

PRE-PLEISTOCENE DIFFERENTIATION AMONG CHAT-TYRANTS¹

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Abstract. Most species of chat-tyrants (*Ochthoeca*, *Silvicultrix*) are widespread along the length of the Andes, with broadly overlapping ranges, but segregated in different altitudinal zones or habitats. We used mitochondrial DNA sequences to study their phylogenetic relationships. Our data show considerable divergence and multiple base substitutions at some positions between geographically overlapping species. Such divergence probably predates the period of marked ecoclimatic changes in the Pleistocene, whereas vicariance patterns may reflect events in the lower Pleistocene. Only some of the boundaries between allospecies correspond to marked topographic barriers, and we suggest that sharp species-replacements indicate secondary contact zones. An evolutionary dynamic scenario is suggested where divergence of isolated populations is followed by establishment of sharp geographical replacements and, with development of full ecological compatibility, establishment of broad overlap along the Andes cordillera. The phylogeny suggests a series of adaptive shifts from the ancestral habitat of scrub and river margins in tropical lowlands to bushy highlands and elfin forest, then colonization of edge habitats in the humid montane forest, and finally interior montane and submontane forest. However, the adaptive redistribution has obscured the early vicariance events in the group. The results support the monophyly of all chat-tyrants, but with a distinctive internal structure, where *Silvicultrix* is a monophyletic internal branch within *Ochthoeca*. Support also is given for a close relationship between *Tumbezia salvini* and *Ochthoeca*. We suggest keeping *Tumbezia* as a separate genus, merging *Silvicultrix* with *Ochthoeca*, and elevating *O. frontalis* and *spodionota*, and *O. cinnamomeiventris* and *thoracica* to species rank.

Key words: *Andes*, *biogeography*, *mtDNA*, *ND2*, *Ochthoeca*, *Silvicultrix*, *speciation*.

Resumen. La mayoría de las especies de pitajos (*Ochthoeca*, *Silvicultrix*) tiene distribuciones sobrelapadas a lo largo de los Andes, segregándose en hábitats o zonas altitudinales distintas. Usamos secuencias del ADN mitocondrial para investigar sus relaciones filogenéticas. Nuestros datos muestran gran divergencia entre especies. Al comparar especies cuyas distribuciones se sobrelapan detectamos la ocurrencia de múltiples sustituciones de nucleótidos en algunas posiciones. Dichas especies corresponden a las ramas más profundas de la filogenia, y posiblemente anteceden al período de variaciones climáticas extremas del Pleistoceno, mientras que los patrones de vicariancia visibles reflejan eventos ocurridos en el Pleistoceno inferior. Solamente algunos límites entre aloespecies corresponden con barreras topográficas, y sugerimos que dichas suturas son áreas de contacto secundario. Se propone un escenario evolutivo dinámico. En este, la divergencia de poblaciones aisladas precede al establecimiento de las suturas entre especies que se reemplazan geográficamente, y concluye con el establecimiento de distribuciones sobrelapadas cuando la compatibilidad ecológica es total. La filogenia sugiere una serie de cambios adaptativos del hábitat ancestral en matorrales y márgenes fluviales de las tierras bajas tropicales a los hábitats montañosos, y finalmente al interior de los bosques montano y submontano. La redistribución adaptativa ha oscurecido los eventos tempranos de vicariancia del grupo. Nuestros resultados apoyan la monofilia de los pitajos, aunque con una estructura interna particular, en la que *Silvicultrix* forma una grupo monofilético dentro de *Ochthoeca*. Se apoya una relación estrecha entre *Tumbezia* y *Ochthoeca*. Sugerimos mantener *Tumbezia* como género aparte, fundir *Silvicultrix* con *Ochthoeca*, y otorgarles el rango de especies a *O. frontalis* y *O. spodionota*, y a *O. cinnamomeiventris* y *O. thoracica*.

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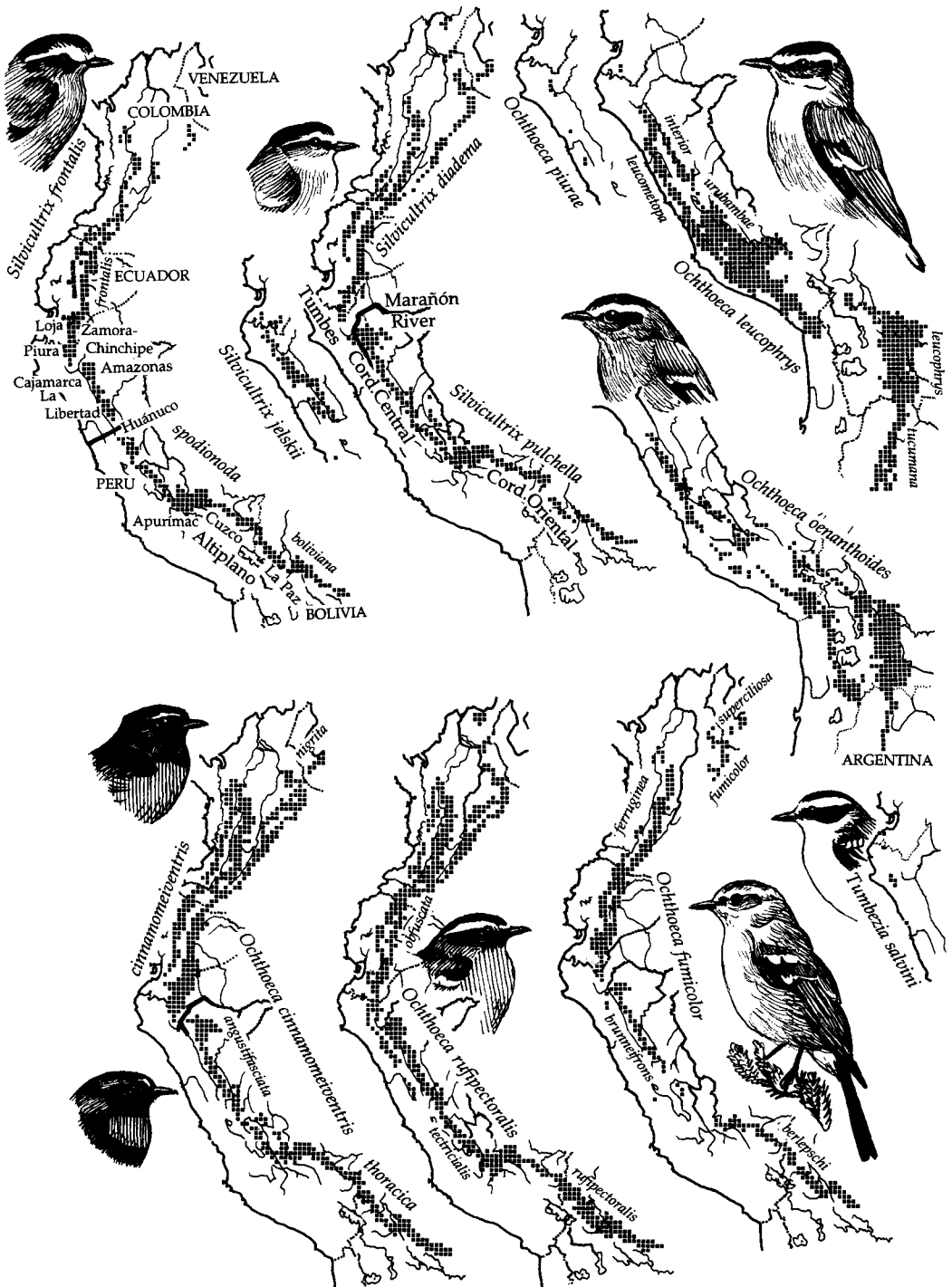


FIGURE 1. Distributions of *Silvicultrix*, *Ochthoeca*, and *Tumbesia* chat-tyrants. 15' × 15' grids (approx. 30 × 30 km), printed by J. FjeldsÅ from a database of the distributions of Andean birds. In order to reduce the sampling, it is assumed that the commoner species are continuously present between the collecting points in suitable habitat. This is defined from a large number of topographic maps and satellite images, and through comprehensive personal travels. Distribution gaps were made where there are negative records in seemingly

INTRODUCTION

Chat-tyrants are small New World flycatchers (Aves: Tyrannidae) distributed in the Andes from Venezuela and Colombia to northwestern Argentina (with one additional species in Patagonia). The 10 species (Ridgely and Tudor 1994) encompass all habitats from the interior of montane cloud-forest to forest edges, tree-line and montane scrub to puna terrain, with scattered rocks and emergent shrubs serving as perches for their aerial sallies. Many species are broadly sympatric along the length of the tropical Andes region, or a significant portion thereof (Fig. 1). However, each species inhabits a particular ecological zone and are thus segregated in different altitudinal zones or habitats. Fitzpatrick (1973) and Traylor (1985) explained their differentiation in terms of temporary isolations driven by large climatic-vegetational changes during the Pleistocene. A Plio/Pleistocene age for most Andean groups was suggested by Fjeldså (1994) using the DNA hybridization data produced by Sibley and Ahlquist (1990). Studies of such young radiations are well within the resolution limits for mitochondrial DNA sequence data used for constructing phylogenies (Edwards et al. 1991), and can contribute to our understanding of the process of diversification in tropical montane forests (see Roy et al. 1997).

Lanyon (1986) proposed the recognition of two genera of chat-tyrants (*Silvicultrix* and *Ochthoeca*), based upon differences in syringeal morphology. Within each group, individual species differ in their conspicuous light supercilia, which vary in shape and color, and in the color of the breast. Other characters, such as pigment saturation and the expression of wingbars, vary between subspecies or even amongst individuals in a population. This has caused considerable confusion over species-level taxonomy (Hellmayr 1927, Fitzpatrick 1973, Traylor 1977, 1979, 1985). Subspecies of currently recognized species may intergrade or replace each other sharply, often where the habitat is interrupted by deep valleys (Fitzpatrick 1973, Traylor 1985).

Detailed accounts of speciation events, based upon morphological data, have been proposed for the chat-tyrants as a whole (Fitzpatrick 1973) and for the *Silvicultrix* group (Traylor 1985).

The present study on chat-tyrants is included, together with several other groups (García-Moreno and Fjeldså, in press; García-Moreno et al., unpubl. data), in a broad study of the role of montane areas for the diversification of tropical avifaunas (see Roy et al. 1997 for a detailed description of the problem and the approach). In the present paper we (1) present a phylogenetic model for chat-tyrants using mtDNA sequence data, (2) compare this model with classifications based upon morphology, (3) interpret the evolution of the group in relation to levels of genetic divergence, degree of range overlap between species, and new knowledge about the Pleistocene ecoclimatic history, and (4) infer a sequence of adaptive shifts that took place during the diversification of chat-tyrants.

METHODS

Tissue samples were analyzed for 26 birds representing 9 species of *Ochthoeca* and *Silvicultrix*, and *Tumbezia salvini*, a Tumbesian lowland endemic considered to be related to chat-tyrants by Lanyon (1986) (but see Ridgely and Tudor 1994; sample from Lambayeque-Piura, Peru). We used samples of *O. cinnamomeiventris* from Pichincha and Zamora-Chinchi in Ecuador and Piura in Peru (ssp. *cinnamomeiventris*), and from Cuzco in Peru and La Paz, Bolivia (ssp. *thoracica*); *O. fumicolor* from Valle in Colombia and Zamora-Chinchi (ssp. *ferruginea*) and Cuzco (two samples, ssp. *berlepschi*); *O. rufipectoralis* from Carchi and Zamora-Chinchi in Ecuador (ssp. *obfuscata*), and Apurímac and Cuzco in Peru (ssp. *tectricialis*); and a sample each of *O. leucophrys* (Cuzco) and *O. oenanthoides* (Arequipa, Peru). *Silvicultrix* was represented by tissues of *S. jelskii* from Loja in Ecuador and Cajamarca in Peru; *S. diadema* from Loja and Carchi in Ecuador; *S. p. pulchella* from Cocapata, La Paz, Bolivia; *S. frontalis* from Pi-

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suitable habitat and, for genuinely rare and local species, where only point data were used. Names of geographical areas mentioned in the text are given mainly on the upper left map. A black thick line intersecting the map grid indicates proposed species barriers (between *S. frontalis* and *S. [f.] spodionota*, *S. diadema* and *S. pulchella*, and *O. cinnamomeiventris* and *O. [c.] thoracica*). Birds drawn by Jon Fjeldså.

chinchu and Zamora-Chinchipe (ssp. *frontalis*), and from Huánuco and Apurímac, Peru (ssp. *spodionota*). Tissues of *S. pulchella similis* from Pasco and Amazonas, Peru were obtained from the tissue collection of the Louisiana State University Museum of Natural Science, Baton Rouge, Louisiana. All other samples were from the Zoological Museum, University of Copenhagen, Copenhagen, Denmark. The only species of *Ochthoeca* (as recognized by Ridgley and Tudor, 1989) missing from the analysis was *O. piurae*, a Peruvian west-slope taxon believed to be closely related to *O. leucophrys* (Fitzpatrick 1985) (and the quite different-looking Patagonian *O. [Colorhamphus] parvirostris*). *Fluvicola nengeta* (Palmas de Monte Alto, Bahia, Brazil) was used as the outgroup (Lanyon 1986). Because a close relationship has been suggested between chat-tyrants and bush-tyrants (Smith and Vuilleumier 1971), we also made comparisons with *Myiotheretes fuscorufus*. This species was rejected as a possible outgroup because of a very high level of sequence divergence (see also Ames 1971, Lanyon 1986).

DNA was extracted from blood samples preserved in EDTA (Arctander 1988) or in DMSO (Arctander and FjeldsÅ 1994). Samples were washed and incubated in CTAB buffer (Doyle and Doyle 1987) at 65°C, and extracted with chloroform:isopentyl alcohol (24:1 volume:volume). The DNA was then precipitated with isopropanol, cleaned twice with ice cold ethanol, and finally dissolved in TE buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA).

A fragment of the mitochondrial ND2 gene was amplified by means of the polymerase chain reaction using primers L5215 and H5578 (Hackett 1996). Single stranded template DNA for the sequencing reactions was obtained by use of a biotinylated primer. The strand attached to the biotinylated primer was separated by means of Dynal® magnetic beads coated with streptavidin following the protocol of the supplier. Dideoxy sequencing was performed following the protocol for Sequenase® v. 2.0 (United States Biochemicals, Cleveland, Ohio).

Sequences were aligned by eye using the program SeqApp 1.9 (Gilbert 1992). We used the program CS3 (H. Siegismund, University of Copenhagen, unpubl.) to calculate the number of substitutions (total, transitions [ts], transversions [tv]) between homologous DNA sequences because it is known that paralogous mitochondrial

and nuclear sequences exhibit differences in their substitution patterns (Arctander 1995). To assess saturation of the sequences, i.e., more than one change occurring at a given position, we plotted the observed number of substitutions (*p* distances) vs. Kimura-2 parameter distances, which adjusts the raw estimate based upon differences in substitution rates between ts and tv. Deviation from the line $y = x$ was taken as indication that multiple substitutions at some positions had taken place (Burns 1997).

Phylogenetic analyses were carried out using Phylip 3.56 (Felsenstein 1996), PAUP 3.1.1 (Swofford 1991), and MEGA 1.01 (Kumar et al. 1993). Maximum-likelihood can become inconsistent, i.e., converge to a wrong tree, if one assumes rate homogeneity and the true evolutionary process exhibits rate variation (Swofford et al. 1996). Therefore, such analyses were performed accounting for three categories of substitution rates (which do not necessarily correspond to codon positions) with values 0.25, 1, and 3, and probabilities of 0.7, 0.25, and 0.05; these values were chosen to approximate a gamma distribution with a parameter of 0.5 (Yang 1996). Parsimony analysis was done assigning equal weights to all characters, up-weighting transversions five times over transitions, down-weighting third codon positions, and combining the last two regimes. Neighbor-joining was performed based upon different distance measures (*p*-distances, Kimura-2 parameter).

RESULTS

Three hundred and twenty base pairs of homologous positions of the mitochondrial ND2 gene were compared between nine species of *Silvicultrix* and *Ochthoeca* chat-tyrants and *Tumbezia salvini*, using *Fluvicola nengeta* as an outgroup. Of these base pairs, 102 were variable and 67 were informative concerning the relationships among chat-tyrants. Transitions (ts) outnumbered transversions (tv) in all pairwise comparisons (Table 1), with a range of 5–39 ts (including subspecies; mean \pm SD = 27.5 \pm 6.9) and 0–10 tv (4.4 \pm 2.2). Intraspecific variation was recorded for those species with more than one subspecies included (*O. cinnamomeiventris*, *O. fumicolor*, *O. rufipectoralis*, *S. frontalis*, and *S. pulchella*). In some cases, variation was detected even within subspecies, with a maximum of 5 ts (1.6%) between samples of *S. pulchella similis*. All sequence comparisons

among different taxa resulted in at least one aminoacid change, except for the pairs *O. rufipectoralis obfuscata*-*O. r. tectricialis*, and *O. oenanthoides*-*O. leucophrys*. Plots of observed substitutions vs. Kimura-2 parameter distance suggested multiple substitutions on third codon positions for most pairwise comparisons, and first codon position for pairwise comparisons between distant taxa. A sequence of *Myiotheretes fusciorufus*, which was intended as a further outgroup, showed a high level of substitutions at both the nucleotide level (Table 1) and the aminoacid level (21–25 substitutions).

Phylogenetic analyses with different algorithms using *Fluvicola* as an outgroup yielded trees with very similar topologies to the one depicted in Figure 2. The optimal trees found disagreed in three nodes: (1) *Tumbezia* was either basal to all the chat-tyrants or paired with *O. fumicolor* at the base of the tree, (2) the position of *O. rufipectoralis* varied with respect to the clade formed by *O. oenanthoides*-*O. leucophrys* (basal to it, derived from it, or sister to it), and (3) *S. pulchella pulchella* was grouped either with *S. p. similis*, or the most basal branch in the clade grouping members of the *S. diadema* group (*S. pulchella*, *S. jelskii*, and *S. diadema*). Using *Myiotheretes* as an outgroup did not alter the topologies of the trees, and *Fluvicola* remained basal to all chat-tyrants. None of the optimal topologies recovered with different algorithms was significantly better than the others according to the test of Kishino and Hasegawa (1989). Conversely, the topology where *Silvicultrix* and *Ochthoeca* were forced to be sister clades was considered a much worse hypothesis by the same test ($P < 0.001$).

We used divergence data (Table 1) to provide a relative timing of speciation events. We considered two mammalian cytochrome *b* rates (Irwin et al. 1991) and assumed that avian ND2 does not differ significantly from these rates (although Mindell et al. 1996 suggest that avian genomes may evolve at a slower rate than mammalian ones). The first rate was 10% third codon position substitutions per million years. This only was applicable to those comparisons that did not show signs of saturation for third codon positions; in our data set this corresponds to a third position Kimura distance ≤ 0.1 according to the substitutions vs. Kimura distance plots (not shown). For all other comparisons we used a rate of 0.5% third codon tv per million years.

However, this approach also is prone to errors because our estimations are based upon short sequence fragments (320 base pairs) and tv are relatively rare among closely related taxa. There are three comparisons which show third codon position saturation but zero or one tv (Table 1). We therefore tried to get a time estimate based upon an average mtDNA rate of 2% (Klicka and Zink 1997) assuming that ND2's rate does not differ significantly from that of the mtDNA as a whole. Our estimates using either a 2% rate or third position tv agree for the six comparisons where third positions were saturated and tv were present, but not for a comparison showing complete saturation (*Ochthoeca-Fluvicola*).

DISCUSSION

PHYLOGENETIC RELATIONSHIPS AMONG CHAT-TYRANTS

Phylogenetic analyses based upon 320 base pairs of the mitochondrial ND2 gene suggest that the chat-tyrants form a monophyletic assemblage when compared to *Fluvicola*. The molecular data support the monophyly of *Silvicultrix*, but not Lanyon's (1986) view that *Ochthoeca* and *Silvicultrix* are sister taxa.

Relationships between different *Ochthoeca* lineages are deep in the phylogeny and not completely resolved (Fig. 2, Table 1). Smith and Vuilleumier (1971) suggested a close relationship between *Ochthoeca* and *Tumbezia salvini*, and Lanyon (1986) included it in *Ochthoeca*. Our sequence data place this Tumbesian endemic either as the most basal taxon of the chat-tyrant assembly, or forming a basal lineage together with *O. fumicolor*. This latter view seems possible in view of the paucity of transversions between the two taxa (Table 1). In any case, *O. fumicolor* is the most basal among all those taxa which traditionally have been assigned to the chat-tyrants. It is somewhat disjunctly distributed in humid tree-line habitat (Fig. 1) and presents a punctuated genetic differentiation. Samples from Colombia and Ecuador (ssp. *brunneifrons*) gave identical sequences, but were different from those from Cuzco (ssp. *berlepschi*; uncorrected distance 0.030).

The associations between *O. rufipectoralis* and the clade formed by *O. leucophrys* (together with *O. piurae*) and *O. oenanthoides* are not completely clear. This is because all possible topologies appeared in our results depending upon the algorithm employed. Of these species, only

TABLE 1. Pairwise comparisons among chat-tyrant ND2 sequences. Kimura-2 parameter distances (above diagonal) and number of transitions (ts)/transversions (tv) (below diagonal).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|-------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 <i>S. diadema</i> | *** | 0.067 | 0.057 | 0.066 | 0.111 | 0.117 | 0.134 | 0.171 | 0.157 | 0.156 | 0.170 | 0.176 | 0.135 | 0.137 | 0.169 | 0.227 | 0.335 |
| 2 <i>S. jelskii</i> | 19/1 | *** | 0.057 | 0.059 | 0.088 | 0.100 | 0.134 | 0.153 | 0.148 | 0.138 | 0.131 | 0.149 | 0.110 | 0.119 | 0.143 | 0.199 | 0.347 |
| 3 <i>S. p. similis</i> | 16/1 | 15/2 | *** | 0.040 | 0.102 | 0.085 | 0.117 | 0.140 | 0.116 | 0.111 | 0.111 | 0.114 | 0.095 | 0.104 | 0.133 | 0.192 | 0.348 |
| 4 <i>S. p. pulchella</i> | 18/2 | 15/3 | 11/1 | *** | 0.088 | 0.081 | 0.106 | 0.133 | 0.119 | 0.114 | 0.119 | 0.137 | 0.123 | 0.124 | 0.135 | 0.190 | 0.358 |
| 5 <i>S. f. frontalis</i> | 30/2 | 23/3 | 26/3 | 22/4 | *** | 0.062 | 0.122 | 0.145 | 0.127 | 0.117 | 0.106 | 0.153 | 0.138 | 0.131 | 0.164 | 0.216 | 0.386 |
| 6 <i>S. f. spodiota</i> | 30/2 | 25/3 | 21/3 | 19/4 | 14/4 | *** | 0.108 | 0.135 | 0.100 | 0.096 | 0.119 | 0.143 | 0.128 | 0.124 | 0.148 | 0.192 | 0.358 |
| 7 <i>O. c. cinnamomeiventris</i> | 34/4 | 33/5 | 28/5 | 25/6 | 29/6 | 24/6 | *** | 0.056 | 0.111 | 0.106 | 0.123 | 0.138 | 0.140 | 0.133 | 0.150 | 0.193 | 0.366 |
| 8 <i>O. c. thoracica</i> | 41/6 | 36/7 | 32/7 | 30/8 | 33/8 | 29/8 | 15/2 | *** | 0.123 | 0.118 | 0.140 | 0.138 | 0.130 | 0.128 | 0.149 | 0.200 | 0.419 |
| 9 <i>O. r. obfusca</i> | 39/3 | 36/4 | 30/2 | 30/3 | 30/5 | 23/5 | 28/3 | 29/5 | *** | 0.017 | 0.088 | 0.084 | 0.112 | 0.085 | 0.114 | 0.164 | 0.345 |
| 10 <i>O. r. tectricialis</i> | 39/3 | 34/4 | 29/2 | 29/3 | 28/5 | 22/5 | 27/3 | 28/5 | 5/0 | *** | 0.092 | 0.095 | 0.100 | 0.081 | 0.093 | 0.155 | 0.353 |
| 11 <i>O. leucophrys</i> | 39/6 | 29/7 | 26/5 | 27/6 | 22/8 | 25/8 | 28/6 | 32/6 | 20/5 | 21/5 | *** | 0.044 | 0.108 | 0.100 | 0.135 | 0.186 | 0.386 |
| 12 <i>O. oenanthoides</i> | 39/8 | 32/9 | 25/7 | 30/8 | 32/10 | 29/10 | 32/6 | 32/6 | 19/5 | 22/5 | 11/2 | *** | 0.099 | 0.092 | 0.137 | 0.180 | 0.378 |
| 13 <i>O. fumicolor brunneifrons</i> | 33/4 | 26/5 | 24/3 | 30/4 | 32/6 | 29/6 | 36/2 | 32/4 | 30/1 | 27/1 | 26/4 | 24/4 | *** | 0.030 | 0.102 | 0.160 | 0.379 |
| 14 <i>O. fumicolor bertepschi</i> | 33/4 | 28/5 | 26/3 | 30/4 | 30/6 | 28/6 | 34/2 | 31/4 | 23/1 | 22/1 | 24/4 | 22/4 | 9/0 | *** | 0.087 | 0.144 | 0.355 |
| 15 <i>Tumbezia salvini</i> | 40/6 | 33/7 | 32/4 | 32/6 | 37/8 | 32/7 | 37/4 | 35/6 | 29/2 | 24/2 | 31/5 | 32/5 | 27/1 | 23/1 | *** | 0.168 | 0.363 |
| 16 <i>Fluiccola nengeta</i> | 48/12 | 41/13 | 40/11 | 40/12 | 44/14 | 38/12 | 42/10 | 42/12 | 35/9 | 33/9 | 37/12 | 36/12 | 35/8 | 31/8 | 36/10 | *** | 0.377 |
| 17 <i>Myiotheretes fuscus</i> | 50/32 | 52/32 | 53/31 | 54/32 | 57/34 | 54/30 | 52/36 | 59/38 | 51/32 | 52/33 | 55/38 | 52/38 | 56/33 | 52/31 | 54/34 | 55/34 | *** |

Boxed comparisons correspond to Lanyon's (1986) *Sibicultrix* (1-6) and *Ochthoeca* (7-15).

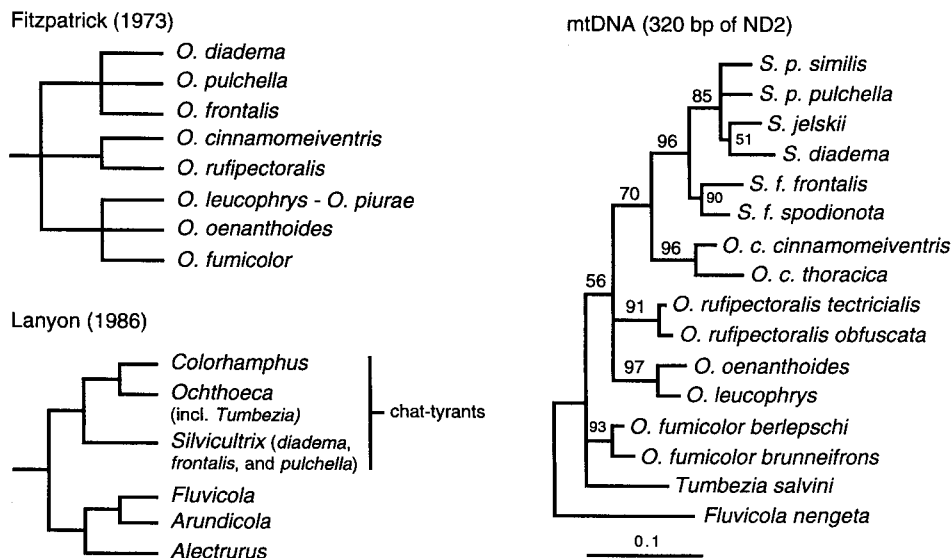


FIGURE 2. Left: Fitzpatrick's (above) and Lanyon's (below) hypotheses of the relationships among chat-tyrants. Notice that Fitzpatrick considered all chat-tyrants as one genus (*Ochthoeca*), whereas Lanyon split them into three genera. Right: Maximum likelihood tree obtained using 320 base pairs of the mitochondrial ND2 gene. Branches are drawn proportionally to the change along them. Figures above the branches represent percentage bootstrap values (under parsimony criterion) after 500 replicates. Branches with bootstrap value less than 50% have been collapsed. Optimal trees found with other algorithms shared the overall topology with the one depicted (see Results for details).

O. rufipectoralis was studied at the subspecific level. Contrary to the picture shown by *O. fucicolor*, the small differences detected between the haplotypes associated with each subspecies (0.016) could well be related to the 2,000 km separating the locations from which the different samples originated (Ecuador and Cuzco; Fig. 1).

The most recent *Ochthoeca* proper to branch out in the mtDNA phylogeny is *O. cinnamomeiventris*. It is the most basal taxon in a clade that also includes the species which Lanyon (1986) assembled in *Silvicultrix* (Fig. 2). The genetic differentiation between *cinnamomeiventris* and *thoracica* (0.053) is of the same magnitude as that between other sister species (e.g., 0.039–0.063 within the *S. diadema* group, 0.042 between *O. leucophrys* and *O. oenanthoides*; Table 1).

Lanyon (1986) split the genus *Silvicultrix* from *Ochthoeca* based upon peculiarities of the nest and on the nasal septum in *Silvicultrix*, as well as the presence of two derived syringeal characters absent in his *Ochthoeca* group. These characters can indeed be regarded as characteristic of the assemblage, because *Silvicultrix* also forms a monophyletic group according to mt-

DNA evidence, but the molecular data show that *Ochthoeca* is paraphyletic with respect to it (Fig. 2). Within Lanyon's (1986) *Silvicultrix*, the branching conforms well with Traylor's (1985) recognition of a *S. diadema* group and a *S. frontalis* group (Fig. 2). The former group comprises three forms which have been given species rank both by Traylor (1985) and Ridgely and Tudor (1994), namely *S. diadema*, *S. jelskii*, and *S. pulchella*. *S. diadema* is replaced by *S. pulchella* south and east of the Marañón River on the eastern Andean slope (Fig. 1), whereas *S. jelskii* inhabits the cloud-forest of southwestern Ecuador and northwestern Peru, where sharp parapatric replacements are found between all three species (Fig. 1). Traylor (1985) considered *S. jelskii* to be more related to *S. frontalis* than to *S. diadema*, but our analyses suggest the opposite (Fig. 2 and Table 1).

TAXONOMIC IMPLICATIONS

Because of the incomplete resolution of the phylogeny, we do not think that a sufficiently strong case exists for including *Tumbezia salvini* in *Ochthoeca* (as done by Lanyon 1986). Instead, we suggest maintaining it as a monobasic genus

in the chat-tyrant assembly. *Tumbezia salvini* shows some distinctive plumage and habitat characteristics not shared by the Andean chat-tyrants.

The large genetic differentiation between *Ochthoeca c. cinnamomeiventris* and *O. c. thoracica* presents a good case for elevating them to different species (unfortunately we lack genetic material to discuss the Venezuelan *O. c. nigrita* in this context). Different populations of *O. cinnamomeiventris* differ dramatically in plumage characteristics. Whereas the Venezuelan subspecies *nigrita* is completely slate-gray with a white eyebrow, the nominate subspecies is chestnut below the breast, and the southern subspecies *angustifasciata* and *thoracica* have only a pectoral chestnut bar (Fig. 1). Based upon plumage characters and the different vocalizations north and south of the Marañón River, Ridgely and Tudor (1994) suggest the possibility that more than one species are involved.

The two taxa of the *S. frontalis* clade differ at a genetic level comparable to those of fully recognized species, and of an order of magnitude greater than normally found in intergrading populations of Andean birds (García-Moreno and FjeldsÅ, in press; García-Moreno et al., unpubl. data). These two taxa replace each other in a similar way as *S. pulchella* replaces *S. diadema*, with an important difference. Although the replacement of *S. diadema* and *S. pulchella* corresponds to a physical barrier, the dry upper Marañón River, the replacement between *S. f. frontalis* and *S. f. spodionota* is farther south, in an area without obvious physical barriers or habitat discontinuities. Traylor (1985) identified a gap of 150 km between the southernmost *frontalis* in La Libertad and the northernmost *spodionota* a little north of the Huallaga Gap in Huánuco, and decided to treat them as a single species until information was obtained about how they interact in a zone of sympatry. This segment of the Cordillera Central remains poorly explored, thus the taxa are recognized currently as subspecies. However, the genetic differences between them (0.059, including 4 tv) is of a level comparable to that of fully recognized species (e.g., *S. diadema* and *S. jelskii*: 0.063, *O. leucophrys* and *O. oenanthoides*: 0.042; Table 1). Although we do not think that species status can be diagnosed solely on the grounds of a quantity of molecular or morphological divergence, we believe that the mtDNA divergence

together with biogeographic separation and plumage differentiation suggest that they are different species: *S. frontalis* (including subspecies *albidiadema* and *orientalis*) and *S. spodionota* (including subspecies *boliviana*). It should be noted that the southern taxa (*spodionota* and *boliviana*) are phenetically very similar to *S. jelskii*, whereas the northern *S. frontalis albidiadema* is characterized by the absence of wingbars and rufous fringes on the tertials (this could be a derived character state; however, a similar lack of wing-pattern also is found within *S. spodionota* in western Cuzco and Ayacucho).

SPECIATION, SEQUENCE OF EVENTS, AND ECOLOGICAL PHYLOGENETICS

Fitzpatrick (1973) and Traylor (1985) proposed detailed sequences of events (isolation and dispersal) related to the Pleistocene ecoclimatic changes. However, Fitzpatrick referred to an outdated climatic model (see Roberts 1992), whereas Traylor adapted Fitzpatrick's model to the group discussed by him (*Silvicultrix*). Unfortunately, their suggested time-scales are not in agreement with the divergence at the molecular level (Table 1).

Divergence data (Table 1) can be used to provide a relative timing of speciation events. Because many Andean bird groups have been postulated to be of a Plio/Pleistocene age (FjeldsÅ 1994), it would be interesting to determine whether chat-tyrants conform to this pattern. However, although it is useful to add a time framework for the discussion, date estimations from molecular data alone are problematic. There are several difficulties with assuming a molecular clock, such as nonlinearity or unequal evolutionary rates between lineages (Hillis et al. 1996). Our data cover a large time window and thus comparisons among sequences cover the whole range of saturation, from unsaturated to extensively saturated (and fully saturated for third codon positions). Because of this, it would be wrong to use a "universal" evolutionary rate for all comparisons. We therefore used different rates for estimating times according to the level of saturation shown by the sequences of the taxa in question.

We estimated *Ochthoeca* to have separated from the *Fluvicola* group between 10 and 20 million years ago (mya). This corresponds well with the extensive mountain building in the Andes during the upper Miocene (Coltrinari 1993).

The deep branches of *Ochthoeca* have an age of 4–8 mya, which corresponds to the Miocene/Pliocene transition. We estimated that *Tumbezia* and *O. fumicolor* split around 5 mya, but there are no tv between the sequences, which is a typical feature of closely related taxa. Even the split between *O. cinnamomeiventris* and the *Silvicultrix* lineage might have taken place during the Miocene/Pliocene transition, whereas the *S. frontalis* and *S. diadema* lineages apparently already diverged in the Pliocene. We reckon that the most recent speciation events, those leading to the tips of the different lineages in both *Ochthoeca* and *Silvicultrix*, would have taken place in the Pleistocene, some perhaps as recently as 0.5 mya.

It is now well documented that the entire geological period discussed here has been characterized by global cooling. However, the transition from a time with a high frequency of very brief cold events to a time with large climatic cycles involving long glacial periods happened as recently as 800,000 years ago (Hooghiemstra 1993, Hooghiemstra and Ran 1994). These cycles can be associated with changes in the earth's orbital eccentricity around the sun, which varies with a period of 100,000 years (Bartlein and Prentice 1989). Thus, the molecular data place the speciation in chat-tyrants well before the period of major ecoclimatic changes. Although the increased ecoclimatic instability in the Pleistocene necessarily caused high levels of vegetational changes, this apparently was not a significant cause of speciation in chat-tyrants (assuming that no extensive extinction episodes have occurred). Perhaps the qualitative and quantitative vegetational changes during the Pleistocene (Colinvaux 1993, Hooghiemstra and Cleef 1995) provided opportunities for redistribution of common Andean birds, and circumvention of deep valleys which today act as barriers. Such changes may have led to a situation in which it is possible to find up to four different chat-tyrants syntopically (Traylor 1985). Low levels of endemism and broad overlap among many congeneric species is a characteristic feature of New World flycatchers compared with other large South American bird families (Fjelds  and Rahbek, in press).

Because most chat-tyrant species groups have more or less filled up a certain ecological zone (Fig. 1), biogeographic signals which could have related individual speciation events to specific

areas or ecoclimatic events have been lost. DNA studies on other Andean groups (Patton and Smith 1992, Arctander and Fjelds  1994) showed that species which are segregated in different altitudinal zones of a single Andean slope originated by isolation on different slopes. Unfortunately, because of the large distribution ranges and the small number of samples per chat-tyrant species, we have no firm evidence for tracing the population structure and patterns of redistribution in individual species.

As a result of the long time for redistribution, we cannot tell whether the initial speciation events are related to topographic barriers formed during mountain building, or to bottleneck effects in relation to ecoclimatic instability (Vrba 1985, Carson 1990, Walter 1995) and high risks of fragmentation of populations inhabiting a narrow habitat band (Graves 1988). However, a detailed review of the morphological variation illustrates the role of random changes in local populations. As an example, the populations of *S. (f.) spodionota* in western Cuzco and adjacent Ayacucho lack the wing-bars and patterning of the tertials that otherwise distinguish *spodionota* from *frontalis* (Traylor 1985). Such variation resembles the drastic changes in pigment saturations among, for example, *Atlapetes* brush-finches (Remsen 1993, Garc a-Moreno and Fjelds , in press) and *Cranioleuca* spinetails (Garc a-Moreno et al., unpubl. data). A dynamic scenario of local isolation and dispersal would explain why the most marked geographical barrier in the Andes, the Mara on Valley, is reflected only in the differentiation of *S. diadema/pulchella* and *O. cinnamomeiventris/thoracica*. Other species (*S. frontalis/spodionota*, *S. fumicolor brunneifrons/berlepschi*) are distributed across this gap with the principal genetic dichotomy farther to the south, in an area without any major topographic features. The Mara on Gap is an erosion gap which predates the uplift of the eastern Andean ridge (R s nen et al. 1993), and therefore represents a barrier which is too old to explain many of the taxon displacements. The total range overlap between the *S. frontalis* and *S. diadema* groups, and between *O. cinnamomeiventris*, *O. rufipectoralis*, and *O. fumicolor* (Fig. 1), indicates that initial speciation events were followed by range expansions along the entire length of the tropical Andes region. A dynamic scenario would be needed to explain the extensive sympatry of *O. leucophrys* and *O. oenan-*

thoides (Fig. 1). We suggest that sharp replacements of taxa which are not yet fully compatible are most easily maintained where there is a physical barrier, although this does not necessarily mean that this barrier was the initial cause of isolation and divergence of taxa (Vuilleumier 1969, 1977, O'Neill 1992).

The northwest Peruvian (Tumbes) region is particularly interesting, because the three endemic chat-tyrants inhabiting this region (Fig. 1) represent different lineages (Fig. 2). *Tumbezia salvini*, known from a few lowland sites, may be interpreted as a geographic relict which could well date back to the period in the upper Miocene when the Andean uplift blocked the Amazonian drainage into the Pacific Ocean. *O. piurae* (for which we lacked genetic material) is a very local species considered to be close to *O. leucophrys*. *S. jelskii* is widespread in the western Andes and in mountains fringing the upper Marañón Valley, where it inhabits similar closed humid forests as its east slope relatives. According to the analysis of Best and Kessler (1995), the Tumbes region retained relict taxa of a wide range of biogeographic origins and on many different time scales.

ADAPTIVE TENDENCIES

By using phylogeny (Fig. 2) and habitat data (FjeldsÅ and Krabbe 1990, Ridgely and Tudor 1994), we can comment on the ecological phylogeny of chat-tyrants (because of the limited number of taxa, we have not employed existing computer software). According to Lanyon (1986), the sister group of chat-tyrants comprise the genera *Fluvicola*, *Arundicola*, and *Alecturus*. All inhabit marshy and shrubby vegetation in tropical lowlands. *T. salvini* also is a lowland bird, adapted to *Acacia*-like scrub and mesquite bushes along seasonal streams. We therefore assume a lowland ancestry for *Ochthoeca*. Following the uplift of the tropical Andes and the isolation on the Pacific slope, the chat-tyrants colonized highlands with scattered bushy vegetation, ranging from dry bushy slopes (*O. leucophrys* and *O. piurae*), rocky places and *Polytepis* patches in the puna zone up to 4,500 m (*O. oenanthoides*) to humid bushy páramo vegetation and edges of elfin forests (*O. fumicolor*). Habitat data suggest that the adaptive zone of *O. leucophrys*, *O. piurae*, and *O. oenanthoides* pre-dates that of *O. rufipectoralis*, which is a bird of bushy areas and broken canopies in the humid

montane forest. This adaptive zone, in turn, would be basal to that of *O. cinnamomeiventris*, which inhabits shady forest edges in stream ravines, and *Silvicultrix*, which inhabits the dark interior of the montane forest and bamboo habitat, with *S. diadema*, *S. jelskii*, and *S. pulchella* adapting to lower montane and submontane forests.

Thus, as the chat-tyrants had colonized and filled up the rather barren highland which developed in the early Pleistocene (Hooghiemstra and Cleef 1995), new species adapted to progressively denser and more humid vegetation and lower altitudinal zones. Our study does not support the view of a particularly recent diversification among chat-tyrants, because several branches in our phylogeny extend back in time well beyond the Pleistocene. In order to resolve the initial speciation events, a much more fine scaled sampling scheme is needed.

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