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PIGMENTATION CHARACTERS: AN EXAMPLE OF SANKOFF CHARACTERS FROM TINAMOU PHYLOGENY

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Resumen. – Caracteres de pigmentación: ejemplos de caracteres de Sankoff en una filogenia de perdices. – En un análisis cladístico de perdices, se prestó especial atención a la definición y codificación de caracteres tradicionalmente cuestionados para la filogenia en aves. Por ejemplo, se definieron costos de transformación entre estados (caracteres de Sankoff) para analizar los colores de los huevos en base a la composición de los pigmentos. En este esquema, costos menores fueron asignados a los cambios entre estados más similares. Este criterio es parecido al aplicado en el caso de los caracteres de plumaje (superposición de patrones de pigmentación) en un estudio anterior. El presente estudio muestra que los cambios obtenidos en los caracteres de pigmentación no han sido una consecuencia de la penalidad impuesta en el régimen de costos sino de su interacción con otros caracteres. Esto es interpretado como evidencia que los caracteres multiestados con costos complejos entre sus estados (como en el caso de los caracteres de pigmentación) no son un artefacto y deberían ser usados para describir grados relativos de similitud.

Abstract. – In a cladistic analysis of tinamous, special attention was paid to character definition and scoring of non-traditional characters for phylogenetic reconstruction of birds. For example, transformation costs between states were applied (Sankoff characters) to analyze the egg coloration on the basis of pigment composition. In this scheme, lesser costs were assigned to transformations between more similar states. This approach is similar to that applied to plumage characters (overlap of pigmentation patterns) in a previous study. The present study shows that changes between several states of pigmentation characters are not a consequence of the imposed penalty in the cost regime, but an interaction with other characters. This is interpreted as evidence that multistate characters with complex costs among characters states (as in pigmentation characters) are not an artifact and should be used to describe relative degrees of similarity.
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Key words: Morphology, cladistics, Sankoff characters, Tinamidae.

INTRODUCTION

It has been traditionally accepted that integumentary and behavioral characters are reliable only for alpha taxonomy of birds, but that they cannot be trusted for their systematic value to establish relationships at higher taxonomic levels (e.g., Lowe, 1915, Atz, 1970, Hodos, 1976). Chu (1998) was one defendant of the use of integumentary characters in ornithological systematic. A more recent example of the phylogenetic value of integumentary characters is the first phylogenetic analysis of Tinamidae based on integumentary characters of Bertelli *et al.* (2002).

Behavioral characters (songs, nidification, etc.) could be as informative as morphological characters (de Queiroz & Wimberger 1993, Paterson *et al.* 1995, Irwin 1996, Kennedy *et al.* 1996, Zyskowski & Prum 1999). To have a robust reconstruction of the phylogeny of tinamous, and to test the hypothesis obtained

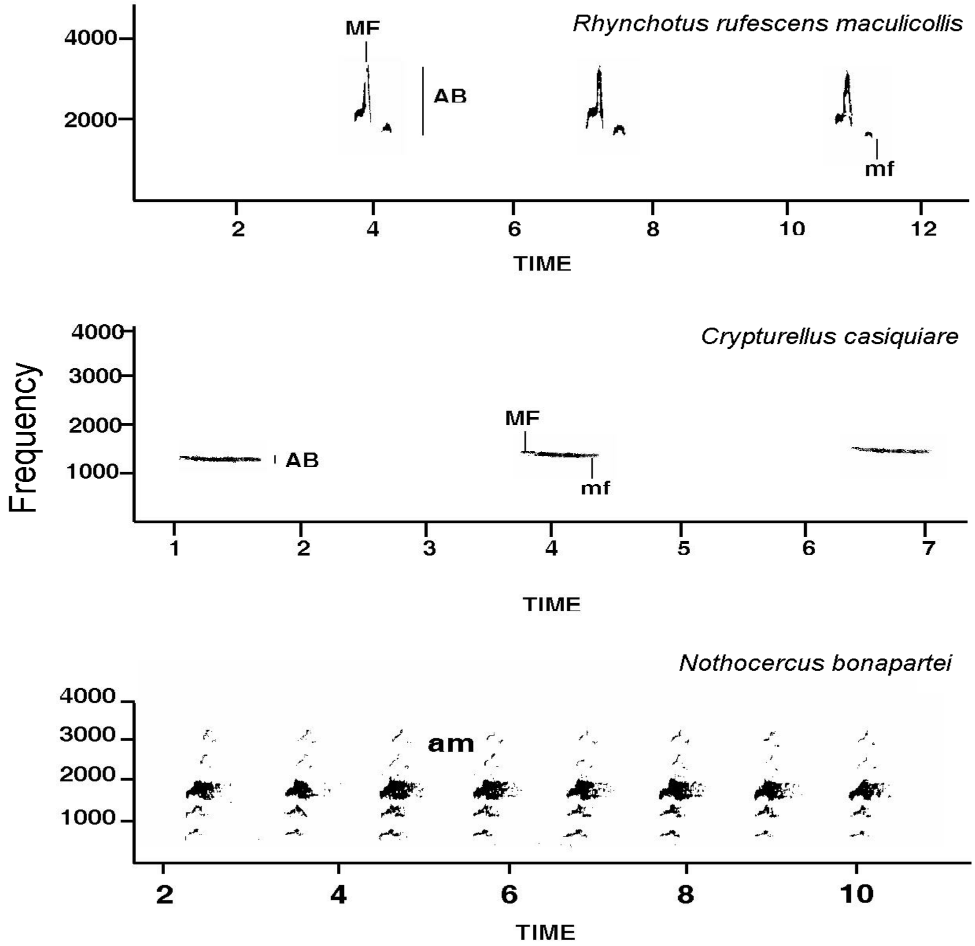


FIG. 1. Songs of *Rhynchotus rufescens maculicollis* and *Crypturellus casiquiare* (showing two character states: modulated frequency (*R. r. maculicollis*) and constant frequency (*C. casiquiare*). Song of *Nothocercus bonapartei* showing another example of coded variation in tinamous: presence of armonics. Abbreviations: MF = Maximum frequency, mf = minimum frequency, AB = range (AB), and am = armonics.

by using integumentary characters, additional types of evidence, such as characters of internal anatomy (osteology and myology) and of behavior and reproduction were included in a comprehensive cladistic analysis by Bertelli (2002).

While tinamou relationships were analyzed in Bertelli (2002), the focus of the current paper will be on character definition and scoring of non-traditional characters (e.g., reproductive, integumentary, behavioral characters). Most of these are multistate pigmentation characters in which several conditions are related in complex form. The use of generalized (Sankoff) parsimony to analyze these characters is reported herein. This was assessed in the context of the phylogenetic analysis by comparing the optimizations derived from alternative methods with characters under generalized parsimony and without Sankoff matrices. Therefore, the consequences of applying transformation costs to multistate characters with complex costs among characters states were evaluated.

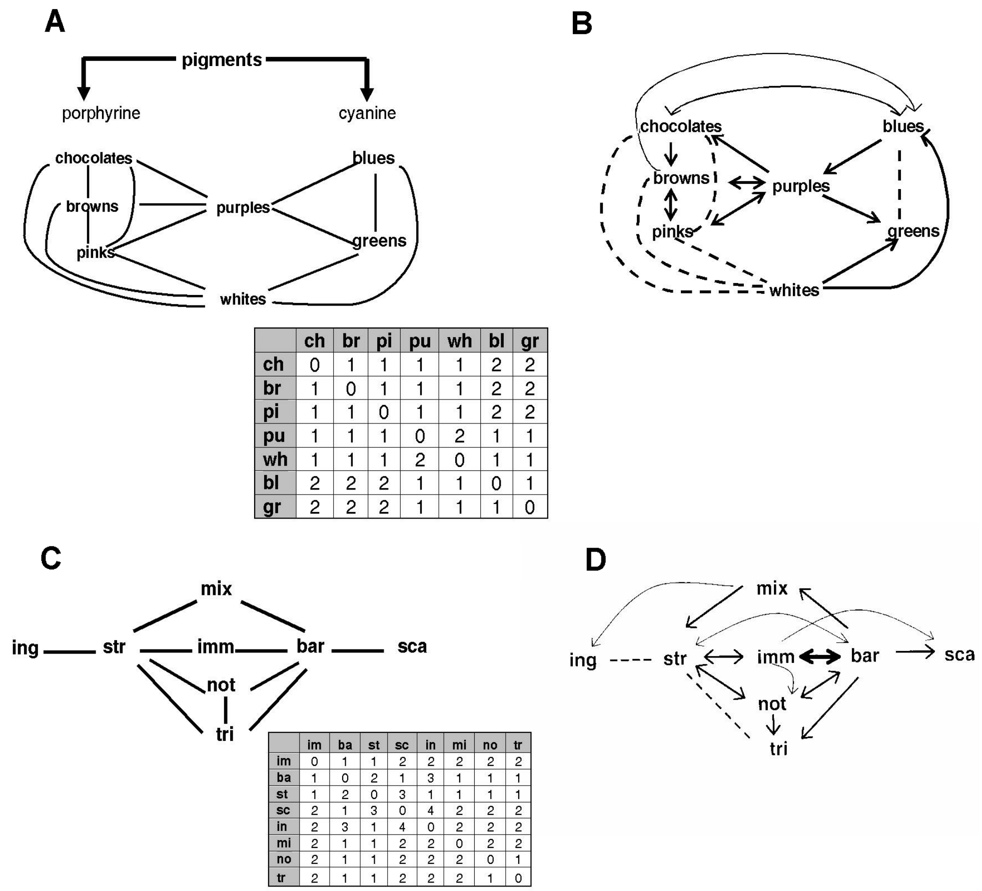
METHODS

The data set used in the phylogenetic analysis of Bertelli (2002) included 148 characters of internal anatomy (osteology and myology) and non-traditional characters derived from two different sources: 80 integumentary characters contributed by Bertelli *et al.* (2002), and 9 characters of behavior and reproduction (Salvadori 1895, Oates 1901, Miranda-Ribeiro 1938, Blake 1977, Sick 1985, de la Peña 1987, Fjelså & Krabbe 1990, Cabot 1992, Hardy *et al.* 1993). Ethological characters were based on the acoustic structure of the song and nesting behavior. Songs are combinations of fundamental sounds and their structure is defined by quantitative (maximum and minimum frequency, range, etc.) and qualitative variables (syntactic structure, acoustics, modulation, etc.) that characterize each type of song (Fig. 1). Therefore, songs have vocal characters in their structure that are comparable across bird species (McCracken & Sheldon 1997). Nest structures is the record of a behavior and so is a part of the phenotype of the birds (Collias 1986, Hansen 1979) and several studies have demonstrated that nest characters could provide evidence of phylogenetic relationships (Kiff 1977, Lanyon 1986, Prum 1993, Whitney *et al.* 1996).

Characters analysis. Characters were selected from the comparative study of Tinamidae and related birds (ratites), considering the morphological variation observed in attributes primarily homologous. Topographical and morphological correspondence was used to postulate the structural identity of attributes (primary homology *sensu* Pinna 1991). The morphological variation is represented in several conditions that constitute character states. Most of the characters have two alternative conditions (binary characters), but other characters have up to seven states. In these cases, the costs of transforming from one state to another were considered identical (characters not ordered) or different (ordered), according to established approaches (see below).

In additive characters, the order of the series of transformation determined the cost between each pair of states. Character ordering sometimes does not conform to a lineal structure, but to a ramified one (e.g., some myology characters). In those cases, the variation was recodified into two or more characters, such that the costs between the transformations express the magnitudes of the observed differences (using non-redundant linear coding, Mickevich 1982).

In more complex cases step matrices are applied (using generalized parsimony, Sankoff & Rosseau 1975), in which the cost of transformation between any two states is represented as a function of the observed similarity between states. This may include asymmetric costs, in which the transformation in one direction (e.g., from state A to B) does not cost the same as the inverse (from B to A). The criteria used here to determine the relative transformation costs is purely observational and does not involve evolutionary assumptions (Lipscomb 1992). In the step matrix, the transformations that are less costly are favored and occur between most similar states. Other pathways are relatively penalized but not prohibited. In this way the transformations between similar states have a lower cost than those between radically different states, which will reflect better the relationships between observation and phylogeny



	ch	br	pi	pu	wh	bl	gr
ch	0	1	1	1	1	2	2
br	1	0	1	1	1	2	2
pi	1	1	0	1	1	2	2
pu	1	1	1	0	2	1	1
wh	1	1	1	2	0	1	1
bl	2	2	2	1	1	0	1
gr	2	2	2	1	1	1	0

	im	ba	st	sc	in	mi	no	tr
im	0	1	1	2	2	2	2	2
ba	1	0	2	1	3	1	1	1
st	1	2	0	3	1	1	1	1
sc	2	1	3	0	4	2	2	2
in	2	3	1	4	0	2	2	2
mi	2	1	1	2	2	0	2	2
no	2	1	1	2	2	2	0	1
tr	2	1	1	2	2	2	1	0

FIG 2. A) Transformations among characters states in eggs colors: each direct connection represents one step between the two character states under the pigment composition criterion (modified from Bertelli 2002); B) Transformations among character states in adult plumage: each direct connection represents one step between the two character states under the overlap criterion (modified from Bertelli *et al.* 2002); C) Transitions implied by the results of the analysis with no step matrix characters in egg colors (modified from Bertelli 2002); and D) adult plumage (modified from Bertelli *et al.* 2002). Straight solid lines indicates the more similar states; thin curved lines indicate transitions between less similar states; dashed lines indicate transitions between similar states that never occur; arrows indicate direction of transformations. Abbreviations for 2C and 2D: bar or ba = bicolored barred, imm or im = immaculate, ing or in =ingoufi-like, mix or mi = mixed, not or no = nothura-like, sca or sc = scalloped, str or st = streaked, and tri or tr = tricolored barred. Abbreviations for 2A: bl = blue, br = brown, ch = chocolate, gr = green, pi = pink, pu = purple, wh = white.

reconstruction (relative degrees of homology by Lipscomb 1992).

This type of character analysis was applied in the study of egg color. The tinamous present a great variety of colors that can be grouped into seven main types: blues, greens, purples, chocolates, browns, pinks, and whites. According to Völker (*fide* Makatash

1952), egg colors are of two main types: pigments derived from hemoglobin (porphyrine) that are responsible for the brown and red colors and those derived from bile or decomposed products of hemoglobin (cyanine) that are responsible for the blue and green colors. Transformation costs between the main colors were assigned based on this classification so that transformations between most similar colors (based on the type of predominant pigments: cyanines or porphyrines) were less costly (see Fig. 2A).

Sankoff step matrices were also applied to the analysis of feather design (Bertelli *et al.* 2002). A feather pattern can be seen as several distinct elements superimposed and the complexity of feather patterns was analyzed by an overlap criterion of pigmentation. Three main feather designs were identified: the first one included transverse patterns, called "bicolored barred". The second main pattern, "streaked," corresponded to a longitudinal stripe of varying width along the sides of the rachis (this does not include the rachis itself, which is always darker). The third main pattern was "immaculate". The situation becomes more complex when other patterns are considered (Fig. 2C; see Bertelli *et al.* 2002).

Cladistic analysis. The program SPA (Goloboff 1996) was used because it permits the treatment of characters under generalized parsimony. An analysis without Sankoff matrices (carried out only for comparative purposes) was performed using NONA (Goloboff 1993) (see Bertelli 2002). This program was used to assess the consequences of applying equal transformation costs to those characters in which the states are related in complex form. The trees produced in the alternative (NONA) analysis were used to examine the transformations in the characters of plumage and egg coloration characters without step-matrices (i.e., considering these characters with unweighted tree states). In this way it could be determined whether the penalized transformations not obtained under Sankoff analysis (being hypothesized as less related to each other) were obtained when such cost was eliminated.

RESULTS AND DISCUSSION

The levels of homoplasy of the osteology + myology data set (Ci 0.48) are similar to those of the integument + behavior data set (Ci 0.44) although the number of taxa is lower (45 taxa in the first and 54 in the second). This value is basically maintained in the total analysis; for the shortest tree including the 54 terminals, the consistency index is 0.46 (see Bertelli 2002).

These results demonstrate that behavioral and integumentary characters have similar levels of homoplasy in relation to those of internal anatomy. When these types of characters were analyzed separately, both types of data presented similar degrees of homoplasy contradicting the perception among ornithological systematists that integumentary features are too labile to be historically informative (see Chu 1998).

Few direct transformations between less similar states were observed in the unweighted analysis (Figs 2B and 2D) and most of the transformations pertained to changes between similar states (i.e., adjacent in the state tree or separated by one intermediate state in the tree). This means that the transformations that were penalized under generalized parsimony (by being less related in the pattern of feather design or the type of egg shell color) were rarely obtained under equal costs. The same changes were also observed, with approximately the same frequency, in the Sankoff optimal trees. This is interpreted as evidence that the reduced number of transformations between several states is not a consequence of the imposed penalty but an interaction with other characters.

The results of an analysis in which all state transformations are equally likely suggest that transformations costs, as observed and coded in step matrices are not artifacts, and that transformations indeed occurred more frequently between the most similar states. Some authors have strongly advocated the use of non-additive characters because they argue that assigning costs to some transformations implies strong evolutionary assumptions (e.g., Scotland & Williams 1993). In contrast, Lipscomb (1992) suggests that the information on relative degrees of similarity, whenever available, should be used to decide relative costs between different states in the same manner as it is used to decide primary homology. Results of this analysis show that using Sankoff cost matrices representing more complex observations such as overlap in feather design analysis and egg pigment composition results in a better fit between the observations and the transformations among more similar states implied in the cladogram.

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