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Major Divisions in Oscines Revealed by Insertions in the Nuclear Gene c-*myc*: A Novel Gene in Avian Phylogenetics

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The order Passeriformes is a monophyletic group consisting of more than half of all living birds species (Raikow 1982). The major split of the passerines into the suboscines and oscines is well supported by morphological characters, although a few taxa (e.g. Acanthisittidae, New Zealand wrens) defy allocation to either suborder (see Sibley and Ahlquist 1990). Molecular analyses corroborate this dichotomy in passerines (Sibley and Ahlquist 1990, Edwards et al. 1991).

Some oscine families are distinct, but convergent evolution apparently is common and has obscured phylogenetic relationships, making the subdivision of this group based on morphology difficult (Beecher 1953, Tordoff 1954, Ames 1971, Raikow 1978, Bledsoe 1988). In fact, even the delimitations of most families are uncertain, and only two families, the Alaudidae (larks) and the Hirundinidae (swallows and martins), are unambiguously defined (Mayr 1958). Consequently, oscine relationships at the family level and above are insufficiently known, and all taxonomic arrangements are controversial.

Besides the larks and swallows, three main groups of oscines have been recognized based on morphology: (1) Old World insect-eaters and their relatives; (2) New World insect-eaters and finches; and (3) crows, birds-of-paradise, and associated families (Mayr and Greenway 1956, Voous 1985). Before the advent of quantitative biochemical methods, most systematists recognized these groups, and the major debate concerned how they were related (Voous 1985). Although all combinations of the three groups have been advocated at one time or another, a major issue is whether the crows and their allies constitute the sister group to all other oscines, or are nested within them. The fully developed double pneumatic fossae in the proximal end of the humerus present in many oscines, but not in crows and allies or in the suboscines (Bock 1962), suggests the existence of a clade including all oscines except crows and their allies. This dichotomy has been supported by DNA-DNA hybridization studies (Sibley and Ahlquist 1990, Harshman 1994, Sheldon and Gill 1996). In the classification of Sibley and Monroe (1990), the dichotomy is reflected by the division of the oscines into the parvorders Corvida and Passerida. The Passerida is further divided into the superfamilies Muscicapoidea, Sylvioidea, and Passeroidea.

The DNA-DNA hybridization method as applied by Sibley and Ahlquist has been criticized on several grounds, and doubts concerning the validity of some of their results have been raised (Cracraft 1987, Houde 1987, Sarich et al. 1989, Sheldon and Bledsoe 1993). However, the currently favored method in molecular systematics, the comparison of nucleotide sequences, so far has generated few phylogenetic hypotheses at this high taxonomic level in oscines (but see Edwards et al. 1991, Groth 1998).

Here, we present a hypothesis of phylogenetic relationships among oscines based on two previously undescribed insertions in exon 3 of c-*myc*. This hypothesis defines major groups of songbirds. C-*myc* is a nuclear proto-oncogene that encodes a protein transcription factor that plays a crucial role in the regulation of cell proliferation and apoptosis (Bouchard et al. 1998). The sequence of c-*myc* is highly conserved throughout the vertebrates, especially compared with the more rapidly evolving mitochondrial genes. Although no dates are known for splits between the evolutionary lineages studied herein, some of them might be very old, perhaps even of early Tertiary age (Feduccia 1995). Mutational saturation can reduce the resolving power of gene sequences and might be a problem when using mitochondrial genes to study ancient branching events in birds. In contrast, dissimilarities between c-*myc* sequences increase nearly linearly for evolutionary divergences well beyond 100 million years ago (Graybeal 1994). To investigate early avian divergences, we have sequenced about 500 base pairs of exon 3 of this gene for more than 150 species representing 65 nonpasserine and 36 passerine families. Our results confirm the slow rate of evolution of c-*myc* in birds. The maximum sequence divergence observed was about 11%, and only three indels occurred. Only one indel, an insertion of four amino acids relative to the published chicken sequence, has been observed outside the passerines.

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TABLE 1. Distribution of taxa used in this study. Family names and lower taxonomic categories follow Morony et al. (1975), and higher categories follow Sibley and Monroe (1990). AM = Australian Museum, NRM = Swedish Museum of Natural History, ZMCU = Zoological Museum of the University of Copenhagen, and NCBI = National Center for Biotechnology Information (GenBank).

Parvorder or superfamily		Family or subfamily	Species	Sample no.	Locality
		Suborder Tyranni, infraorder Eurylaimides			
	Pittoidea				
		Pittidae	*Pitta angolensis*	ZMCU S1027	Tanzania
	Eurylaimoidea				
		Eurylaimidae	*Smithornis capensis*	ZMCU S967	Tanzania
		Philepittidae	*Philepitta castanea*	ZMCU S458	Madagascar
		Suborder Tyranni, infraorder Tyrannides			
Tyrannida					
		Tyrannidae	*Muscivora tyrannus*	NRM 976722	Paraguay
			Gubernetes yetapa	NRM 976700	Paraguay
			Idioptilon margaritaceiventer	NRM 966959	Paraguay
			Xolmis irupero	NRM 937154	Paraguay
		Phytotomidae	*Phytotoma rutila*	ZMCU S466	Bolivia
		Cotingidae	*Tityra cayana*	NRM 956584	Paraguay
		Pipridae	*Pipra fasciicauda*	NRM 947271	Paraguay
Thamnophilida					
		Formicariidae	*Thamnophilus caerulescens*	NRM 967007	Paraguay
Furnariida					
	Furnarioidea				
		Furnariidae	*Furnarius cristatus*	NRM 966772	Paraguay
		Dendrocolaptidae	*Lepidocolaptes angustirostris*	NRM 937184	Paraguay
	Formicarioidea				
		Conopophagidae	*Conopophaga lineata*	NRM 956653	Paraguay
		Rhinocryptidae	*Rhinocrypta lanceolata*	NRM 966793	Paraguay
		Suborder Passeri			
Corvida					
	Menuroidea				
		Menuridae	*Menura novaehollandiae*	AM LAB1112	Australia
	Corvoidea				
		Laniidae	*Lanius collurio*	NRM 986403	Sweden
		Vireonidae	*Vireo olivaceus*	NRM 976766	Paraguay
		Vireonidae	*Cychlaris gujanensis*	NRM 966964	Paraguay
		Grallinidae	*Corcorax melanoramphos*	AM LAB1059	Australia
		Paradisaeidae	*Ptiloris magnificus*	AM O64926	Australia
		Cracticidae	*Cracticus torquatus*	AM LAB1110	Australia
		Oriolidae	*Oriolus oriolus*	ZMCU O1376	Denmark
		Campephagidae	*Campephaga phoenica*	ZMCU O11	Kenya

TABLE 1. Continued.

Parvorder or superfamily	Family or subfamily	Species	Sample no.	Locality
	Dicruridae	*Dicrurus balicassius*	ZMCU O352	Philippines
	Platysteirinae	*Batis mixta*	ZMCU O2953	Tanzania
Passerida				
Muscicapoidea				
	Bombycillidae	*Bombycilla garrulus*	NRM 986044	Sweden
	Turdinae	*Erithacus rubecula*	NRM 976377	Sweden
	Muscicapinae	*Ficedula hypoleuca*	NRM 976132	Sweden
	Sturnidae	*Sturnus vulgaris*	NRM 966615	Sweden
	Mimidae	*Mimus saturinus*	NRM 966912	Paraguay
Sylvioidea				
	Sittidae	*Sitta europea*	NRM 976163	Sweden
	Panurinae	*Panurus biarmicus*	NRM 966576	Sweden
	Sylviinae	*Sylvia atricapilla*	NRM 976380	Sweden
	Certhiidae	*Certhia familiaris*	NRM 976184	Sweden
	Troglodytidae	*Troglodytes troglodytes*	NRM 986416	Sweden
	Paridae	*Parus major*	NRM 956363	Sweden
	Aegithalidae	*Aegithalos caudatus*	NRM 976089	Sweden
	Remizidae	*Remiz pendulinus*	NRM 966576	Sweden
	Hirundinidae	*Hirundo rustica*	NRM 976238	Sweden
	Pycnonotidae	*Chlorocichla flaviventris*	ZMCU O1789	Kenya
	Zosteropidae	*Zosterops nigrorum*	ZMCU O2663	Philippines
Passeroidea				
	Alaudidae	*Alauda arvensis*	NRM 966614	Sweden
	Dicaeidae	*Dicaeum australe*	ZMCU O3737	Philippines
	Nectariniidae	*Aethopyga flagrans*	ZMCU O1346	Philippines
	Passerinae	*Passer montanus*	NRM 976359	Sweden
	Ploceinae	*Ploceus velatus*	—	Kenya
		Quelea quelea	—	Kenya
	Motacillidae	*Anthus trivialis*	NRM 976393	Sweden
		Motacilla alba	NRM 976193	Sweden
	Prunellidae	*Prunella modularis*	NRM 976138	Sweden
	Estrildidae	*Lonchura malacca*	ZMCU O1716	Philippines
	Fringillidae	*Carduelis chloris*	—	Sweden
		Carpodacus erythrinus	NRM 976373	Sweden
		Coccothraustes coccothraustes	NRM 976374	Sweden
		Loxia curvirostra	NRM 976546	Sweden
		Pinicola enucleator	NRM 996030	Sweden
		Pyrrhula pyrrhula	NRM 986379	Sweden
		Serinus canaria	NCBI 64252	—

TABLE 1. Continued.

Parvorder or superfamily	Family or subfamily	Species	Sample no.	Locality
	Emberizinae	*Ammodramus humeralis*	NRM 966958	Paraguay
		Calcarius lapponicus	NRM 976550	Sweden
		Emberizoides herbicola	NRM 976735	Paraguay
		Oryzoborus angolensis	NRM 947261	Paraguay
		Paroaria coronata	NRM 976781	Paraguay
		Plectrophenax nivalis	NRM 986392	Sweden
		Volatinia jacarina	NRM 966961	Paraguay
	Cardinalinae	*Saltator atricollis*	NRM 966978	Paraguay
	Tersininae	*Tersina viridis*	NRM 976669	Paraguay
	Thraupinae	*Eucometis penicillata*	NRM 966968	Paraguay
		Euphonia chlorotica	NRM 956750	Paraguay
		Tangara seledon	NRM 956580	Paraguay
	Parulidae	*Conirostrum speciosum*	NRM 976671	Paraguay
		Geothlypis aequinoctialis	NRM 956574	Paraguay
		Parula pitiayumi	NRM 947170	Paraguay
	Icteridae	*Agelaius cyanopus*	NRM 966916	Paraguay
		Amblyramphus holosericeus	NRM 966856	Paraguay
		Icterus cayanensis	NRM 967139	Paraguay
		Molothrus badius	NRM 976783	Paraguay
		Pseudoleistes guirahuro	NRM 976736	Paraguay

Methods.—Representatives of 46 passerine families were selected for study (Table 1). Special emphasis was placed on sampling the superfamily Passeroidea sensu Sibley and Ahlquist (1990). If not stated otherwise, the usage of family and subfamily names follows Morony et al. (1975). However, at higher levels, i.e. superfamilies and parvorders, we use the terminology of Sibley and Ahlquist (1990) to facilitate comparisons between our results and their phylogenetic hypotheses.

We extracted genomic DNA from tissue or blood using standard techniques of Proteinase K/SDS digestion followed by phenol chloroform extraction and ethanol precipitation, or by QIAamp DNA extraction kits following manufacturer's recommendations. Amplification was performed with primer pairs *myc*EX3A (CAAGAAGAAGATGAGGAAAT) and R*myc*EX3A (TTAGCTGCTCAAGTTTGTG), or *myc*EX3D (GAAGAAGAACAAGAAGAAGATG) and R*myc*EX3D (ACGAGAGTTCCTTAGCTGCT), developed by Thomas J. Parsons. Sequencing was performed with primers *myc*EX3A and R*myc*EX3A using Perkin Elmer Applied BioSystems 373 or 377 automated fluorescent sequencing instruments, and Perkin Elmer Applied BioSystems PRISM terminator cycle sequencing kits with AmpliTaq FS polymerase (either standard rhodamine and BigDye chemistries were employed). Sequence assembly was performed using the Perkin Elmer Applied BioSystems Sequence Navigator or the DNASTAR SeqMan II programs. Alignments of completed sequences were performed by eye. Indications of sequence positions throughout this report are relative to the numbering of the full-length protein-coding sequence of the chicken (Watson et al. 1983).

Results.—Nucleotide sequences of exon 3 of c-*myc* have been studied in 80 species of suboscine and oscine passerines, representing 46 traditional families (Table 1). The sequences vary from 498 to 510 bases (corresponding to 166 to 170 amino acids) in length as a consequence of the presence or absence of two insertions consisting of one and three amino acids, respectively. These two insertions have not been observed among 65 nonpasseriform families, but they appear to exhibit consistent taxonomic distributions within the Passeriformes (with no reversals inferred on the portions of the tree where relationships are well established). Thus, they presumably represent unique and significant evolutionary events in passerine evolution.

The ancestral state in passerines of no insertions was observed in all nonpasseriforms investigated and also was found in all suboscine and Corvida families (Table 2). All oscine families representing the parvorder Passerida that we examined possessed an insertion of a single amino acid at nucleotide position 793 relative to the chicken c-*myc* sequence (Watson et al. 1983). The occurrence of this insertion in all oscine passerines except the Corvida supports the hypothesis based on DNA-DNA hybridization of a sister-group relationship between the Corvida and all other oscines. In most families, this extra amino acid is a threonine. However, it is a proline in *Hirundo* and *Sylvia* and a serine in *Certhia, Carduelis,* and *Icterus.*

At position 991, the Motacillidae, Fringillidae, Emberizidae, Parulidae, and Icteridae share an additional insertion of three amino acids relative to the chicken (Table 2). The first two of these are always a serine and a glycine. The third amino acid varies more among the families. Most taxa have a serine, but motacillids (*Motacilla* and *Anthus*) have threonine; *Geothlypis, Parula, Carpodacus,* and *Icterus* have leucine; *Conirostrum* has phenylalanine; and *Carduelis* has tryptophan. Some silent third-position variation in codon coding also occurs for this third inserted amino acid.

Discussion.—We consider the passerine c-*myc* insertions described here to represent two unique evolutionary events, with no reversals evident in the taxa studied. This pattern is strongly suggested by the extreme rarity of indels in c-*myc* exon 3 throughout avian taxa. For example, among 102 nonpasserine species studied, representing 65 families, only one indel has been observed. This insertion of four amino acids relative to the chicken sequence occurs at position 796, i.e. at a different position than the passerine insertions reported here. The conservation in sequence length of c-*myc* may be due to the fact the *myc* protein has a helix-loop-helix structure that must form a heterdimeric complex with the regulatory Max protein. The central regulatory role of *myc* in cell division and development likely would tolerate little functional variation (Bouchard et al. 1998, Eilers 1999). Length changes may be rare owing to a requirement for radical compensatory changes in other genes, with reversals encountering an evolutionary hurdle of equivalent magnitude. Table 2 indicates that multiple amino-acid substitutions have occurred within the single amino-acid insertion, with possibly three substitutions of proline for threonine and two substitutions of serine for threonine. This further supports the low rate of indel mutations compared with the already slow rate of amino-acid sequence substitution. Likewise, the third amino acid of the three that are inserted displays substantial variation within related groups, whereas the length of insertion remains constant.

The insertion involving a single amino acid observed in the c-*myc* sequence is a synapomorphy for all oscines that we studied, except species in the parvorder Corvida (Fig. 1). This observation supports the sister-group relationship of the corvids and their allies relative to other oscines, as suggested by DNA-DNA hybridization (Sibley and Ahlquist 1990, Harshman 1994, Sheldon and Gill 1996). Unfortunately, only one representative of the superfamily Menuroidea was available to us.

TABLE 2. Taxonomic distribution of the two insertions of amino acids in exon 3 of the nuclear c-*myc* gene in a survey of passerines. The insertions occur at positions 793 and 991 in the published c-*myc* sequence of the chicken (Watson et al. 1983). Genera, families, and subfamilies are based on the "traditional" classification of Morony et al. (1975), and superfamilies for oscines are from Sibley and Monroe (1990) based on DNA-DNA analysis.

Genus	Family/subfamily	Superfamily	Position in chicken sequence: 792 793	Position in chicken sequence: 991 992
Gallus	Phasianidae		T C C A G C A C A — G A A G C A T C A	C C C C G C A C G —— T C A G A C T C A
Smithornis	Eurylaimidae		—	——
Philepitta	Philepittidae		—	——
Pitta	Pittidae		—	——
Muscivora	Tyrannidae		—	——
Gubernetes	Tyrannidae		—	——
Idioptilon	Tyrannidae		—	——
Xolmis	Tyrannidae		—	——
Phytotoma	Phytotomidae		—	——
Tityra	Cotingidae		—	——
Pipra	Pipridae		—	——
Thamnophilus	Formicariidae		—	——
Furnarius	Furnariidae		—	——
Lepidocolaptes	Dendrocolaptidae		—	——
Conopophaga	Conopophagidae		—	——
Rhinocrypta	Rhinocryptidae		—	——
Menura	Menuridae	Menuroidea	—	——
Lanius	Laniidae	Corvoidea	—	——
Vireo	Vireonidae	Corvoidea	—	——
Cychlaris	Vireonidae	Corvoidea	—	——
Corcorax	Grallinidae	Corvoidea	—	——
Ptiloris	Paradisaeidae	Corvoidea	—	——
Cracticus	Cracticidae	Corvoidea	—	——
Oriolus	Oriolidae	Corvoidea	—	——
Campephaga	Campephagidae	Corvoidea	—	——
Dicrurus	Dicruridae	Corvoidea	—	——
Batis	Platysteirinae	Corvoidea	—	——
Bombycilla	Bombycillidae	Muscicapoidea	A C A	——
Erithacus	Turdinae	Muscicapoidea	A C A	——
Ficedula	Muscicapinae	Muscicapoidea	A C G	——
Sturnus	Sturnide	Muscicapoidea	A C A	——
Mimus	Mimidae	Muscicapoidea	A C A	——
Sitta	Sittidae	Sylvioidea	A C A	——
Panurus	Panurinae	Sylvioidea	A C A	——
Sylvia	Sylviinae	Sylvioidea	C C A	——
Certhia	Certhiidae	Sylvioidea	T C A	——

TABLE 2. Continued.

Genus	Family/subfamily	Superfamily	Position in chicken sequence 792 793	Position in chicken sequence 991 992
Troglodytes	Troglodytidae	Sylvioidea	A C A	——
Parus	Paridae	Sylvioidea	A C A	——
Aegithalos	Aegithalidae	Sylvioidea	A C A	——
Remiz	Remizidae	Sylvioidea	A C A	——
Hirundo	Hirundinidae	Sylvioidea	C C A	——
Chlorocichla	Pycnonotidae	Sylvioidea	A C A	——
Zosterops	Zosteropidae	Sylvioidea	A C A	——
Alauda	Alaudidae	Passeroidea	A C A	——
Dicaeum	Dicaeidae	Passeroidea	A C A	——
Aethopyga	Nectariniidae	Passeroidea	A C A	——
Passer	Passerinae	Passeroidea	A C A	——
Ploceus	Ploceinae	Passeroidea	A C A	——
Quelea	Ploceinae	Passeroidea	A C A	——
Prunella	Prunellidae	Passeroidea	A C A	——
Lonchura	Estrildidae	Passeroidea	A C A	——
Anthus	Motacillidae	Passeroidea	A C A	T C A G G C A C A
Motacilla	Motacillidae	Passeroidea	A C A	T C A G G C A C G
Carduelis	Fringillidae	Passeroidea	T C A	T C A G G C T C G
Carpodacus	Fringillidae	Passeroidea	A C A	T C A G G C T T A
Coccothraustes	Fringillidae	Passeroidea	A C A	T C A G G C T C G
Loxia	Fringillidae	Passeroidea	A C A	T C A G G C T C G
Pinicola	Fringillidae	Passeroidea	A C A	T C A G G C T C A
Pyrrhula	Fringillidae	Passeroidea	A C A	T C A G G C T C A
Serinus	Fringillidae	Passeroidea	A C A	T C A G G C T C G
Ammodramus	Emberizinae	Passeroidea	A C A	T C A G G C T C C
Calcarius	Emberizinae	Passeroidea	A C A	T C A G G C T C C
Emberizoides	Emberizinae	Passeroidea	A C A	T C A G G C T C C
Oryzoborus	Emberizinae	Passeroidea	A C A	T C A G G C T C C
Paroaria	Emberizinae	Passeroidea	A C A	T C A G G C T C C
Plectrophenax	Emberizinae	Passeroidea	A C A	T C A G G C T C G
Volatinia	Emberizinae	Passeroidea	A C A	T C A G G C T C C
Saltator	Cardinalinae	Passeroidea	A C A	T C A G G C T C C
Tersina	Tersininae	Passeroidea	A C A	T C A G G C T C C
Eucometis	Thraupinae	Passeroidea	A C A	T C A G G C T C C
Euphonia	Thraupinae	Passeroidea	A C A	T C A G G C T CC
Tangara	Thraupinae	Passeroidea	A C A	T C A G G C T CC
Conirostrum	Parulidae	Passeroidea	A C A	T C A G G C T T C

TABLE 2. Continued.

Genus	Family/subfamily	Superfamily	Position in chicken sequence	
			792 793	991 992
Geothlypis	Parulidae	Passeroidea	A C A	T C A G G C T T G
Parula	Parulidae	Passeroidea	A C A	T C A G G C T T G
Agelaius	Icteridae	Passeroidea	A C A	T C A G G C T C G
Amblyramphus	Icteridae	Passeroidea	A C A	T C A G G C T C G
Icterus	Icteridae	Passeroidea	T C A	T C A G G C T T G
Molothrus	Icteridae	Passeroidea	A C A	T C A G G C T C G
Pseudoleistes	Icteridae	Passeroidea	A C A	T C A G G C T C A

Passerines typically have 10 primaries, which is generally agreed to be the ancestral condition. In several oscine families, the outermost primary is secondarily reduced or lost, and species in these groups are effectively nine-primaried. Which families are nine-primaried has been a matter of considerable confusion, however. Some families that are regarded as "nine-primaried" include species in which the tenth primary is in fact present, although vestigial. A long-recognized group of truly nine-primaried families is the so-called "New World nine-primaried oscines" that consist of the Parulidae, Emberizidae (Emberizinae, Thraupinae, Cardinalinae), and Icteridae (Raikow 1978, Feduccia 1996). Although not all of these families are confined to the New World, they are concentrated there.

All representatives of the New World nine-primaried oscines that we analyzed (Parulidae, Emberizinae, Thraupinae, Cardinalinae, and Icteridae) possess the insertion of three amino acids at position 991 in the chicken sequence. This is a strong indication of the shared common ancestry of this group. Moreover, the Fringillidae and Motacillidae also share this insertion. The fringillids and motacillids are included in the Passeroidea by Sibley and Ahlquist (1990), along with the New World nine-primaried oscines. However, in other families in Passeroidea and studied herein (Alaudidae, Nectariniidae, Dicaeidae, Estrildidae, Passeridae, and Prunellidae), this insertion is absent. The c-*myc* data thus support a clade consisting of the New World nine-primaried oscines, the primarily Old World finches, and the wagtails and pipits. The Motacillidae have a vestigial tenth primary and traditionally have not been thought to be closely related to the New World nine-primaried oscines, although cytochrome-*b* sequence data suggest them to be closer to the Emberizidae than are the Fringillidae (Groth 1998). Cytochrome-*b* sequence data also suggest that the ten-primaried Passeridae are nested within this clade of emberizids, fringillids, and motacillids (Groth 1998). This arrangement is not supported by c-*myc* data, because the three species of Passeridae (=Ploceidae sensu Morony et al. 1975) we studied do not share the insertion of three amino acids with the rest of the group.

It could be argued that the insertions reported herein, as single characters, should not be afforded more weight than other molecular characters. However, we believe that these insertions represent unique evolutionary events of unequivocal homology, with no reversal. As such, they present powerful evidence regarding relationships within passerines that have been difficult to resolve based on other potentially quite homoplastic characters. The greatly increased significance of unique molecular rearrangements has been recognized elsewhere (Batzer et al. 1996), and shared indels in protein-coding genes previously have been interpreted as strong markers for monophyly as long as the observations

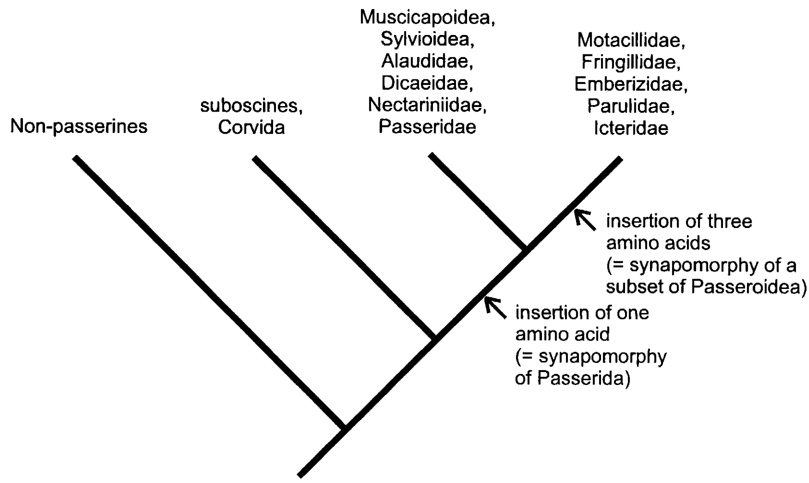

FIG. 1. Major divisions of passerines as indicated by insertions of amino acids in the nuclear gene c-*myc*. The first insertion is synapomorphic for the parvorder Passerida (sensu Sibley and Ahlquist 1990), whereas all representatives of the New World nine-primaried oscines, the primarily Old World finches, and the Motacillidae share a second insertion of amino acids.

are based on wide taxonomic sampling (van Dijk et al. 1999). We studied sequences from more than 110 families of passerines and nonpasserines. The extreme low frequency of indels in c-*myc*, and the taxonomic distribution of insertions that we report, indicate that these should be considered highly significant characters for elucidating the evolution of passerines.

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Specialized Extrapair Mating Display in Western Bluebirds

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Western Bluebirds (*Sialia mexicana*) are socially monogamous, maintain long-term pair bonds, and share equally in biparental care (Dickinson et al. 1996). Females often have extrapair young in their nests even though males exhibit kin-based winter sociality and sometimes help at the nests of relatives (Dickinson and Akre 1998). DNA fingerprinting has revealed that more than 45% of females have at least one offspring sired by a male outside the family group and that 19% of offspring are sired by extrapair males (Dickinson and Akre 1998). Paired males follow their mates closely during the receptive period, a behavior that dramatically reduces the frequency of extrapair copulation (EPC) attempts (Dickinson and Leonard 1996, Dickinson 1997). As a con-

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