Chapter Three

The Phylogeny of Some Thrushes of the Genus *Catharus* Derived from Control Region III Sequence Data

Introduction

The thrushes of the genus *Catharus* have a complex taxonomic history characterized by lack of recognition of species for long periods, and difficulty in defining intraspecific taxa. Indeed some putative subspecies within *Catharus* may still deserve species rank, e.g., *C. ustulatus ustulatus* and *C. u. swainsoni*, (Phillips 1991). The causes of this confusion include conservative plumage variation among species, and the distant wilderness ranges of some of the more troubling species, e.g., *C. minimus* and *C. ustulatus* (Todd 1963)*C. frantzii* and C. *occidentalis*(Phillips 1991).

Catharus thrushes are relatively small in comparison to other thrushes, ranging in length from 16 to 21.5 cm and with masses ranging from 25 to 45 gm; including the Wood Thrush, still officially in the monotypic genus *Hylocichla* (American Ornithologists' Union 1998). The generic status of the Wood Thrush (*H. mustelina*) remains controversial, although persuasive arguments exist for its inclusion in *Catharus* (Avise *et al.* 1980; Winker and Rappole 1988). All *Catharus* are brown-backed, whitish below, and many show dark spotting on the breast. These thrushes also have long pale legs, medium length pick-like bills used for digging in leaf litter, and spectacularly complex, flute-like songs with mixed

harmonics. The genus has 13 representatives breeding from eastern Siberia to northwestern Argentina (American Ornithologists' Union 1998; Ridgely and Tudor 1989). All live in wooded habitats ranging from scrubby and secondary growth to mature forest in mesic to wet climates. The group is dominated by ground feeding birds that occasionally feed as high as the shrub layer, with the exception of Swainson's Thrush (*C. ustulatus*), which is arboreal (Sabo and Holmes 1983; Holmes and Robinson 1988). Most species in the genus also nest on or near the ground.

The genus *Catharus* has received considerable attention from taxonomists because of its morphologically similar species (Rowley and Orr 1964; Ouellet 1993), its subtle intraspecific variation (Phillips 1991), its mix of migratory and non-migratory species (D. Outlaw, pers. comm.), and the complex biogeography of its many montane species. In spite of all this interest, only one paper employing biochemical methods to explore phylogeny has been published to date (Avise *et al.* 1980). *Catharus* has also been studied from a variety of other perspectives including interspecific competition (Noon 1981), comparative behavior (Dilger 1956b), vocalizations (Dilger 1956a; Weary *et al.* 1987), response to habitat fragmentation (Robinson *et al.* 1995), comparative metabolism (Holmes and Sawyer 1975), flight behavior during migration (Cochran *et al.* 1967; Diehl and Larkin 1998), and migratory stopover ecology (Yong and Moore 1997). Such a commonly studied group should have a better-specified phylogeny to promote accurate comparative study.

It has been inferred for some time that the Nearctic members of *Catharus* are members of a species group distinct from the Neotropical representatives (Mayr and Short 1970). Furthermore, Todd (1963) proposed that the Veery (*C. fuscescens*) and the Gray-cheeked Thrush (*C. minimus*) were likely each other's closest relatives, this being before the elevation of Bicknell's Thrush (formerly *C. minimus bicknelli*) to species status. This inference was based largely on general morphological and, particularly, vocal similarities (they have rapidly descending songs of stepped notes in series). There are also broad similarities in habitat structure, and nesting biology (Todd, 1963; W.G. Ellison, pers. observ.). Recent work has shown that Bicknell's and Gray-cheeked thrushes are allopatric in Québec and the Canadian Maritime provinces, have distinct wintering ranges in the West Indies (Bicknell's) and South America (Gray-cheeked), and differ consistently, if subtly, in morphology, plumage, and voice (Ouellet 1993). A restriction enzyme analysis of mtDNA by Gilles Seutin also revealed 1.7% divergence between Gray-cheeked Thrush and Bicknell's Thrush (Ouellet 1993).

The Bicknell's Thrush has no recognized subspecies, but the Veery and Gray-cheeked Thrush are considered polytypic, with at least two subspecies each (Phillips 1991). The eastern subspecies of the Veery (*fuscescens*), is distinctive, with warm cinnamon brown upperparts and indistinctly spotted breast, whereas the western subspecies, (*salicicola*), with its more olive brown back and discrete breast spotting, is similar to Gray-cheeked and Bicknell's (Moskoff 1995). The Gray-cheeked Thrush's subspecies include the Bicknell's-like *minimus* of insular Newfoundland and adjacent coastal Labrador and Québec,

and the grayer *aliciae* of subarctic Canada, Alaska and the Chukchi Peninsula of Russia (Godfrey 1986).

Gray-cheeked Thrush, Veery, and Bicknell's Thrush are essentially allopatric. Details of the approach of the range of Bicknell's Thrush to that of Gray-cheeked Thrush in southeastern Québec and the Maritimes are not completely worked-out, but it appears that they seldom, if ever, come into contact on the breeding grounds (Ouellet 1993). Veery and Gray-cheeked Thrush come into scant contact in southwestern Newfoundland. Although Veery and Gray-cheeked have different habitats in Newfoundland, there are rumors of rare hybridization between them (Phillips 1991). Although Bicknell's Thrush and Veery share a coextensive geographic range they are so well separated by elevation and habitat that they are functionally allopatric with respect to breeding (Levine 1998).

Although no formal hypothesis of the relationships among these three thrushes has been proposed, the opinion that Bicknell's and Gray-cheeked thrushes must be sister species may be traced to Ridgway's original description including *bicknelli* within the Gray-cheeked Thrush as a subspecies (Ridgway 1882). The assumption that Gray-cheeked and Bicknell's Thrushes are sister species is therefore based on the Bicknell's original classification as a subspecies and not rigorous quantitative assessment. In order to generate hypotheses about the geography of speciation in this group and its comparative ecology, history, and origin it would be well to have a hypothesis based on a more rigorous analysis.

The standard hypothesis of relationships among Bicknell's Thrush, Veery and Gray-cheeked Thrush is illustrated in cladogram A in Figure 3.1, showing Bicknell's and Gray-cheeked thrushes as sister species with a common ancestor shared with the Veery. Other plausible hypotheses are shown in cladograms B, wherein Gray-cheeked Thrush is shown as the sister species to Veery with Bicknell's basal, and C, with Bicknell's and Veery illustrated as sister species arising from a common ancestor with Gray-cheeked Thrush.

As part of this comparison of gene flow between Bicknell's Thrush and a close congener, I have collected blood samples from it and Gray-cheeked Thrush and Veery. I perceived this as an opportunity to examine the relationships among Bicknell's Thrush and its close relatives. Because details of the restriction enzyme fragment study of mtDNA mentioned above have not been published, this research could also provide some independent support for the species status of Bicknell's Thrush. I therefore obtained samples from the two other Nearctic *Catharus* thrushes, Hermit Thrush (*C. guttatus faxoni*) and Swainson's Thrush (*C. ustulatus swainsoni*) in order to have outgroups for the analysis, and to be able to examine molecular distances in this putative species group proposed by Mayr and Short (1970). In order to root the phylogenetic relationships within *Catharus*, I obtained from GenBank control region III sequence of another thrush, the European Robin (*Erithacus rubecula*; Questiau, 1998), to serve as the outgroup.

I have aligned a 395 base pair section of control region III (the right hand or 3' end of the light strand) to study relationships among these thrushes.

Although this is only a small sample of the mtDNA sequence available for analysis, it is a potentially useful segment. This is because it represents the most variable portion of the control region in many species, exceeding both control region I and the highly conserved central domain, also called control region II, in studies of other passerine birds (Baker and Marshall 1997; Zink and Blackwell 1998). Examination of control region I sequence from single Hermit and Bicknell's thrushes showed only about half as much divergence within this region as in control region III. As such, control region III is likely one of the shortest available sequences containing a notable amount of molecular evolutionary signal. At a minimum, these sequences may allow me to propose a useful working hypothesis of the relationships among Veery, Gray-cheeked and Bicknell's thrushes, and their general relationship to other species in the genus. Control region sequences have produced phylogenies that show reasonable agreement with morphological phylogenies in other studies of relationships within avian genera (Zink and Blackwell 1998; Marshall and Baker 1998; Freeland and Boag 1999b). These results suggest a close relationship among Veery, Gray-cheeked and Bicknell's thrushes, very recent divergence of the three species, and deeper divergences with other Nearctic members of the genus.

Methods

To investigate phylogenetic relationships among Swainson's, Hermit, Gray-cheeked, and Bicknell's thrushes and Veery, I selected one sample each for the first two species, two samples from Gray-cheeked Thrush representing

both haplotypes in that species, and three samples each from Bicknell's Thrush and Veery representing the most common and most divergent haplotypes in each species. A published control region III sequence from the European Robin (*Erithacus rubecula*) from GenBank was selected as an outgroup for examining the phylogeny of *Catharus* (Questiau *et al.* 1998). The complete collection of thrush sequences was used to generate an overall tree to examine within species variation in the focal taxa Bicknell's Thrush and Veery.

I obtained control region III sequences via amplification of DNA extracted from blood with the PCR primers L817, BTL800, and H1248 followed by dye terminator cycle sequencing on an ABI 373A automated sequencing machine (see Chapter 2 above). Representative sequences have been deposited in GenBank, accession numbers are listed in Tables 2.1, 2.2 and 2.3.

Because the control region does not code for a protein, analyses of sequence variation in terms of codon structure, and the relative positions of substitutions within codons was not possible. I conducted analyses of the two types of nucleotide substitution, transitions between cytosine and thymine, and between adenine and guanine, and tranversions among all other pairings of pyrimidine and purine bases. I determined the ratio of transitions, which are far more frequent in mtDNA, to tranversions, and related rates of transition divergence to transversion divergence to see if transitions were saturated and thus subject to multiple hits. I weighted transversions over transitions by ten times and weighted gaps by twenty times. I calculated Kimura two parameter distances among sequences with PAUP*.

I conducted phylogenetic analyses on the sequences with the program PAUP*v. 4.0b8 (PPC/Altivec) (Swofford 2001), running an exhaustive maximum parsimony search, a heuristic maximum likelihood search, and a minimum evolution neighbor-joining search. Bootstrap and jackknife replication were applied to determine the dependence of tree topology upon character and taxon composition of the data, respectively (Felsenstein 1985; Lanyon 1985). Bootstrap and jackknife replicates (10,000 each), were generated using maximum parsimony with the branch and bound search algorithm in PAUP*. Ten thousand bootstrap replicates were also run using the neighbor-joining algorithm in PAUP*. Lastly, a heuristic neighbor-joining search was used to generate a single tree with all thrush sequences.

Results

Sequence Variation and Comparisons

Aligned sequences are shown in Table 3.1. Within *Catharus* thrushes, I found 69 sites showing nucleotide substitutions among individuals (including intraspecific variation) in 395 base pairs, including 61 transitions and 12 transversions (four sites showed both transitions and transversions). I also found four insertions and deletions (indels) among species; these were only detected in Swainson's and Hermit thrushes versus Bicknell's and Gray-cheeked thrushes and Veery. Within *Catharus*, invariant sites numbered 321 (81%), however, European Robin contributed 94 additional variable sites to the sequence analysis. Of the 163 variable sites, 34 were parsimony informative.

The frequency of substitutions varied along the sequence with the 5' end, adjacent to and including some of the strongly conserved central domain, showing the least variation, and sites near the 3' end, showing the most (Table 3.2). The frequency of transitions increased from 5' to 3', whereas transversion frequency changed relatively little, being slightly higher from sites 133 to 264. The transition to transversion ratio was very high at the 3' end of the sequence.

The matrix in Table 3.3 shows the number of transitions (upper diagonal) versus the number of transversions (lower diagonal) among *Catharus* species. There were no transversions observed among Veery, Bicknell's, and Gray-cheeked thrushes and the number of transitions is low among these taxa. The overall ratio of transitions to transversions is 4.9:1. The most frequent transitional substitution is between thymine and cytosine (72.4%). Fifty-three percent of transversions were shifts between adenine and thymine or cytosine.

Kimura two-parameter distances among all sequences are presented in Table 3.4. Distances within Bicknell's and Gray-cheeked thrushes and Veery were very small, ranging from 0.26 to 1.29%. Distances among the three species were also small, ranging from 1.03 to 2.91%. By contrast, distances for these three species to Hermit Thrush (average 8.73%) and Swainson's Thrush (average 13.33%) were much larger. The distance between Hermit and Swainson's thrushes was also relatively large (10.64%). The outgroup species, European Robin, averaged 50.65% divergent from *Catharus* thrushes in control region III.

Phylogenetic analyses

Maximum parsimony analysis using the exhaustive search algorithm generates a single shortest tree of 1089 steps (consistency index = 0.975, retention index = 0.807). Figure 3.2A illustrates this tree, showing the Bicknell's Thrush and Veery as sister species with Gray-cheeked Thrush as the sister species to this grouping. The heuristic maximum likelihood search produced two shortest trees (Length = 1230.755). One of these trees is identical with the shortest maximum parsimony tree (Figure 3.2A), the second is similar save for leaving the relationship of Hermit and Swainson's thrushes indeterminate (Figure 3.2B).

Results of 10,000 bootstrap replicates using maximum parsimony and neighbor-joining are shown in Figure 3.3. Both methods generate the same shortest tree as the exhaustive search. The trees show 100% support for a clade uniting Gray-cheeked Thrush with Bicknell's Thrush and Veery. There is strong support for retaining the Bicknell's Thrush and Veery samples within species clusters in the maximum parsimony tree, but less than 70% support in the distance tree. There is also less than 70% support in the neighbor-joining tree for a Bicknell's-Veery clade, but there is 75% support for this grouping in the parsimony tree. There is excellent support, 87% in the parsimony bootstrap and 93% in the neighbor-joining tree, for a monophyletic Gray-cheeked Thrush clade. Hermit Thrush also appears more closely related to the other *Catharus* than it is to Swainson's Thrush based on support in excess of 80% on both bootstrap trees (as well as on the jackknife tree). Among clades supported by less than 50% of

the bootstrap replicates none received more than 21% support. Clades uniting Bicknell's and Gray-cheeked thrushes occurred in less than 5% of replicates for each method. Jackknifing recovers the same phylogeny with similar levels of support for all branches (Figure 3.4).

The neighbor-joining tree for all 90 thrush samples, including the full intraspecies samples for Bicknell's Thrush and Veery (Figure 3.5), reproduces the relationships shown in the phylogenetic analyses with Gray-cheeked-Bicknell's-Veery united and a nested clade uniting Bicknell's Thrush with Veery. Relationships among haplotype designations (indicated by letter code) in Bicknell's Thrush and Veery are generally supported in the tree (see also Figures 4.1 and 4.3 in Chapter 4). However, the most common, and presumably ancestral, A haplotypes in each species have the less common haplotypes arising paraphyletically within them on the tree. Because this is a gene tree with presumably ancestral haplotypes still present within it, paraphyly is not unexpected (Avise and Wollenberg 1997).

Discussion

The phylogenies retrieved from these data provide strong support for the grouping of Veery, Gray-cheeked and Bicknell's thrushes into a clade. The shortest topologies reflect this relationship, and there is 100% bootstrap and jackknife support for this arrangement. Thus Todd's (1963) assertion that Veery and Gray-cheeked Thrush (*sensu lato*) are sister taxa is supported by my data. The only other published molecular phlyogeny of the genus, the allozyme study

of Avise *et al.* (1980), did not provide much resolution because the four *Catharus*, presumably excluding Bicknell's Thrush (samples were from Florida panhandle tower kills where Bicknell's is likely very rare), differed by only 3% via Nei's D. Avise *et al.*'s UPGMA tree places Veery closest to Swainson's Thrush and "distant" from Gray-cheeked Thrush. Their parsimony tree is more similar to my trees, ascribing a unique synapomorphy uniting Swainson's Thrush to Gray-cheeked Thrush and Veery, but not sorting out the relationship among the three. The trees generated here suggest that Hermit Thrush is more closely related to the Veery-Gray-cheeked clade than is Swainson's Thrush. It would be well to examine the Wood Thrush and Neotropical *Catharus* thrushes to further clarify the relationship between Swainson's Thrush and Hermit Thrush.

The alliance of Veery, Bicknell's Thrush and Gray-cheeked Thrush was not very surprising, but the low mtDNA divergence among them was. The closeness of Bicknell's and Gray-cheeked thrushes is sensible given their classification as members of the same species for over a century, but although the Veery was allied by some to Gray-cheeked Thrush it was not believed to be as closely related to it as Bicknell's Thrush. The unexpectedly close relationship between Veery and Bicknell's Thrush provides serendipitous support for my decision to use the Veery in comparison to Bicknell's Thrush in population genetic analyses. I also suggest these taxa probably constitute a superspecies (Mayr 1963), this in spite of the tenuous geographic separation of breeding Bicknell's Thrushes and Veeries by elevation and habitat.

Resolution of the relationships among Veery, Gray-cheeked and Bicknell's thrushes is more elusive than establishing them in a single clade. However some useful lessons may be drawn from this study. First, and unexpectedly, my data cast doubt on the traditional grouping of Bicknell's Thrush with Gray-cheeked Thrush. This arrangement had less than 5% bootstrap and jackknife support. Levels of support were much higher for allying Bicknell's Thrush to Veery (Figures 3.3 and 3.4).

Lack of strong phylogenetic resolution among Veery, Bicknell's and Gray-cheeked thrushes is attributable to the very low genetic distances among them and the small number of synapmorphies supporting the arrangement. Further molecular analyses of these species with other markers should further clarify the situation. The vocal and morphological similarities of the Veery and Bicknell's Thrush are considerable, but they are far less similar than Bicknell's Thrush is to the Gray-cheeked. Perhaps isolating mechanisms between Veery and Bicknell's Thrush have been reinforced due to their close geographic approach and brief periods of contact early and late in interglacials, whereas the clear geographic separation between Bicknell's and Gray-cheeked has led to far less morphological divergence between them.

This study supports the conclusions of Ouellet (1993), Seutin (cited by Ouellet 1993), and Phillps (1991) that Bicknell's Thrush is distinct from Gray-cheeked Thrush. Bicknell's Thrush exhibits four fixed differences with Veery, and six with Gray-cheeked Thrush. The Veery shows five fixed differences with Gray-cheeked Thrush, plus one haplotype in a single bird that shows a homoplasy at

site 331 with Gray-cheeked (see Tables 3.1 and 4.1). There are long-recognized species, e.g. *Geospiza* finches (Freeland and Boag 1999a; Petren *et al.* 1999) with less molecular divergence. Bicknell's Thrush also has the traits of a biological species, besides showing a discrete mitochondrial lineage. Among the other arguments in favor of Bicknell's Thrush's status as a species include discrete breeding and non-breeding distributions from both Veery and Gray-cheeked Thrush, different songs that do not elicit territorial responses from either Veery or Gray-cheeked Thrush, and an extensively bright yellow lower mandible (Ouellet 1993). This last is apparently important for interspecific recognition in *Catharus*, as demonstrated by the case of Ruddy-capped (*C. frantzii*) versus Russet Nightingale-Thrushes (*C. occidentalis*) in Mexico. These species were long considered conspecific due to lack of strong morphological differences save lower mandible pattern, but they occur sympatrically, with no interbreeding, over much of their Mexican ranges (Rowley and Orr 1964; Phillips 1991). Other differences between Bicknell's Thrush and Veery or Gray-cheeked Thrush include upperpart color, and small size (Ouellet 1993). There is also no good evidence in the present that Bicknell's Thrushes ever contact or hybridize with Veeries in spite of completely coextensive geographic distributions with separation only via elevation and breeding habitat.

The mitochondrial control region is too variable among species to be useful for applying a clock calibration to molecular divergence data. However, it is possible to postulate a divergence date for Bicknell's and Gray-cheeked thrushes based on estimates of mitochondrial coding region divergence of

2%/MY for birds (Shields and Wilson 1987; Tarr and Fleischer 1993), and Seutin's restriction fragment distance of 1.7% for the entire mitochondrial genome (cited in Ouellet 1993). This yields an estimate of ca. 850,000 years before present, a date within the Pleistocene. Because the Veery appears equidistantly related to Gray-cheeked and Bicknell's thrushes, it seems appropriate to assume that it diverged from the ancestral stock during the same approximate period. On the other hand, the other species examined here, Hermit and Swainson's thrushes, probably diverged much earlier, before the Pleistocene. This is in line with the observations of Klicka and Zink (1997) regarding other groups of similar species in North America. The North American *Catharus* species are considered members of a species group exclusive of neotropical *Catharus* and Wood Thrush (Mayr and Short 1970; American Ornithologists' Union 1998).

The postulated Pleistocene origin of the Gray-cheek-Bicknell's-Veery trio appears to contradict the dismissal of the putative paradigm of Pleistocene speciation by Klicka and Zink (1997). However these authors did not present evidence that discarded Pleistocene glacial vicariance as a source of avian speciation. Indeed they included nine cases of species that likely arose in the Pleistocene (see Table 1 in Klicka and Zink 1997), over 27% of the cases presented by them. A major late Tertiary burst of avian speci[illegible] the Pliocene seems to reflect a shift to a drier, colder climate, followed by a more modest burst of speciation in the Pleistocene related to glacial effects (Lovette and Bermingham 1999; Klicka and Zink 1997). It is clear that many intraspecific

lineages have originated in the Pleistocene apparently due to isolation in glacial refugia (Avise and Walker 1998; Buerkle 1999; Holder *et al.* 1999), but it seems uncommon for these lineages to achieve species status.

A single widespread progenitor of the modern Gray-cheek-Bicknell's-Veery trio was likely sundered by glaciation several times early in the Pleistocene leading to independent lineages in the second half of the epoch. Whether this bird was more Veery or Gray-cheeked-Bicknell's like is an open question. During the most recent glaciation, large ice-free areas of the treeline habitat of Gray-cheeked Thrush existed in Beringia and on the Newfoundland Grand Banks and coastline (reviewed in Pielou 1991). These areas almost certainly harbored the ancestral forms of the two current Gray-cheeked Thrush subspecies *aliciae* (continental subarctic) and *minumus* (largely insular Newfoundland). I also see no reason why such refugia were not present in prior glaciations. If this was so, continental ice frequently separated the ancestral Gray-cheeked Thrush from the ancestral Bicknell's Thrush allowing their lineages to diverge. How the Veery diverged from its ancestral progenitor is less obvious and may have something to do with the cool and dry climate south of glacial ice in eastern North America; essentially restricting the Veery's ancestor to pockets of mesic habitat.

In conclusion, I have established the close relationship of Veery, Gray-cheeked and Bicknell's thrushes. I have shown that these three species are so closely related and recently diverged that there is only moderate bootstrap support for an apparent but counterintuitive sister species relationship between Bicknell's Thrush and Veery. Further analyses with more rapidly evolving

markers such as microsatellites (Petren *et al.* 1999), or more mitochondrial DNA sequence, including the less variable, but potentially useful, control region I (Baker and Marshall 1997), or the newly described non-coding region originating in the duplication of the control region in muscicapid birds (Bensch and Härlid 2000) may help clear up relationships. I have also found that Bicknell's Thrush is a discrete lineage from Gray-cheeked Thrush, as well as from Veery (this latter point is not contested). Finally a molecular clock estimate derived from Seutin's restriction enzyme data (in Ouellet 1993) suggest that these three thrushes may represent a Pleistocene diversification, whereas Hermit and Swainson's thrushes likely arose before the Pleistocene as have many other recent bird species (Klicka and Zink 1997; Lovette and Bermingham 1999).

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                  10         20         30         40         5
                   |          |          |          :
GCTHa  GATGCACTTT GACCCCATTC ACGAGGGGGA GGCTATTTAC CTCTTAAGTA
GCTHb  .G........ .......... .?........ .......... ..........
BITHa  .......... .......... .......... .......... ..........
BITHb  .......... .......... .......... .......... ..........
BITHc  .......... .......... .......... .......... ..........
BITHd  .......... .......... .......... .......... ..........
BITHe  .......... .......... .......... .......... ..........
BITHf  ?......... .......... .......... .......... ..........
BITHg  .......... .......... .......... .......... ..........
VEERa  .......... .......... .......... .......... ..........
VEERb  .......... .......... .......... ........G. ..........
VEERc  .......... .........T .......... .......... ..........
VEERd  ????...... .......... .......... ...C...... ..........
VEERe  .......... .......... .......... .......... ..........
VEERf  .......... .......... .......... ..?....... ..........
VEERg  ?......... .......... .......... .......... ..........
VEERh  .......... .......... .......... ........?. ..........
VEERi  ?????????. .......... .......... .......... ..........
VEERj  ?......... .......... .......... .......... ..........
HETHa  ?????????? .......... .......... .......... ..........
SWTHa  .......... .......... .......... .......... ..........
EURO   .......... .......... .......... .......... ..........
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Table 3.1. Control region III sequences used for phylogenetic analysis. Species identification codes: GCTH = Gray-cheeked Thrush, BITH = Bicknell's Thrush, VEER = Veery, HETH = Hermit Thrush, SWTH = Swainson's Thrush. Letter suffixes appended to species codes designate unique sequences identified in this study. EURO = European Robin (*Erithacus rubecula*). Outgroup sequence from Questiau *et al.* (1998), GenBank accession number Y08057.

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                60          70          80          90          100
                |           |           |
GCTHa  TGCAGATAGT GTAATGGTCA CCGCACATAT TTAGATTGTT TCCCCTTTCT
GCTHb  .......... .......... .......... .......... ..........
BITHa  .......... .......... .......... .......... ..........
BITHb  .......... .......... .......... .......... ..........
BITHc  .......... .......... .......... .......... ..........
BITHd  .......... .......... .......... .......... ..........
BITHe  .......... .......... .......... .......... ..........
BITHf  .......... .......... .......... .......... ..........
BITHg  .......... .......... .......... .......... ..........
VEERa  .......... .......... .......... .......... ..........
VEERb  .......... .......... .......... .......... ..........
VEERc  .......... .......... .......... .......... ..........
VEERd  .......... .......... .......... .......... ..........
VEERe  .......... .......... .......... .......... ..........
VEERf  .......... .......... .......... .......... ..........
VEERg  .......... .......... .......... .......... ..........
VEERh  .......... .......... .......... .......... ..........
VEERi  .......... .......... .......... .......... ..........
VEERj  .......... .......... .......... .......... ..........
HETHa  ........A. .C........ ...G...... .......... ..........
SWTHa  ........A. .C........ ...G...... .......... ..........
EURO   -A......A. .C........ ...G.....C ..GAT..A.. ....T..C..
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Table 3.1. Control region III sequences used for phylogenetic analysis.
(continued)

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                110         120         130         140         150
                 |           |           |           |           |
GCTHa  AGGAACTTCC ATCTAAACCC CTAAAAATCA TCATTTTTTT CGTTCGTTTA
GCTHb  .......... .......... .......... .......... ..........
BITHa  .....T.... .......... .......... .......... ..........
BITHb  .....T.... .......... .......... .......... ..........
BITHc  .....T.... .......... .......... .......... ..........
BITHd  .....T.... .......... .......... .......... ..........
BITHe  .....T.... .......... .......... .......... ..........
BITHf  .....T.... .......... .......... .......... ..........
BITHg  .....T.... .......... .C........ .......... ..........
VEERa  .....T.... .......... .......... .......... ..........
VEERb  .....T.... .......... .......... .......... ..........
VEERc  .....T.... .......... .......... .......... ..........
VEERd  .....T.... .......... .......... .......... ..........
VEERe  .....T.... .......... .C........ .......... ..........
VEERf  .....T.... .......... .......... .......... ..........
VEERg  .....T.... .......... .......... .......... ..........
VEERh  .....T.... .......... .......... .......... ..........
VEERi  .....T.... .......... .......... .......... ..........
VEERj  .....T.... .......... .......... .......... ..........
HETHa  .....T.... .......... .C........ ..........- .A........
SWTHa  .....T.... .........A .C........ .......... TA........
EURO   ..AG.....T .C.......T TC.TTTT..C AT.C...A.G ..........
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Table 3.1. Control region III sequences used for phylogenetic analysis. (continued)

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                160         170         180         190         200
                 |           |           |
GCTHa  TTTTTATCAT GACATTTTCG TTTAAAATTA ACCAAATATT CTTAGACATC
GCTHb  .......... .......... .......... .......... ?.........
BITHa  .......... .......... .........G .......... ..........
BITHb  .......... .......... .........G .......... ?.........
BITHc  .......... .......... .........G .......... ..........
BITHd  .......... .......... .........G .......... .........?
BITHe  .......... .......... .........G .......... ..........
BITHf  .......... .......... .........G .......... ..........
BITHg  .......... .......... .........G .......... ..........
VEERa  .......... .......... .......... .......... ..........
VEERb  .......... .......... .......... .......... ..........
VEERc  .......... .......... .......... .......... ..........
VEERd  .......... .......... .......... .......... ..........
VEERe  .......... .......... .......... .......... ..........
VEERf  .......... .......... .......... .......... ..........
VEERg  .......... .......... .......... .......... ..........
VEERh  .......... .......... .......... .......... ..........
VEERi  .......... .......... .......... .......... ..........
VEERj  .......... .......... .......... .......... ..........
HETHa  .......... .T........ .......... ..T....... T.A.T.....
SWTHa  .......... .........A .AC....... G.T....... T.A.T.....
EURO   .....C..T. .......... ...C..TCAT CAA..TAT.. AAGTA.ATCT
```

Table 3.1. Control region III sequences used for phylogenetic analysis. (continued)

```
                 210         220         230         240         250
                  |           |           |           |           |
GCTHa  TCCCTACCTT TAACCAAAGC ATTCATCATC ACAAAACTAA CGAACAAACT
GCTHb  .......... .......... .......... .......... ..........
BITHa  .......... .......... .......... .......... ..........
BITHb  .......... .......... .......... .......... ..........
BITHc  .......... .......... .......... .......... ..........
BITHd  .......... .......... .......... ......?... ..........
BITHe  .......... .......... .......... .......... ..........
BITHf  .......... .......... .......... .......... ..........
BITHg  .......... .......... .......... .......... ..........
VEERa  .......... .......... .......... .......... ..........
VEERb  .......... .......... .......... .......... ..........
VEERc  .......... .......... .......... .......... ..........
VEERd  .......... .......... .......... .......... ..........
VEERe  .......... .......... .......... .......... ..........
VEERf  .......... .......... .......... .......... ..........
VEERg  .......... .......... .......... .......... ..........
VEERh  ....?..... .......... .......... .......... ..........
VEERi  .......... .......... .......... .......... ..........
VEERj  .......... .......... .......... .......... ..........
HETHa  .........A .......... .CC....... ....G..... .A........
SWTHa  .........C .......... ....G.T... .TT.C.T.T. .A........
EURO   ...-...AAC A..A....A. .C........ .TTTC.TC.- .A.CT..TA.
```

Table 3.1. Control region III sequences used for phylogenetic analysis. (continued)

```
                260         270         280         290         300
                 |           |           |           |           |
GCTHa  TTCTCTATTT TCCCCCTATT TATCAGAACC GAAAATACAA CAAACTTCTC
GCTHb  .......... .......... .......... .......... ..........
BITHa  .C........ .......... .......... ....G..... .......T..
BITHb  .C....G... .......... .......... ....G..... .......T..
BITHc  .C....G... .......... ..C....... ....G..... .......T..
BITHd  .C........ .......... .......... A...G..... .......T..
BITHe  .C........ .......... C......... A...G..... .......T..
BITHf  .C........ .......... C......... A...G..... .......T..
BITHg  .C........ .......... C......... A...G..... .......T..
VEERa  .C........ .......... .......... .......... .......TC.
VEERb  .C........ .......... .......... .......... .......TC.
VEERc  .C........ .......... .......... .......... .......TC.
VEERd  .C........ .......... .......... .......... .......TC.
VEERe  .C........ .......... .......... .......... .......TC.
VEERf  CC........ .......... .......... .......... .......TC.
VEERg  .C........ .......... ..C....... .......... .......TC.
VEERh  .C?....... .......... .....A.... .......... .......TC.
VEERi  .C........ .......... .......... .......... .......TC.
VEERj  .C........ .......... .......... .......... .......TC.
HETHa  .C........ ....T..... A.CT.....T .....C.... ..........
SWTHa  .C........ .......... A.A..-...T A......... ....A-...T
EURO   .C........ ...T..C.C. ..A.TT...A CT....C..C AC..AAATAA
```

Table 3.1. Control region III sequences used for phylogenetic analysis. (continued)

```
                310         320         330         340         35
                 |           |           |
GCTHa  CATCTTT--A CACACACACA CAAACAGCAA TCCCCCTGAC AAACCACCC
GCTHb  .......--. ....T..... .......... .......... .........
BITHa  .......--. .......... .......... C......... .........
BITHb  .......--. .......... .......... C......... .........
BITHc  .......--. .......... .......... C......... .........
BITHd  .......--. .......... .......... C......... .........
BITHe  .......--. .......... .......... C......... .........
BITHf  .......AC. .......... .......... C......... .........
BITHg  .......--. .......... .......... C......... .........
VEERa  .......--. .......... .......... C......... ....T....
VEERb  .......--. .......... .......... C......... ....T....
VEERc  .......--. .......... .......... C......... ....T....
VEERd  .......--. .......... .......... C......... ....T....
VEERe  .......--. .......... .......... C......... ....T....
VEERf  .......--. .......... .......... C......... ....T....
VEERg  .......--. .......... .......... C......... ....T....
VEERh  .......--. .......... .......... C......... ...?T....
VEERi  .......--. .......... .......... .......... ....T....
VEERj  .......--. .......... .......... C......... ....T....
HETHa  ....C.C--. .......... ......A... .T........ ..........
SWTHa  T.C..CCAC. .....GT... .......T.. .T........ ...T......
EURO   ACATAAA--. .C..A.TCA. TCCTT.C.C. A..TAA.CC. ..CTA...A.
```

Table 3.1. Control region III sequences used for phylogenetic analysis. (continued)

```
                360         370         380         390    395
                 |           |           |           |
GCTHa  AACTAAAACC AAACAAAAAC ACAACGCATG TTCTTGTAGC TTAAC
GCTHb  .......... .......... .......... .......... .....
BITHa  .......... .......... .......... .......... .....
BITHb  .......... .......... .......... .......... .....
BITHc  .......... .......... .......... .......... .....
BITHd  .......... .......... .......... .......... .....
BITHe  .......... .......... .......... .......... .....
BITHf  .......... .......... .......... .......... .....
BITHg  .......... .......... .......... .......... .....
VEERa  .......... .......... .......... .......... .....
VEERb  .......... .......... .......... .......... .....
VEERc  .......... .......... .......... .......... .....
VEERd  .......... .......... .......... .......... .....
VEERe  .......... .......... .......... .......... .....
VEERf  .......... .......... .......... .......... .....
VEERg  .......... .......... .......... .......... .....
VEERh  .......... .......... .......... .......... .....
VEERi  .......... .......... .......... .......... .....
VEERj  ..T....... .......... .......... .......... .....
HETHa  .......... .......... ....T..... .C........ .....
SWTHa  .......... .......... .....A.... .C.C...... ....T
EURO   T.TCTCCCTA GC.A.TC..T C...ATT
```

Table 3.1. Control region III sequences used for phylogenetic analysis. (continued)

Region	number bases	number subst.	subst. per site	transitions per site	transversions per site	transitions: transversions
1-132	132	10	0.0758	0.0606	0.0152	4.0:1
133-264	132	26	0.1970	0.1591	0.0530	3.0:1
265-395	131	33	0.2519	0.2443	0.0229	10.7:1
All	395	69	0.1747	0.1519	0.0304	5.1:1

Table 3.2. Variation in base pair substitutions along a 395 base pair segment of sequence in control region III for five *Catharus* thrushes.

	GCTH	BITH	VEER	HETH	SWTH
GCTH	–	6	5	22	34
BITH	0	–	4	23	35
VEER	0	0	–	22	34
HETH	6	6	6	–	28
SWTH	11	11	11	9	–

Table 3.3. Transitions and transversions along a 395 base pair segment of sequence in control region III for five *Catharus* thrushes.
The number of transitions appears on the upper diagonal and the number of transversions is on the lower diagonal of the data matrix. GCTH=Gray-cheeked Thrush; BITH=Bicknell's Thrush; VEER=Veery; HETH=Hermit Thrush; SWTH=Swainson's Thrush.

	GCTHa	GCTHb	BITHa	BITHc	BITHg	VEERa	VEERb	VEERc	HETH	SWTH	EURO
GCTHa	—										
GCTHa	0.00516	—									
BITHa	0.01551	0.02093	—								
BITHc	0.02078	0.02632	0.00512	—							
BITHg	0.02344	0.02906	0.00769	0.01289	—						
VEERa	0.01551	0.02098	0.01028	0.01551	0.01814	—					
VEERb	0.01814	0.02365	0.01289	0.01814	0.02078	0.00255	—				
VEERc	0.01814	0.02365	0.01289	0.01814	0.02078	0.00255	0.00512	—			
HETH	0.08180	0.08279	0.08801	0.08801	0.08789	0.08792	0.09106	0.09104	—		
SWTH	0.12748	0.13165	0.13405	0.13734	0.12749	0.13393	0.13724	0.13721	0.10639	—	
EURO	0.50512	0.50961	0.50517	0.51036	0.50490	0.49970	0.50504	0.50508	0.53113	0.48852	—

Table 3.4. Genetic distances among thrushes in control region III. Kimura two parameter distances (adjusted percent sequence divergence) are shown for each individual sequenced among 10 individuals of five *Catharus* thrush species and European Robin. See Table 3.1 for abbreviations for species names.

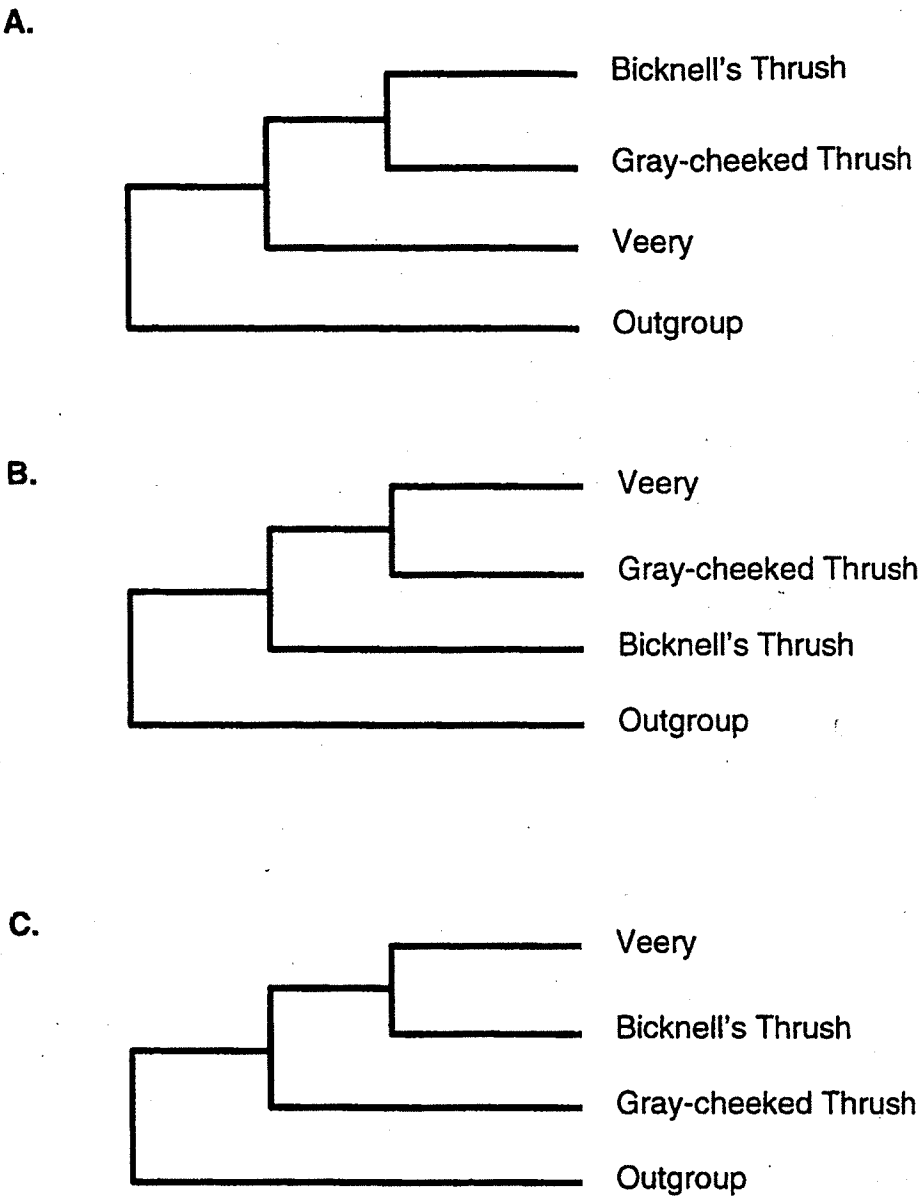


Figure 3.1. Hypothetical relationships of species within the Bicknell's Thrush, Gray-cheeked Thrush, Veery clade. Figure A denotes the hypothesis that the morphological sibling species Bicknell's and Gray-cheeked thrushes are also true sister species (Ridgway 1882; Todd 1963). Figures B and C show alternative hypotheses of relationships among Veery, Bicknell's, and Gray-cheeked thrushes.

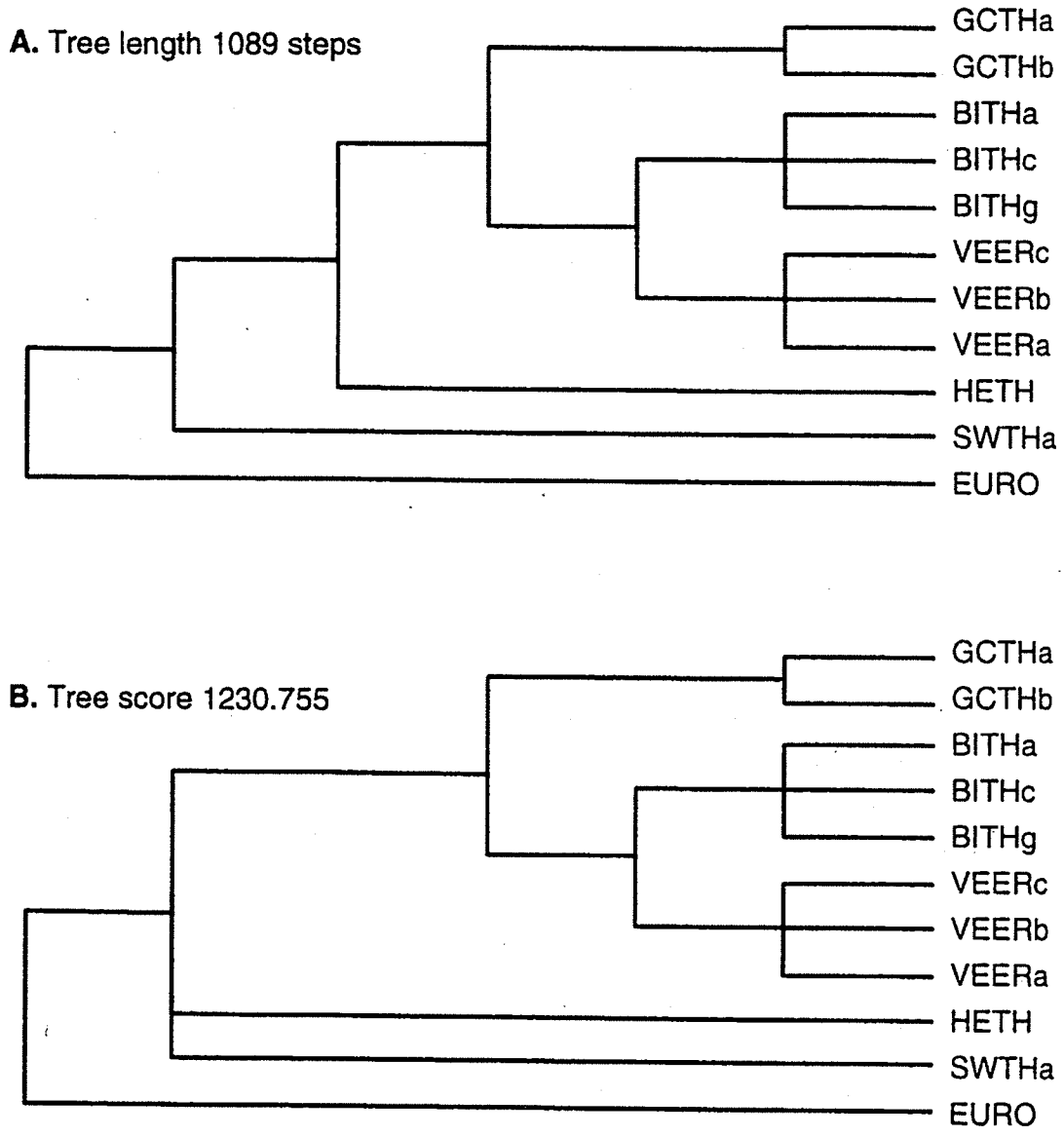
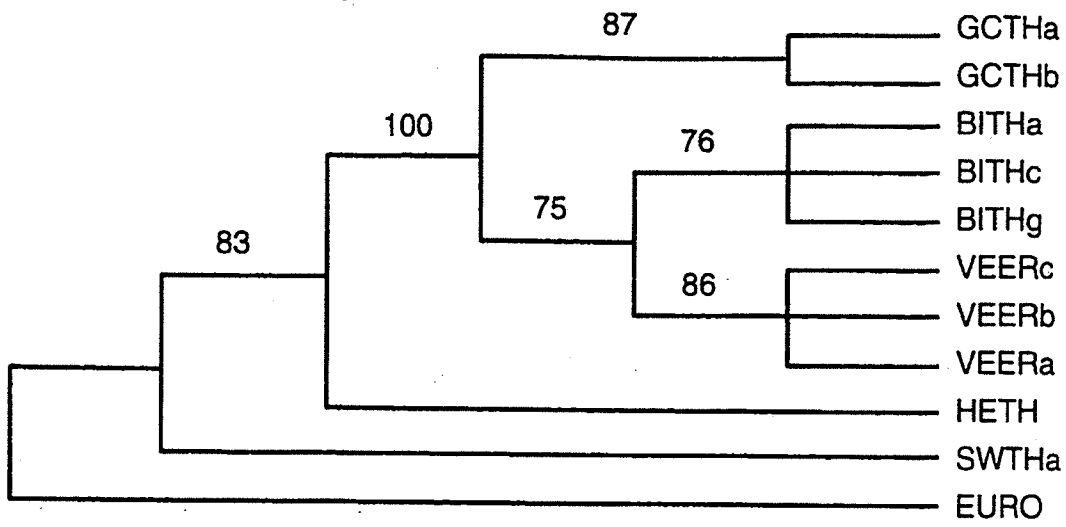


Figure 3.2. A. Maximum parsimony exhaustive search. The shortest tree produced via maximum parsimony analysis with the program PAUP* (Swofford 2001) based on 395 bp of control region III in five *Catharus* thrush species. **B. Heuristic maximum likelihood search.** This search produced two trees with the same lowest score, the first had the same topology as Tree A above, the second is shown in B. Transition:transversion ratio 1.3:1; base frequency A=0.335, C=0.271, G=0.095, T=0.299; shape parameter (alpha)=0.0945. In all analyses, transversions were weighted over transitions 10:1, gaps were given a weight of 20. Species abbreviations follow those in Table 3.1, letters appended represent the unique sequences used from each species.

A. Maximum parsimony



B. Neighbor-joining

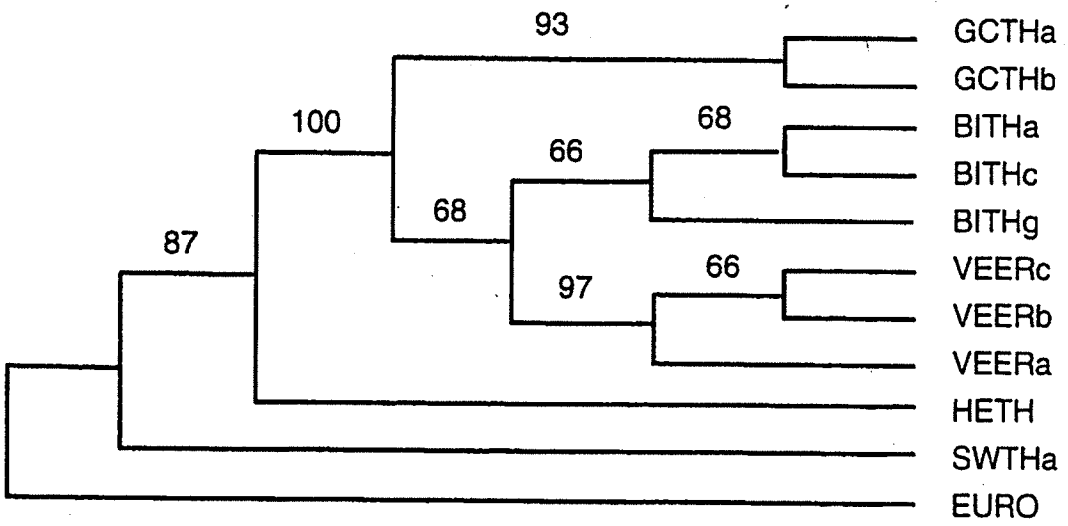


Figure 3.3. Bootstrap 50% majority consensus trees. A. Branch and bound maximum parsimony search. B. Neighbor-joining search. Bootstrap trees derived from 10,000 replicate trees with the program PAUP* (Swofford 2001), (**B**) employing minimum evolution as its objective function. See Table 3.1 for species abbreviations, appended letters represent the samples used from each species.

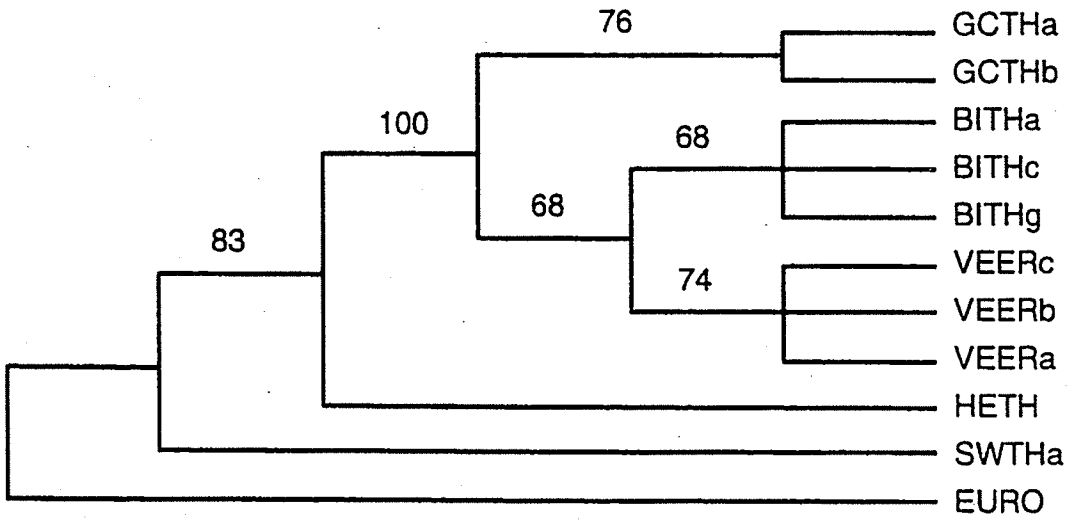


Figure 3.4. Maximum parsimony tree with jackknife values, 10,000 replicates. Jackknife tree produced via maximum parsimony branch and bound analysis of 395 bp of control region III in five *Catharus* thrush species. In all analyses, transversions were weighted over transitions 10:1, gaps were given a weight of 20. Species abbreviations follow Table 3.1.

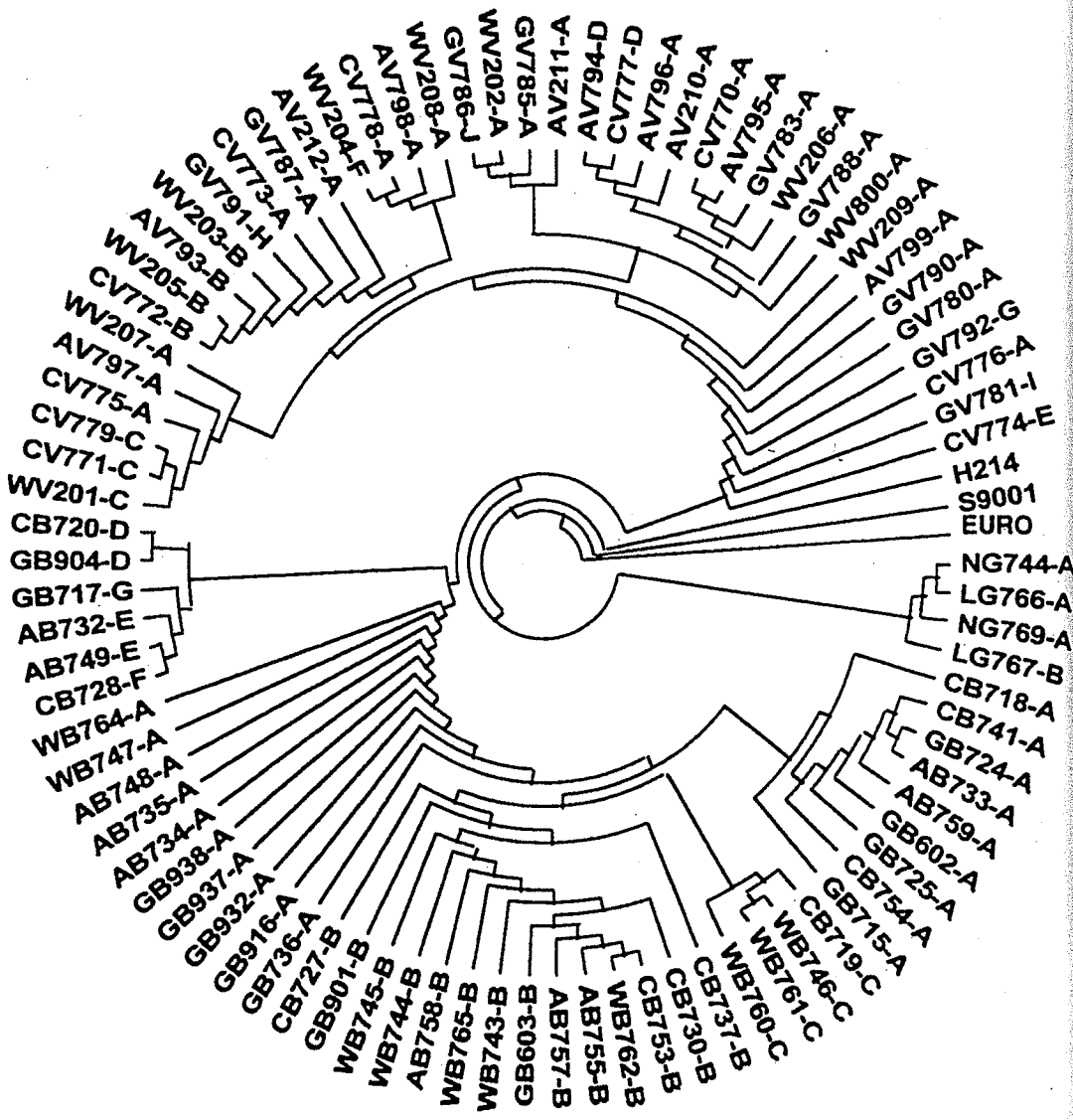


Figure 3.5. Circular neighbor-joining tree incorporating 90 thrush sequences. *Catharus* sample ID codes are derived from a single letter locality designation (G=Green Mt; C=Catskill Mt; A=Adirondack Mt; W=White Mt; L=Labrador; N=Newfoundland), a single letter species code (G=Gray-cheeked; B=Bicknell's; V=Veery; H=Hermit; S=Swainson's), a three digit numerical code for each bird, and the single letter within-species haplotype code. Outgroup: EURO=European Robin.

Chapter Four

The Population Structure of Bicknell's Thrush and Veery

Introduction

The division of a species into sub-populations requires barriers to the free exchange of breeders across its distribution. Because birds can fly and can efficiently find their way across landscapes they tend to show little population structure (Rockwell and Barrowclough 1987). Factors that might affect the genetic structure of bird populations include isolation by distance, where gene flow is attenuated by the very small number of birds that are exchanged between the end points of a range, strong natal philopatry, and sub-divided ranges with hostile intervening barriers, e.g. islands isolated by oceans.

The differing distributional patterns of Bicknell's Thrush and Veery imply that there could be differences in the genetic structure of their respective populations. The scatter of apparently discrete populations that characterizes the range of the Bicknell's Thrush might promote population structure in that species. By contrast, the Veery should show no genetic subdivision except via isolation by distance because there are no large gaps in its distribution. Alternatively the high mobility conferred by flight and long-distance migratory behavior may overcome the apparent disjunctions in the Bicknell's Thrush's range, leading to similar population structures in the two species in spite of the differences in the contiguousness of their geographical distributions. Strong natal philopatry with

concomitantly little among-population dispersal could promote population structure in spite of migratory behavior in both species.

An estimate of among sub-population genetic variation in relation to within sub-population variation also allows an estimate of gene flow to be made. The greater the F_{ST} or analogous statistic, the lower the gene flow among sub-populations unless mutation rates are very high or selection has a disproportionate effect on gene frequencies (Wright 1978; Slatkin 1987). Gene flow is generally represented by the number of effective migrants among sub-populations per generation (Neigel 1996; Slatkin 1987). The average number of effective migrants among sub-populations for mtDNA data may be calculated via the equation $Nm = 1/2\ (1/F_{ST} - 1)$ (Takahata and Palumbi 1985). I report estimates of gene flow based on the F_{ST} analogs, Φ_{ST}, and N_{ST} in this chapter.

Drawbacks to using F_{ST} and its analogs to estimate gene flow include the assumptions of both population size and drift being in equilibrium, and symmetric bi-directional gene flow among sub-populations (Beerli 1998). The assumption regarding symmetric genetic exchange is particularly unrealistic, because it appears unlikely that the genetic contribution of immigrants to one sub-population from another is balanced. This is especially so when there are differing sub-population sizes, great discrepancies in the size of geographic regions occupied by sub-populations, or the sub-populations are of different ages (Beerli 1998). Because of these objections to estimates of gene flow from fixation indices, I also used a second method for estimating gene flow based on coalescence theory (Beerli 1997) that addresses these objections. I report my results for the

coalescence method in the succeeding chapter on the population history of Bicknell's Thrush and Veery.

Methods

I generated DNA sequence data for 43 Bicknell's Thrushes and 40 Veeries using methods and PCR primers described in Chapter 2. I defined haplotypes based on nucleotide sequence differences along 395 base pairs of mitochondrial control region III sequence among individual birds for both Bicknell's Thrush and Veery (see Table 4.1).

Because the techniques I used to estimate F_{ST} and *Nm* assume that selection has not affected the frequency of haplotypes in the populations I sampled, it is necessary to test sequences for the likelihood of detectable natural selection. I used the population genetics package Arlequin (Schneider *et al.* 1997) to calculate values of Tajima's D statistic to test the assumption of sequence neutrality (Tajima 1989). Neutrality tests also allow inferences about population history, therefore I also discuss the results of these tests in the following chapter.

I defined the samples from each mountain range as sub-populations for the population structure analyses. I defined each haplotype by nucleotide sequence differences attributable to substitutions and insertions/deletions. I used Arlequin's program AMOVA (analysis of molecular variance) (Schneider *et al.* 1997) to calculate Φ_{ST}, using within and among population variance in genetic distances among sub-populations and haplotypes (Excoffier *et al.* 1992). I used

the program HAPLO2 (Lynch and Crease 1990) to produce estimates of the fixation index, N_{ST}, which is derived via partitioning of the per site nucleotide divergence or nucleotide diversity into within and among sub-population components for sub-populations and haplotypes (Lynch and Crease 1990). Thus nucleotide diversity and genetic distance are used in both Arlequin and HAPLO2 as substitutes for heterozygosity as a measure of genetic diversity within and among sub-populations.

Results

Haplotype Diversity and Distribution

Bicknell's Thrush

I generated 395 base pairs of control region III sequence for 43 Bicknell's Thrushes including 10 each from the Catskill and Adirondack Mountains of New York State, and White Mountains of New Hampshire, and 13 from the Green Mountains of Vermont. I detected seven haplotypes based on five variable sites (all transitions) and one two base pair insertion (Table 4.1A). Five haplotypes were found in multiple individuals. Two haplotypes, designated A and B (Figure 4.1), accounted for 33 birds (over 75% of all sequences). Five haplotypes of the seven were found in the Catskills, four were in the Green Mountains, and three each were in the Adirondacks and White Mountains (Figure 4.2). No Tajima's D statistic (Table 4.2A) achieved significance at $p \leq 0.05$, implying that the Bicknell's Thrush sequences were probably not influenced by natural selection.

Veery

I produced 40 sequences of 395 base pairs for Veery with 10 individuals sampled from each of the four of the mountain ranges. Veery sequences revealed nine variable sites (all transitions) defining 10 haplotypes (Table 4.1B). Four haplotypes were detected in multiple birds with one, designated A (Figure 4.3), accounting for over 62% of the birds in the sample. Half of the haplotypes in the total sample were seen in the Catskills, an equivalent number were found in the Green Mountains of Vermont, four were in the White Mountains, and only three were in the Adirondacks (Figure 4.4).

Most of the Tajima's D statistics indicate that Veery samples do not vary significantly from neutrality (Table 4.2B). However for all 40 sequences Tajima's D reaches $P<0.05$. The significance of Tajima's D for the entire Veery sample indicates that the populations may not be in equilibrium with respect to growth or drift, or that selection may be acting on the mitochondrial genome of the Veery at a regional scale (Tajima 1989). This begs the question of why selection does not appear to operate at a local scale as well, because most selective variation should be related to local environmental variation.

Population Structure

Bicknell's Thrush

Analysis of population structure with AMOVA suggests that the Bicknell's Thrush exhibits modest structuring with $\Phi_{ST} = 0.0674$, however this is not significantly distinguishable from zero at $P<0.05$ (although it is at $P<0.1$) with a permutation test (1000 permutations). The results of HAPLO2 analysis produce

an N_{ST} of 0.116 (SE = ±0.185), which was also not significant at P<0.05. Table 4.2 shows values of *Nm* among all mountain ranges derived from Φ_{ST} and N_{ST} estimates. All of these effective migration rates are in excess of the 1.00 migrant per generation cutoff for effective panmixis (c.f. Hartl and Clark 1997). Only the *Nm* of 1.66 between the White Mountains and Green Mountains even approaches 1.00.

Veery

In contrast to Bicknell's Thrush the Veery showed absolutely no structure with $\Phi_{ST} = -0.0185$, not significantly different from zero. The result produced by HAPLO2 was comparable with $N_{ST} = -0.014$ (SE = ±0.220). This is attributable to the low average differentiation within Veery and the predominance of haplotype A. As one might expect, values of *Nm* are very high among mountain ranges in the Veery indicating effective panmixis in New England and New York State (Table 4.2).

Discussion

If any conclusion might be drawn from these results it is that the Veery has no obvious population structure whereas Bicknell's Thrush has a tendency to show structure, albeit weakly, in northern New England and eastern New York State. That there is only a tendency toward population structuring in Bicknell's Thrush is emphasized by lack of significance at the standard P<0.05 alpha level in the permutation test employed by AMOVA.

Migration and post-natal dispersal appear to effect considerable gene flow among mountain ranges. This is shown by the lack of significant structure, and the levels of *Nm* derived from inter-range N_{ST} and Φ_{ST}. Hobson and others (2001) showed that there was a high variance in depletion of deuterium residues in tail feathers within collection localities for Bicknell's Thrush, a result they interpreted as indicative of high levels of dispersal among populations. There are several examples of migratory species with little or no population subdivision including Red-winged Blackbird (*Agelaius phoeniceus*) (Ball *et al.* 1988), Song Sparrow (*Melospiza melodia*) (Zink and Dittmann 1993), and Snow Goose (*Chen caerulescens*) (Avise *et al.* 1992). By contrast many sedentary species tend to show significant population structure. Although Gray-crowned Babblers (*Pomatostomus temporalis*) in Australia show high gene flow within New South Wales (*Nm* = 12) they had far lower levels between New South Wales and Queensland (*Nm*= 0.5 to 1.3) (Edwards 1993). The Rock Ptarmigan (*Lagopus mutus*) shows strong population subdivision across its range presumably due to a combination of Pleistocene glacial vicariance and its largely sedentary habits (Holder *et al.* 1999). The flightless Brown Kiwi (*Apteryx australis*) shows even more dramatic population subdivision, as one might expect given its limited dispersal capabilities (Baker *et al.* 1995).

It was not surprising that the Veery showed no sign of population structure given its near universal distribution in the northeastern United States, and its much larger population size. Indeed it is obviously artificial to attempt to impose sub-populations on maternally transmitted lineages of the Veery in the northeast.

Whether population structure is low or non-zero throughout the range of Bicknell's Thrush is an open and worthwhile question. The sub-populations in Nova Scotia, northern New Brunswick, and south central Québec are much more distant from the mountains I studied and have greater gulfs between them than northeastern United States mountain ranges. For instance, northern New Brunswick and northeastern Nova Scotia Bicknell's Thrushes are 390 km apart versus a 280 km gap between the White Mountains and the Catskills. It is plausible that southeastern Canadian populations show more isolation.

Larger scale studies of the Veery might also be informative. However, the near uniformity of its distribution in the northeastern United States and southeastern Canada hold little hope for detecting much structure there. The Veery is limited to upper elevations in the southern Appalachians, e.g., largely above 750 m in West Virginia (Hall 1983), and 1050 m in the Great Smoky Mountains (Stupka 1963). It may be more useful to look into genetic differences among these Veery populations, which appear to be similarly distributed to Bicknell's Thrush. Another possible study could be made of apparently isolated mountain and riparian Veery populations in western North America, where Veery habitat is more patchily distributed. It would also be worthwhile to look into the relationships and status of the western Veery subspecies *salicicola* versus eastern subspecies.

The conservation implications of a lack of structure and concomitantly high gene flow in Bicknell's Thrush are related to the implied existence of a highly mobile population capable of metapopulation dynamics (Hanski 1998; Hastings

and Harrison 1994). The Bicknell's Thrush probably shows fairly rapid recolonization or rescue of small, unstable mountaintop populations from larger nearby source populations. If Bicknell's Thrushes have frequent exchange of breeding birds among mountains and mountain ranges there is less likelihood of local adaptation of sub-populations, and it is also unlikely genetic drift has reduced genetic variation in any sub-population or the species as a whole. The high rate of gene flow and lack of structure in the Veery also suggests that gaps between reserves are of less concern than reserve size for maintaining a genetically diverse Veery population. Concerns with habitat fragmentation for the Veery, it seems, should be focussed on mechanistic problems caused by predation, brood parasitism, and other edge effects (c.f. Robinson *et al.* 1995).

Summary

Bicknell's Thrush and Veery appear to differ slightly in population structure but not significantly so, with the former showing a trend to non-zero population structure opposed to its total absence in the latter. This difference has little or no impact on the ability of Bicknell's Thrush to maintain gene flow among the mountain ranges of the northeastern United States. It is plausible that larger scale studies including southeastern Canadian populations of Bicknell's Thrush, or southern Appalachian or western North American populations of Veery might reveal structure, but this seems doubtful because long-distance migration appears to promote gene flow among populations in these species.

A. Bicknell's Thrush Haplotypes

	Site Number						
	122	257	271	273	281	308	309
A. n=19	T	A	T	T	G	-	-
B. n=14	•	G	•	•	•	-	-
C. n= 4	•	G	•	C	•	-	-
D. n= 2	•	•	•	•	A	-	-
E. n= 2	•	•	C	•	A	-	-
F. n= 1	•	•	C	•	A	A	C
G. n= 1	C	•	C	•	A	-	-

B. Veery Haplotypes

	Site Number								
	20	34	39	122	251	273	276	331	353
A. n=25	C	T	A	T	T	T	G	C	C
B. n= 4	•	•	G	•	•	•	•	•	•
C. n= 3	T	•	•	•	•	•	•	•	•
D. n= 2	•	C	•	•	•	•	•	•	•
E. n= 1	•	•	•	C	•	•	•	•	•
F. n= 1	•	•	•	•	C	•	•	•	•
G. n= 1	•	•	•	•	•	C	•	•	•
H. n= 1	•	•	?	•	•	•	A	•	•
I. n= 1	•	•	•	•	•	•	•	T	•
J. n= 1	•	•	•	•	•	•	•	•	T

Table 4.1. Bicknell's Thrush and Veery haplotypes. Haplotype definitions based on variable sequence sites in control region III with numbers of birds carrying each haplotype and the nucleotide state at each variable site along the sequence (total sites number 395). At each site the letters stand for the nucleotide bases adenine (A), guanine (G), thymine (T), and cytosine (C), and periods indicate identity with the topmost nucleotide. Complete aligned sequences for all haplotypes are presented in Table 3.1 and for all individuals in Appendix 2.

	Tajima's D	
	D statistic	Significance
A. Bicknell's Thrush		
Total	1.8247	*
Adirondack	1.4351	N.S.
Catskill	1.7376	*
Green Mt.	0.1270	N.S.
White Mt.	0.5655	N.S.
B. Veery		
Total	-1.8373	* *
Adirondack	-1.4009	N.S
Catskill	-1.2447	N.S.
Green Mt.	-1.5622	*
White Mt.	-1.0345	N.S.

Table 4.2. Results of Tajima's D test for all samples and for each sub-population for Bicknell's Thrush and Veery. Under significance, * denotes $p \leq 0.1$, * * denotes $p \leq 0.05$, and N.S. stands for "not significant".

A. Φ_{ST}

Bicknell's Thrush

	Catskill	Green Mt.	Adirondack	White Mt.
Catskill	—	0.0899	– 0.0198	– 0.0485
Green Mt.	5.059	—	0.0040	0.2314
Adirondack	infinite	124.0493	—	0.0815
White Mt.	Infinite	1.6607	5.6364	—

Veery

	Catskill	Green Mt.	Adirondack	White Mt.
Catskill	—	0.0899	– 0.0198	– 0.0485
Green Mt.	5.059	—	0.0040	0.2314
Adirondack	infinite	124.0493	—	0.0815
White Mt.	Infinite	1.6607	5.6364	—

B. N_{ST}

Bicknell's Thrush

	Catskill	Green Mt.	Adirondack	White Mt.
Catskill	—	0.0903	– 0.0097	0.0210
Green Mt.	5.04	—	– 0.0119	0.3623
Adirondack	infinite	infinite	—	0.2488
White Mt.	23.32	0.88	1.51	—

Veery

	Catskill	Green Mt.	Adirondack	White Mt.
Catskill	—	– 0.4434	– 0.0696	– 0.0369
Green Mt.	infinite	—	– 0.0437	0.0325
Adirondack	infinite	infinite	—	– 0.0214
White Mt.	infinite	14.88	infinite	—

Table 4.3. Fixation index values and rate of gene flow per generation for Bicknell's Thrush and Veery in northeastern mountain ranges.
A. Among sub-population values of Φ_{ST} (above diagonal) and estimates of rate of gene flow (*Nm*; below diagonal) derived from Φ_{ST}.
B. Among sub-population values of N_{ST} (above diagonal) and estimates of rate of gene flow (*Nm*; below diagonal) derived from N_{ST}.

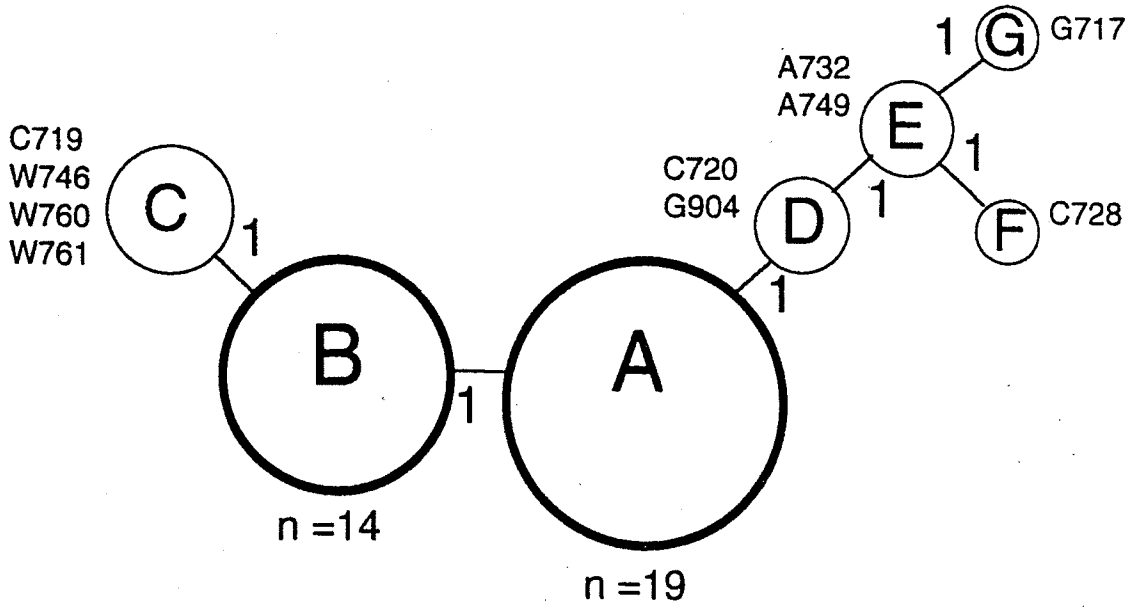


Figure 4.1. Bicknell's Thrush haplotype network. Circle sizes reflect relative abundance of haplotypes; letters correspond to haplotypes defined in Table 4.1A. Numbers adjacent to connecting lines represent the number of nucleotide substitutions between haplotypes. Identifying numbers of individuals bearing less common haplotypes are listed adjacent to each smaller circle. Complete sequences of all individuals are presented in Appendix 2.

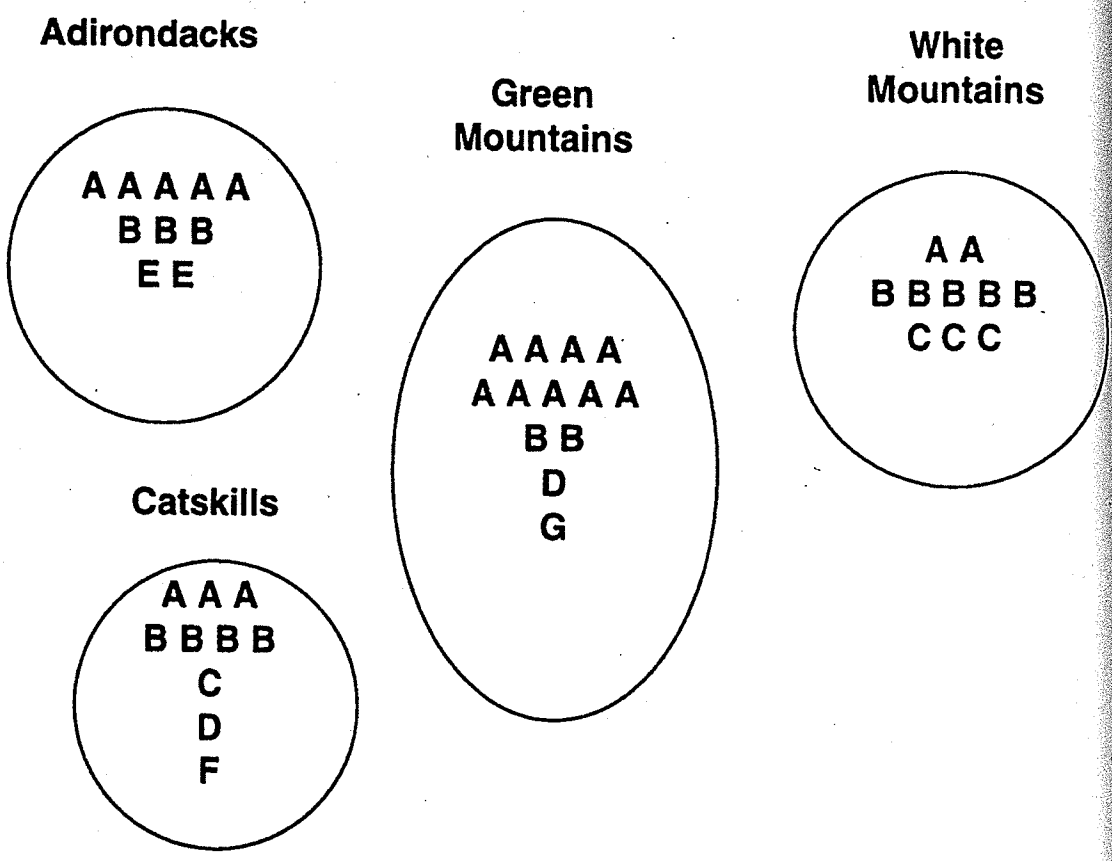


Figure 4.2. The distribution of the seven Bicknell's Thrush haplotypes among sub-populations from four northeastern United States mountain ranges. The letters represent each haplotype as defined in Table 4.1A. Each copy of a letter represents one individual carrying that haplotype. Note the widespread occurrence of the two most common haplotypes (A and B) and the variation in their frequencies among mountain ranges.

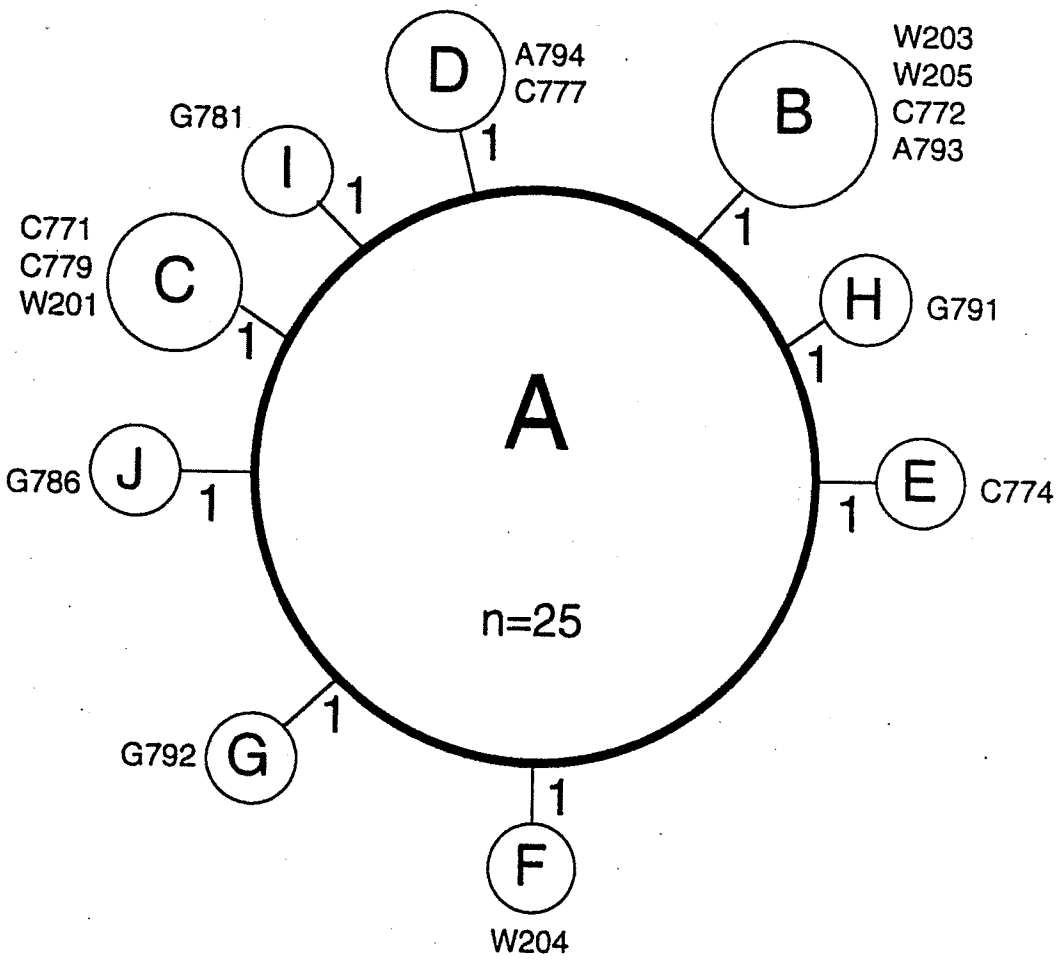


Figure 4.3. Veery haplotype network. Circle sizes reflect relative abundance of haplotypes; letters correspond to haplotypes defined in Table 4.1B. Numbers adjacent to connecting lines represent the number of nucleotide substitutions between haplotypes. Identifying numbers of individuals bearing less common haplotypes are listed adjacent to each smaller circle. Complete sequences of all individuals are presented in Appendix 2.

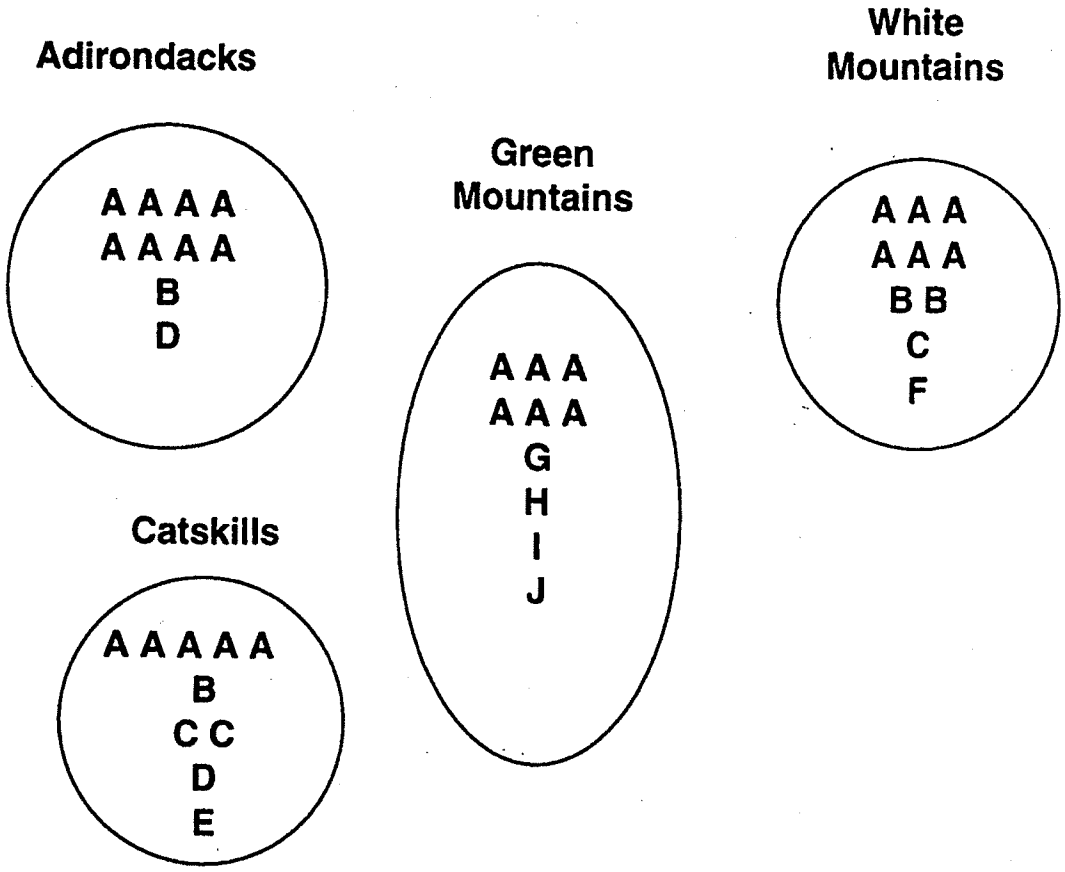


Figure 4.4. The distribution of the 10 Veery haplotypes among sub-populations from four northeastern United States mountain ranges. The letters represent each haplotype as defined in Table 4.1B. Each copy of a letter represents an individual carrying that haplotype. Note the predominance of haplotype A and the occurrence of other mutiple-individual haplotypes (B, C, and D) among mountain ranges.