fold discrepancies in sequence divergence clade depths (0.001–0.008 substitutions per site among northern species compared to 0.01–0.08 for their southern counterparts). For comparative purposes, we approximated evolutionary rates of dispersal for pooled northern vs. southern species by dividing the surface area occupied by a given clade by its time of coalescence which was quantified here by tentatively applying a 'molecular clock' of 2×10^{-8} mutations/site/year. The average evolutionary dispersal rate thus obtained for the southern group was 88 ± 75 km^2/1000 years compared to 9260 ± 811 km^2/1000 years for the northern group. The second prediction of a relatively less significant relationship between occupied surface areas and clade divergence in northern latitudes was also supported by the lower and nonsignificant correlation between these parameters among northern (r^2 = 0.167, P = 0.06) compared to southern species (r^2 = 0.420, P = 0.01).

Although the above values should be considered as relative indicators rather than hard data, the major differences they show between northern and southern species are real, and demonstrate that evolutionary insights such as estimating regional levels of historical gene flow may be obtained through a comparative phylogeographic approach.

Evolutionary processes

The significant latitudinal gradients in patterns of phylogeographic structure demonstrated by the above analyses strongly suggest that the genetic diversity of Nearctic and Palearctic freshwater fishes was affected by Pleistocene glaciations in a predictive manner. None of these treatments, however, directly address the potential influence of such historical events on evolutionary processes. Of particular interest is to determine whether Pleistocene glaciations have influenced speciation rates (Zink & Slowinski 1995). A major tenet of ecological speciation theory is that high ecological opportunity, such as colonization of resource-rich environments free of competitors and/or predators, will promote rapid phenotypic evolution leading to speciation events (e.g. Mayr 1963). Newly available aquatic habitats following glacial retreat in northern latitudes are considered to have provided such opportunities that may have promoted adaptive radiation in colonizing species (Behnke 1972; Schluter & McPhail 1993; Skùlason & Smith 1995). Given the repeated 'founder-flush' cycles of geographical isolation and bottlenecking of northern fishes during glacial advances alternating with expansion and recolonization of newly formed habitats during glacial retreats, one would predict that bursts of speciation events at northern latitudes may have occurred in recent evolutionary times, corresponding with glaciation events. The recentness of speciation events in northern fishes has been indicated by circumstantial evidence and empirical data (e.g. Behnke 1972; Taylor & Bentzen 1993a,b; Bernatchez *et al*. 1996; Chouinard *et al*. 1996). In contrast, fish species in nonglaciated regions would have had less opportunities for ecological release, due to the presumably greater temporal and environmental stability of communities and habitats. It should be noted, however, that recent phenotypic radiations are not unique to glaciated areas, as evidenced by the swarms of closely related but ecologically diverse cichlid species in the African Great Lakes, or Mexican cyprinodonts (Meyer *et al*. 1996; Strecker *et al*. 1996).

In order to test the above predictions, we quantified the relationships of sequence divergence between sister species as a function of their median latitude of distribution, the rationale being that under the neutral theory, smaller divergence estimates reflect younger speciation events. As there is no positive correlation between sister-species divergence and levels of intraspecific diversity (as shown above), this information is independent of the intraspecific diversity vs. latitude relationships. Both linear and negative exponential regression models indicated a highly significant negative relationship between sister-species divergence and their latitudinal distribution ($P < 0.002$), and explained 24% of the total variance in sequence divergence among the 38 species pairs than could be compared (Appendix 2). As with the nucleotide diversity vs. latitude relationship, a piecewise linear regression model proved to be much more powerful, explaining 74% of the total genetic variance. It is again worth noting that the breakpoint between the two linear relationships that composed the piecewise model was at 46° latitude, identical to the previous relationships observed.

As with the nucleotide diversity vs. latitude relationship, case by case comparisons were also suggestive of discrepancies in sister-species divergence values between fishes from the southwestern and southeastern USA at comparable latitudes (Fig. 4). Thus, the sister-species divergence estimates for the three pairwise comparisons from the south west (no. 11, 12, 16) were substantially lower than those generally observed in their south-eastern counterparts (Fig. 4). Excluding these comparisons from the regression models increased the explained proportion of variance by the linear regression model from 24% to 39%. These observations indicate more recent bursts of speciation in southwestern North America compared to the south east. In combination with data documenting the pronounced Pleistocene climatic disturbances in the American south west (Riddle 1996), these data may suggest that disturbance and/or ecological opportunities were involved in promoting speciation in this region.

Further evidence that ecological factors have largely been responsible for promoting postglacial speciation events in northern latitudes can be obtained by assessing phylogeographic relationships of cospecific morphotypes. Sympatric ecomorphs are virtually nonexistent in southern species, but are not uncommon among northern fishes (Skùlason & Smith 1995; Schluter 1996; Schluter & McPhail 1993). The habitats in which these morphs or species pairs occur are typically less than 15 000-years old, setting an upper limit to the duration of their coexistence. Phylogeographic data suggesting ecological diversification and phenotypic differentiation with varying degrees of phylogenetic divergence has been documented in many species, such as lake whitefish (Bernatchez & Dodson 1991), lake trout morphs in the Laurentian Great Lakes (Burnham-Curtis 1994), and arctic charr morphs in Thingvallavatn, Iceland (Danzmann *et al*. 1991; Snorrasson *et al*. 1994), indicating that this phenotypic differentiation has occurred in extremely short spans of evolutionary time.

More advanced cases of genetic divergence through ecological differentiation have led to the formation of reproductively isolated taxa and species pairs. Species pairs from different lakes and/or species complexes usually divide resources in the same way. Typically one species is a pelagic zooplanktivore whereas the other consumes benthic prey. Reproductive isolation between these sympatric forms has been demonstrated with both genetic and ecological data (e.g. Bodaly 1979; McPhail 1993; Vuorinen *et al*. 1993; Taylor & Bentzen 1993b). Phylogeographic analyses also clearly demonstrated that similar species pairs found within a given species complex evolved more than once. For instance, the tandem occurrence of benthic and limnetic ecotypes of lake whitefish in phylogeographic assemblages that are distinguishable by uniquely derived genetic characters provides strong evidence that similar phenotypic patterns evolved in parallel in separate but closely related lineages (Bernatchez *et al*. 1996; Pigeon *et al*. 1997). Similar conclusions have been reached from phylogeographic analyses of other species complexes, namely smelt, *Osmerus* sp. (Taylor & Bentzen 1993a; Bernatchez 1997), threespine sticklebacks, *Gasterosteus* sp. (Taylor *et al*. 1997; Thompson *et al*. 1997), and sockeye salmon, *Oncorhynchus* sp. (Taylor *et al*. 1996; Wood & Foote 1996). The demonstration of parallel evolution of morphotypes within phylogenetically distinct lineages under similar environmental conditions represents the strongest nonexperimental evidence that evolution occurred by natural selection imposed by ecological forces (Schluter & Nagel 1995). The comparative phylogeographic analysis of these young species pairs is therefore indicative that the replicate evolution of phenotypically similar species may be a common phenomenon in northern latitudes, and provides convincing evidence that ecological selective forces may have been largely responsible for promoting postglacial speciation events in northern latitudes.

Relevance for conservation and management

A basic prerequisite for managing biodiversity is the identification of population groups with independent evolutionary histories. While the recognition of evolutionarily distinct groups is more or less straightforward at the species level and above, such delineation at intraspecific levels has been hampered until recently by the lack of clear guidelines to define them. The concept of evolutionarily significant units (ESU) was proposed by Ryder (1986) in the mid-1980s in an effort to better define subunits of species for conservation purposes. While criteria for defining an ESU remain to be firmly established, there is a general consensus that a set of populations with a distinct, long-term evolutionary history (therefore substantially contributing to the overall genetic diversity of the species) merit ESU status. (e.g. Avise & Ball 1990; Dizon *et al*. 1992; Bernatchez 1995; Moritz 1995). It has further been proposed that ESU recognition would be better supported by the demonstration of their reproductive isolation (Waples 1991; Moritz 1994). Given the unique evolutionary and historical insights provided by the phylogeographic approach, its relevance for defining ESUs in fishes is obvious. Indeed, several of the case studies mentioned have provided some of the clearest examples of ESUs in fishes, based on phylogeographic information. In many cases, the unanticipated genetic discontinuities observed allowed the identification of highly distinct population groups that are presently ignored in taxonomy. The evolutionary significance of these population groups has also been supported in many instances (e.g. *Osmerus mordax*, *Coregonus clupeaformis*, *Salmo trutta*) by observations of substantial reproductive isolation when these are found in sympatry (Bernatchez & Dodson 1990a; Bernatchez & Martin 1996; Giuffra *et al*. 1996). When such information leads to the identification of distinct ESUs within multiple species in the same geographical area (e.g. Bermingham & Avise 1986; Avise 1992), it may provide sufficient evidence for defining regions that merit special attention for conservation, even if they do not contain an array of endemic species as recognized by conventional methods (Avise 1992, 1995; Moritz 1994).

Another important asset of the phylogeographic approach from a conservation perspective is in assessing the validity of taxonomically recognized species or population segments (e.g. subspecies). Given the relatively limited resources devoted to conservation of biodiversity, there is a growing concern that priority recognition for protection should be commensurate with the evolutionary distinctiveness of species (Rohlf 1991; Rojas 1992). Currently, recognition of 'evolutionary distinctiveness' largely relies on traditional taxonomy, which has most frequently been derived from the analysis of phenotypic variation. However, increasing evidence indicates that traditional taxonomic designations may not always reflect actual evolutionary relationships, which can detract from conservation efforts for perpetuation of ESUs and maintenance of natural genetic structure within species (discussed in Avise 1994).

Such complications may be acute among newly formed species in which rapidly evolving parallel populations may cluster together as polyphyletic groups when homoplasies are more probable than informative changes. For example, populations of the lake whitefish species complex (*Coregonus* sp.) exhibit extreme phenotypic variation in morphological and life-history characteristics, and have been split into numerous phenotypically based taxa as a consequence (summarized in Bernatchez 1995). Geographic patterns of whitefish population genetic assemblages provided a basis to challenge the validity of traditional taxa, based on the principle that taxonomic designations should identify groups of populations with distinct evolutionary origins. For instance, mtDNA phylogeographic analyses demonstrated that populations from Beringia (recognized as *Coregonus clupeaformis* along with all other North American populations) were much more closely related to Eurasian populations (recognized as *C. lavaretus*) than to any other North American ones (Bernatchez & Dodson 1994). Similarly, mtDNA phylogeographic patterns provided strong evidence for the independent origins of phenotypically similar populations from central European alpine lakes and northern Eurasia which had been lumped together into polyphyletic and therefore artificial taxa (Bernatchez & Dodson 1994). Evidence that traditional taxonomic methods do not always reflect evolutionary distinctiveness of fish populations has also been supported by phylogeographic analysis of other species complexes, particularly smelt (*Osmerus* sp.) in North America and native trout from Eurasia (*Salmo* sp.) (Baby *et al*. 1991; Bernatchez *et al*. 1992; Taylor & Bentzen 1993a; Giuffra *et al*. 1994; Bernatchez & Osinov 1995). Such observations have led to the proposed use of integrating phylogeographic data into the definition of ESUs, along with other types of data (Bernatchez 1995). This approach takes into account the strengths and weaknesses of each data system (namely the limitations of phenotypic data to depict phylogenetic relationships and those of neutral DNA polymorphism to infer local adaptation), overcoming the limitations of individual methods through a synthesis of complementary data types.

These are just a few examples that illustrate the potential offered by the phylogeographic approach to improve conservation practices and policies. Other applications, such as the identification of management units (MU), genetic tags, inferences about population size and connectedness, have been addressed in general terms elsewhere (e.g. Moritz 1994; Avise 1996), and routinely used in fishes over the last decade (e.g. Grewe & Hebert 1988; Billington & Hebert 1988; Bentzen *et al*. 1989; Bernatchez & Dodson 1990b; Bermingham *et al*. 1991; Billington & Hebert 1991; Dodson *et al*. 1991; Bernatchez & Martin 1996).

Future directions

Where can fish phylogeography go from here? Despite the many studies cited in this article, the phylogeographic picture for both North America and Eurasia is far from complete. In particular, more large-scale geographical studies are needed, at both single-species and comparative levels. The relevance of such studies has been convincingly demonstrated in elucidating both multispecies phylogeographic congruence (Bermingham & Avise 1986; Avise 1992) and similarities and contrasts among species in formerly glaciated regions (Hewitt 1996). As the data available grows, the power of comparative approaches will also increase. It is worth noting that one of the earliest phylogeographic structures examining fish mtDNA (Bermingham & Avise 1986), by taking a comparative approach, uncovered significant regional structuring that extended well beyond the species examined and paved the way for subsequent studies (Avise 1992).

Despite the simplifications and associated caveats in the analyses presented here, clear quantitative trends in regional differences emerged. As well as highlighting both qualitative and quantitative differences in phylogeographic structure and evolutionary histories between species in formerly glaciated vs. unglaciated regions, the comparative data indicated major differences between fish species in the southwestern vs. southeastern USA, as well as between teleost and nonteleost fishes. As data collection becomes more standardized through the increased popularity and simplicity of methods such as DNA amplification and sequencing, more detailed comparative analyses will be possible. The growing number of published studies will also enable more detailed comparative studies using historical, ecological, and phylogenetic frameworks to evaluate phylogeographic data.

Phylogeographic studies from other areas of the world should prove equally interesting to those discussed here. Considerable insights could be gained by gathering phylogeographic data from other regions with potentially unique evolutionary histories (e.g. Dodson *et al*. 1995; Lu *et al*. 1997). Given the importance of phylogeographic data for conservation issues (Moritz 1994; Bernatchez 1995), there is a growing urgency for such studies as human impact on aquatic systems increases worldwide.

Studies which examine the interrelationships between ecological and genetic parameters should also prove fruitful (e.g. Turner *et al*. 1996). Comparisons among closely related species or members of ecological guilds may highlight effects of differing demographic traits or historical events on phylogeographic structure. Alternatively, species could be targeted which are not closely related but broadly co-occur and have a shared zoogeographic history. Species could be selected on the basis of variation in body size, dispersal ability, generation time, fecundity, food and habitat requirements, and/or trophic level. These opportunities for experimental and predictive phylogeography could potentially produce huge payoffs for conservation biology and evolutionary ecology.

We hope that phylogeographic data will also be increasingly used to examine other issues in mainstream evolutionary biology. Introgressive hybridization between fish species is a common occurrence, in both natural populations and between native and introduced stocks (e.g. DeMarais *et al*. 1992; Dowling & Childs 1992; Dowling & DeMarais 1993; Wilson & Hebert 1993; Bernatchez *et al*. 1995). Although issues such as reticulate evolution and genetic responses to novel environments can be addressed by population genetics, phylogeographic data is essential for their resolution in an evolutionary context. Where possible, phylogeographic studies should incorporate data from complementary systems such as allozymes, other nuclear genes, VNTR and microsatellite loci (e.g. Tessier *et al*. 1995, 1997; Angers *et al*. 1995; Taylor 1995; Taylor *et al*. 1996; Bentzen *et al*. 1996) with mtDNA to enable more robust inferences (Avise 1989; Moritz 1994). Newly realized complications for using mtDNA as a phylogeographic tool also provide stimulating challenges: the potential non-neutrality of mtDNA or mitochondrial variants (William *et al*. 1995), environmental effects on mutation rates (Rand 1994), incorporation of mtDNA into nuclear genomes (Zhang & Hewitt 1996), and coadaptation and/or interaction between mitochondrial and nuclear genomes may provide new insights into phylogenetic processes and resultant phylogeographic patterns.

In conclusion, phylogeographic studies have significantly contributed to modern fish biology, and will provide increasingly powerful insights as more data accumulates. Both intraspecific studies and comparative intertaxon studies at a variety of scales have produced a wealth of information on microevolutionary patterns, permitting inferences of macroevolutionary processes with some certainty. These and similar studies are enabling us to re-evaluate the evolutionary biology of freshwater and anadromous fishes, and permit the reconstruction of species' evolutionary trajectories across space and time.

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This paper was stimulated by the authors' common research interests in comparative phylogeography, evolution and conservation biology of aquatic organisms. L.B.'s research program focuses on the understanding of pattern and processes of molecular and organismal evolution, as well as their significance to conservation. C.C.W. examined postglacial dispersal of North American salmonids for his PhD, and is currently working on the ecological and evolutionary significance of hybridization in Australian zooplankton.

Appendix 1

Intraspecific genetic data for freshwater and anadromous fishes used in latitudinal analyses, showing median latitudinal range of distribution, overall nucleon diversity (h), maximum intraspecific sequence divergence (d; substitutions/site), nucleotide diversity index ($d \times h$), and evolutionary female effective population size (N_{ef}), estimated by the ratio time of coalescence/generation time. Time of coalescence was estimated by the ratio nucleotide diversity index/0.02×10^6. Numbers to the left of species names refer to identifications in Figs 3 and 4.

Species	Median latitude	Nucleon diversity	d	Nucleotide diversity	N_{ef} (10^3)	Sources
1. *Acipenser oxyrhynchus*	41	0.67	0.0030	0.0020	5.0	Bowen & Avise (1990)
2. *Acipenser transmontanus*	48	0.65	0.0035	0.0023	5.7	Brown *et al.* (1992)
3. *Acipenser fluvescens*	46	0.43	0.0074	0.0032	8.0	Ferguson *et al.* (1993), Guénette *et al.* (1993)
4. *Agosia chrysogaster*	33	0.57	0.0250	0.0140	350	Tibbets & Dowling (1996)
5. *Alosa sapidissima*	39	0.79	0.0037	0.0029	29	Bentzen *et al.* (1989)
6. *Amia calva*	35	0.81	0.0090	0.0073	112	Bermingham & Avise (1986)
7. *Ameiurus nebulosus*	39	0.92	0.0322	0.0296	740	Murdoch & Hebert (1997)
8. *Catostomus commersoni*	50	0.87	0.0240	0.0208	350	Lafontaine & Dodson (1997)
9. *Coregonus autumnalis*	63	0.50	0.0030	0.0015	15	Bernatchez *et al.* (1991), Bickham *et al.* (1992)
10. *Coregonus clupeaformis*	56	0.65	0.0115	0.0075	75	Bernatchez & Dodson (1991, 1994)
11. *Coregonus lavaretus*	59	0.95	0.0050	0.0048	48	Bernatchez & Dodson (1994)
12. *Coregonus nasus*	65	0.50	0.0040	0.0020	20	Bernatchez *et al.* (1991)
13. *Coregonus laurettae*	65	0.99	0.0030	0.0030	30	Bernatchez *et al.* (1991), Bickham *et al.* (1992)
14. *Coregonus artedii*	55	0.59	0.0085	0.0050	50	Bernatchez & Dodson (1990b), Snyder *et al.* (1992)
15. *Culaea inconstans*	49	0.71	0.0640	0.0454	1140	Gach (1996)
16. *Cyprinella lepida*	28	0.86	0.0900	0.0774	1940	Richardson & Gold (1995b)
17. *Cyprinella lutrensis*	35	0.88	0.0680	0.0598	1500	Richardson & Gold (1995a)
18. *Fundulus heteroclitus*	37	0.78	0.0196	0.0153	380	Gonzales-Villasenor & Powers (1990)
19. Gasterosteus aculeatus	48	0.94	0.0300	0.0282	7050	O'reilly *et al.* (1993), Orti et al. (1994)
20. *Lepomis punctatus*	36	0.79	0.0620	0.0489	1220	Bermingham & Avise (1986)
21. *Lepomis gulosus*	34	0.96	0.0630	0.0605	1510	Bermingham & Avise (1986)
22. *Lepomis microlophus*	32	0.68	0.0870	0.0592	1480	Bermingham & Avise (1986)
23. *Micropteus salmoides*	36	0.92	0.0450	0.0414	690	Nedbal & Philipp (1995)
24. *Morone americana*	40	0.33	0.0500	0.0167	278	Mulligan & Chapman (1989)
25. *Morone saxatilis*	36	0.16	0.0004	0.00007	0.7	Wirgin *et al.* (1989, 1993)
26. *Oncorhynchus nerka*	58	0.57	0.0067	0.0038	38	Bickham *et al.* (1995)
27. *Oncorhynchus mykiss*	49	0.91	0.0084	0.0076	76	Danzmann *et al.* (1993)
28. *Oncorhynchus masu*	44	0.82	0.0031	0.0025	25	Kijima & Matsunami (1992)
29. *Oncorhynchus keta*	54	0.42	0.0062	0.0026	25	Cronin *et al.* (1993), Park *et al.* (1993)
30. *O. tshawytscha*	53	0.60	0.0080	0.0048	48	Wilson *et al.* (1987), Cronin *et al.* (1993)
31. *Osmerus mordax*	46	0.83	0.0083	0.0069	172	Taylor & Bentzen (1993a), Bernatchez & Martin (1996)
32. *Osmerus eperlanus*	53	0.84	0.0088	0.0074	185	Taylor & Dodson (1994)
33. *Phoxinus eos*	51	0.70	0.0110	0.0077	192	Toline & Baker (1995)
34. *Poecilipsis occidentalis*	30	0.59	0.0180	0.0106	500	Quattro *et al.* (1996)
35. *Polyodon spatula*	38	0.36	0.0149	0.0052	26	Epifanio *et al.* (1996)
36. *Salmo trutta*	51	0.73	0.0144	0.0105	175	Bernatchez *et al.* (1992), Giuffra *et al.* (1994)
37. *Salmo salar*	51	0.76	0.0072	0.0055	55	Bermingham *et al.* (1991), King *et al.* (1993)
38. *Salvelinus alpinus*	65	0.34	0.0132	0.0045	28	Wilson *et al.* (1996)
39. *Salvelinus namaycush*	58	0.78	0.0086	0.0067	42	Wilson & Hebert (1997)
40. *Salvelinus fontinalis*	48	0.54	0.0130	0.0072	120	Bernatchez & Danzmann (1993), Danzmann *et al.* (submitted)
41. *Stizostedion vitreum*	49	0.67	0.0230	0.0154	140	Billington & Hebert (1988); Billington *et al.* (1992); Billington & Strange (1995)
42. *Xyrauchon texanus*	37	0.83	0.0070	0.0058	58	Dowling *et al.* (1996)

Appendix 2

Interspecific pairwise sequence divergence estimates for sister-species comparisons used in latitudinal analyses, showing median latitudinal range of distribution, and estimated divergence between taxa.

Species	Median latitude	Divergence	Sources
1. *Ameiurus nebulosus – A. natalis*	39	0.060	Murdoch (1992)
2. *Catostomus commersoni- C. catostomus*	50	0.094	Lafontaine & Dodson (1997)
3. *Coregonus artedii – C. laurettae*	63	0.015	Bernatchez *et al*. (1991)
4. *Coregonus lavaretus – C. nasus*	59	0.018	Bernatchez *et al*. (1991)
5. *Coregonus lavaretus – C. clupeaformis*	56	0.013	Bernatchez *et al*. (1991)
6. *Coregonus laurettae – C. autumnalis*	65	0.012	Bernatchez *et al*. (1991)
7. *Coregonus artedii – C. autumnalis*	55	0.015	Bernatchez *et al*. (1991)
8. *Culaea inconstans-G. aculeatus*	49	0.114	Gach (1996)
9. *Cyprinella lepida – C. venusta*	28	0.093	Richardson & Gold (1995b)
10. *Cyprinella lutrensis – C. lepida*	35	0.085	Richardson & Gold (1995a)
11. *Cyprinodon macularius – C. fontinalis*	34	0.027	Echelle & Dowling (1992)
12. *Cyprinodon nevadensis – C. salinus*	34	0.013	Echelle & Dowling (1992)
13. *Gambusia affinis – G. holbrooki*	33	0.037	Lydeard *et al*. (1995)
14. *Gambusia geiseri – G. heterochir*	30	0.047	Lydeard *et al*. (1995)
15. *Gasterosteus aculeatus – G. wheatlandi*	48	0.103	Orti *et al*. (1994)
16. *Gila robusta -G. seminuda*	37	0.033	DeMarais *et al*. (1992)
17. *Lepomis punctatus – L. microlophus*	36	0.070	Avise & Saunders (1984)
18. *Lepomis gulosus – L. cyanellus*	34	0.103	Avise & Saunders (1984)
19. *Lepomis microlophus – L. punctatus*	32	0.070	Avise & Saunders (1984)
20. *Luxilus chrysocephalus – L. cornutus*	37	0.070	Dowling *et al*. (1992)
21. *Luxilus coccogenis – L. zonistius*	35	0.130	Dowling *et al*. (1992)
22. *Luxilus cornutus – L. albeolus*	51	0.020	Dowling *et al*. (1992)
23. *Notropis stramineus – N. topeka*	40	0.083	Schmidt & Gold (1995)
24. *Notropis procne – N. topeka*	39	0.118	Schmidt & Gold (1995)
25. *Oncorhynchus nerka – O. gorbuscha*	58	0.050	Grewe *et al*. (1990)
26. *Oncorhynchus mykiss – O. clarkii*	49	0.030	Grewe *et al*. (1990)
27. *Oncorhynchus masu – O. mykiss*	44	0.064	Phillips *et al*. 1994)
28. *Oncorhynchus keta – O. gorbuscha*	54	0.028	Thomas *et al*. (1986)
29. *Oncorhynchus tshawytscha – O. kisutch*	53	0.027	Thomas *et al*. (1986)
30. *Osmerus mordax – O. dentex*	46	0.062	Taylor & Dodson (1994)
31. *Osmerus eperlanus – O. mordax*	53	0.057	Taylor & Dodson (1994)
32. *Pimephales notatus – P. vigilax*	39	0.068	Schmidt *et al*. (1994)
33. *Pimephales promelas – P. notatus*	45	0.088	Schmidt *et al*. (1994)
34. *Pimephales vigilax – P. tenellus*	35	0.068	Schmidt *et al*. (1994)
35. *Salmo salar – S. trutta*	53	0.062	Gyllenstein & Wilson (1987)
36. *Salvelinus alpinus – S. confluentus*	65	0.017	Grewe *et al*. (1990)
37. *Salvelinus namaycush – S. fontinalis*	58	0.037	Grewe *et al*. (1990)
38. *Stizostedion vitreum – S. canadense*	49	0.081	Billington *et al*. (1990)