

Metachromism and the insight of Wilfred Osgood: evidence of common ancestry for Darwin's fox and the Sechura fox

Metacromismo y la perspicacia de Wilfred Osgood: evidencia de
ancestro común entre el zorro chilote y el zorro de Sechura

CHRISTOPHER J. YAHNKE

Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115, U.S.A.

ABSTRACT

Darwin's fox (*Pseudalopex fulvipes*) is one of the least known canids in South America. Wilfred Osgood was one of the first investigators to address the taxonomic relationship of Darwin's fox to its congeners. This fox was thought to be restricted to the island of Chiloé; however, a population was recently reported on the mainland in Nahuelbuta National Park. The current taxonomic designation of Darwin's fox as a subspecies of the chilla fox (*P. griseus*) does not consider the relevance of this mainland population. One of the initial characters used to determine the presence of Darwin's fox on the mainland was pelage color, since Darwin's fox is darker than both the chilla fox and the culpeo fox (*P. culpaeus*). Analysis of pelage characters was used to determine its effectiveness in generating phylogenetic hypotheses. This analysis suggests a sister group relationship between Darwin's fox and the Sechura fox (*P. sechurae*), which is consistent with observations made by Wilfred Osgood in 1943.

Key words: metachromism, systematics, *Pseudalopex fulvipes*, Chile

RESUMEN

El zorro chilote (*Pseudalopex fulvipes*) es uno de los cánidos menos conocido en Sudamérica. Wilfred Osgood fue uno de los primeros investigadores de las relaciones taxonómicas entre el zorro chilote y sus congéneres. Este zorro se creía restringido a la Isla de Chiloé, aunque se descubrió una población continental en el Parque Nacional Nahuelbuta. La presente designación taxonómica del zorro chilote como una subespecie de la chilla (*P. griseus*) no considera la pertinencia de esta población continental. Uno de los caracteres iniciales que se usó para determinar la presencia del zorro chilote sobre el continente fue el color de la piel, porque el zorro chilote es más oscuro que la chilla y también que el culpeo (*P. culpaeus*). El análisis de los caracteres de la piel se usó para determinar su eficiencia en la generación de hipótesis filogenéticas. Este análisis sugiere una relación entre el zorro chilote y el zorro de Sechura (*P. sechurae*), consistente con las observaciones de Wilfred Osgood en 1943.

Palabras clave: metacromismo, sistemática, *Pseudalopex fulvipes*, Chile.

INTRODUCTION

In 1923 Wilfred Osgood of the Field Museum of Natural History in Chicago collected two specimens of Darwin's fox (*Pseudalopex fulvipes*). These specimens were only the third and fourth known examples of Darwin's fox. This fox had been discovered by Charles Darwin, who obtained a specimen on December 6, 1834, near the mouth of San Pedro channel on the southern end of Chiloé Island (Darwin 1839). Another fox was examined at a museum in Puerto Montt by Phillippi (1869). Neither Darwin nor

Phillipi addressed the relationship of Darwin's fox to its mainland congeners, whereas this relationship perplexed Osgood. Although Darwin's fox is currently recognized as a subspecies of *Pseudalopex griseus* (see Langguth 1969, Clutton-Brock et al. 1976, Wozencraft 1993), Osgood (1943) considered Darwin's fox to be a different species from *P. griseus* (see also Cabrera 1958), and recognized similarities between Darwin's fox and the Sechura fox (*P. sechurae*). In *The Mammals of Chile*, Osgood (1943: 73) states that "In addition to its rather marked peculiarities of color,

fulvipes has cranial characters which distinguish it quite sharply from mainland forms. As compared with that of *domeykoanus* or *maullinicus*, the skull is much shorter and broader in the facial region, the audital bullae are decidedly less inflated, the dentition is slightly heavier, the occlusion of the premolars is more nearly complete, and the angle of the mandible is much deeper and heavier. Some of these characters are at least partially repeated in *sechurae* of northern Peru, which, like *fulvipes*, is a beach fox, whereas *griseus* is mainly a plains animal. It is possible, therefore, to speculate as to a former connection of *fulvipes* with *sechurae* rather than with *griseus*."

The idea that *P. fulvipes* and *P. griseus* were sister taxa was based on biogeography. However, Osgood (1943: 75) recognized the potential for finding mainland populations of *fulvipes*, stating that "In view of its geographical position and its agreement in most general features with *griseus* and subspecies, the conclusion that *fulvipes* is an offshoot of the *griseus* group is certainly the most natural and logical one. However, since it is so well characterized and since the distribution apparently is limited to the southern end of Chiloé Island, its status as a species perhaps should not be disturbed. It is to be remembered, nevertheless, that the mainland coasts in the latitude of Chiloé are practically unexplored zoologically and if foxes should be found there it is not unlikely that they might be nearer to *fulvipes* than is *maullinicus*, in which the approach to *fulvipes* is very slight."

Until recently, the range of Darwin's fox was thought to be restricted to the island of Chiloé. However, Medel et al. (1990) reported a population of Darwin's fox in Parque Nacional Nahuelbuta on the mainland of Chile, 600 km north of the nearest known record on Isla Chiloé (also see Jaksic et al. 1990, Jiménez et al. 1991). Pelage color was one of the characters used to differentiate Darwin's fox from *P. griseus* in Nahuelbuta. Hershkovitz (1967) proposed a method-metachromatic analysis- which used pelage color as a character to distinguish between taxa (see also Hershkovitz 1970, and critique by Lawlor 1969). Hershkovitz (1967) noticed that chromatic variation in a given race (i.e.,

subspecies) began with agouti in some part, or chromogenetic field, of the pelage. The agouti color pattern of alternating color bands on individual hairs then passes through progressive steps to black or brown, then possibly through tones of red, orange, yellow, cream, and finally white. Hershkovitz (1967) postulated that saturation and bleaching of agouti hair occurred through eumelanin and pheomelanin pathways, and that the chromatic succession was both geographic and phylogenetic. Metachromatic analysis is relatively inexpensive, non-destructive, and requires no sophisticated equipment. Furthermore, its character states are inherently ordered allowing the investigator to root cladograms by using hypothetical outgroups. Although very few studies have utilized this technique (see Hershkovitz 1967, 1977), I attempt to show its potential usefulness in inferring phylogenetic relationships. I analyzed the tegumentary pigments of Chilean foxes to determine their potential value in differentiating taxa, in particular between subspecies of *P. griseus*, as well as between species within the genus *Pseudalopex*.

MATERIALS AND METHODS

Individual study skins representing the various subspecies of *Pseudalopex griseus*, including the individuals of *P. fulvipes* collected by Osgood in 1923, were analyzed at the Field Museum of Natural History. Study skins of *P. sechurae*, *P. culpaeus*, and *P. gymnocercus* were also analyzed. A total of 13 skins were examined for this study (Table 1). The geographic range of these collecting sites extended from Peru to Tierra del Fuego (Fig. 1). Individual hairs were scored for the presence of eumelanin, pheomelanin (or both or neither) in ten chromogenetic fields (see Hershkovitz 1967). These fields were chosen to reflect the range of natural color patterns seen in this genus. The fields chosen were: (1) forehead, (2) cheek, (3) throat, (4) shoulder, (5) mid-dorsal, (6) mid-flank, (7) ventral, (8) base of tail, (9) terminus of tail, and (10) rear thigh (numbers correspond to those in Table 1). Representative hairs were chosen in each

TABLE 1

Character matrix showing individual scores of tegumentary pigments in 10 chromogenetic fields (see Methods for further explanation)

Matriz de caracteres mostrando los puntajes individuales de los pigmentos tegumentarios en 10 campos cromogenéticos (ver Métodos para mayor explicación)

Taxa	Collection locality	Chromogenetic field									
		1	2	3	4	5	6	7	8	9	10
<i>P. fulvipes</i>	Chiloé Island	1	3	2	1	2	2	1	2	2	
<i>P. fulvipes</i>	Chiloé Island	1	3	2	1	2	3	1	1	2	2
<i>P. griseus domeykoanus</i>	Limache, Valparaiso	1	3	3	3	3	3	2	3	3	
<i>P. griseus domeykoanus</i>	Limache, Valparaiso	3	3	2	2	3	3	1	3	3	
<i>P. griseus maullinicus</i>	Curacautín, Malleco	3	3	1	2	3	1	1	1	3	
<i>P. griseus maullinicus</i>	Riñihue, Valdivia	3	3	3	2	3	3	1	3	3	
<i>P. griseus</i>	Tierra del Fuego	3	3	3	3	3	3	2	3	3	
<i>P. culpaeus culpaeus</i>	Los Agustinos, Valparaiso	1	3	2	3	3	2	2	3	3	
<i>P. culpaeus andinus</i>	San Pedro, Antofagasta	3	3	1	3	3	3	1	3	3	
<i>P. sechurae</i>	Trujillo, Peru	1	3	2	2	3	1	3	3	3	
<i>P. sechurae</i>	Trujillo, Peru	1	3	2	1	2	3	1	3	3	
<i>P. gymnocercus</i>	Rio Negro, Uruguay	3	4	3	3	3	3	3	3	1	3
<i>P. gymnocercus</i>	Rio Negro, Uruguay	3	3	3	1	3	3	3	3	1	3

field and scores for that field determined by a majority-rule. In most fields, the hairs were uniformly colored, however, agouti guard hairs were present in the mid-dorsal region of most of the specimens. Therefore, the majority-rule criterion was used to eliminate multiple character states in a single field. Individual scores corresponding to pigment types were coded as follows: 0, agouti; 1, black/brown or red/orange; 2, gray/drab; 3, yellow/cream; 4, white (Fig. 2). These codes were implemented to reduce potential errors encountered in distinguishing between subtle pigment differences. Further, character state 1 represents the early stages of bleaching along both pigment pathways, and was chosen to account for switching from the eumelanin to the pheomelanin pathway, particularly from black to red-orange (HersHKovitz 1967).

A character matrix was entered into the computer software package PAUP (Phylogenetic Analysis Using Parsimony, version 3.1; Swofford 1993) using these codes (Table 1). To illustrate that the characters were ordered along two separate pathways (eumelanin and pheomelanin), a stepmatrix was created in the "assumptions" datablock of PAUP (Fig. 2). The function of the stepmatrix was to constrain the phylogenetic analysis by ordering the character state changes from the ancestral state of agouti

(0), through progressive steps (1-2 for eumelanin and 1-3 for pheomelanin) to the derived state of white (4). No character state could change back to a previous state (i.e. white to black) nor could a character state switch pathways late in the bleaching process (i.e. gray to cream). The analysis did not take into account the assertion by HersHKovitz (1967) that the bleaching process may proceed directly to white by skipping one or more intermediate color grades (illustrated by lines in Fig. 2).

These data were analyzed by the maximum parsimony method (branch-and-bound algorithm) using PAUP. Of the existing numerical approaches to inferring phylogenies from character data, maximum parsimony methods have been the most widely used (Swofford & Olson 1990). Parsimony maintains that simpler hypotheses are preferable to more complicated ones. Therefore, parsimony methods used for inferring phylogenies operate by selecting trees that minimize total tree length, or the number of evolutionary steps (transformations from one character to another) required to explain the data (Swofford & Olson 1990). The major assumption is that the shortest tree will also be the best estimate of the actual common ancestry relationship of the taxa analyzed (Wiley et al. 1991). A consistency index (CI) was calculated for each data set. The CI

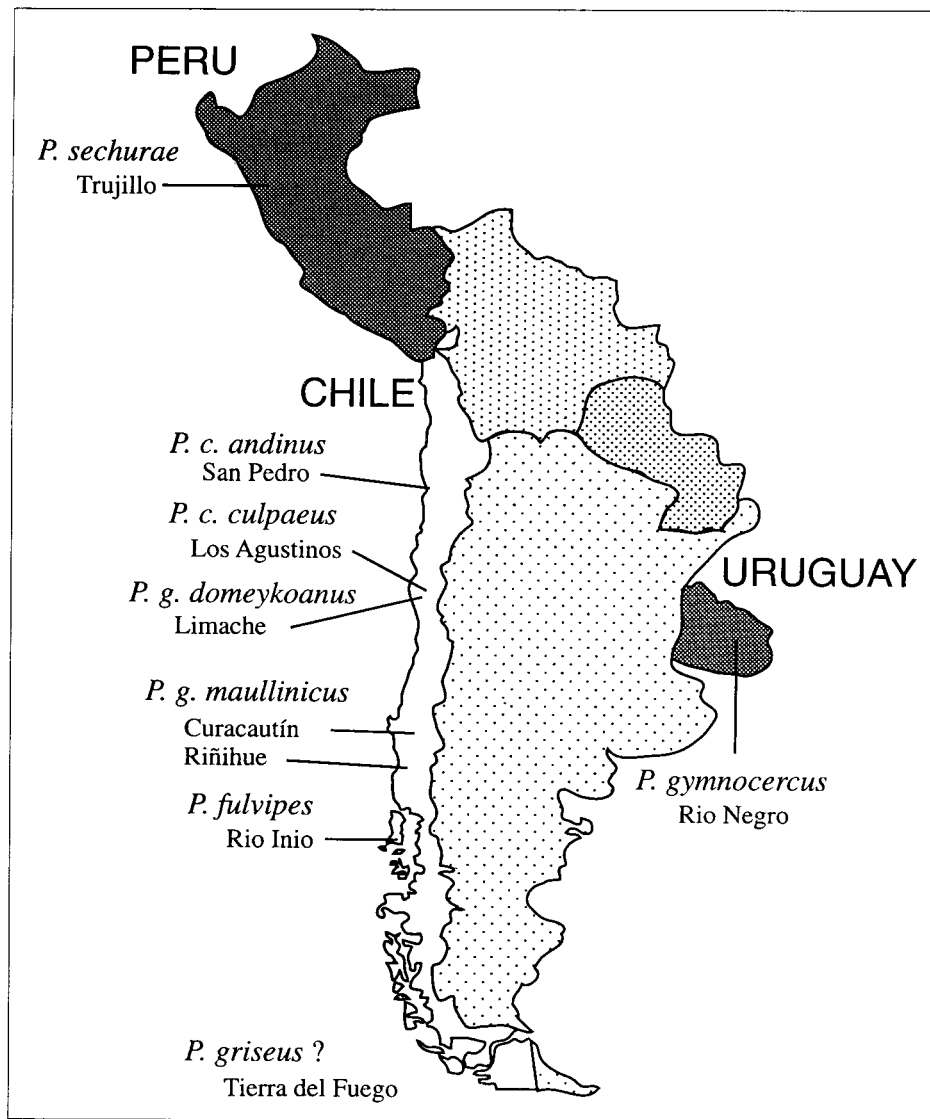


Fig. 1: Map showing the distribution of the individual foxes used in this study. Subspecific designations follow Osgood (1943).

Mapa mostrando la distribución de los zorros usados en este estudio. Designaciones subspecíficas según Osgood (1943).

measures how well the transformation series of the data “fits” a particular tree topology. In general, a transformation series with little or no homoplasy (i.e. convergence, parallelism, reversal) will have a high CI (i.e. 1.0), whereas those that show homoplasy will have lower values (Wiley et al. 1991). Reported values for the consistency index exclude uninformative characters (sites that vary in a single taxon, as well as those that do not discriminate among possible trees, are uninformative in maximum parsimony analysis).

An exhaustive search of all possible trees was done in PAUP which generated a distribution of tree lengths from which a g_1 statistic (skewness) was calculated (Hillis 1991). When all taxa were considered, the g_1 statistic was calculated by surveying 10,000 random trees. A normal distribution ($g_1=0$ or no skew) suggests that the character matrix contains no phylogenetic signal (noise). A bootstrap maximum parsimony analysis with 1000 iterations was performed to see how reliably these data supported the inferred monophyletic groups. With the

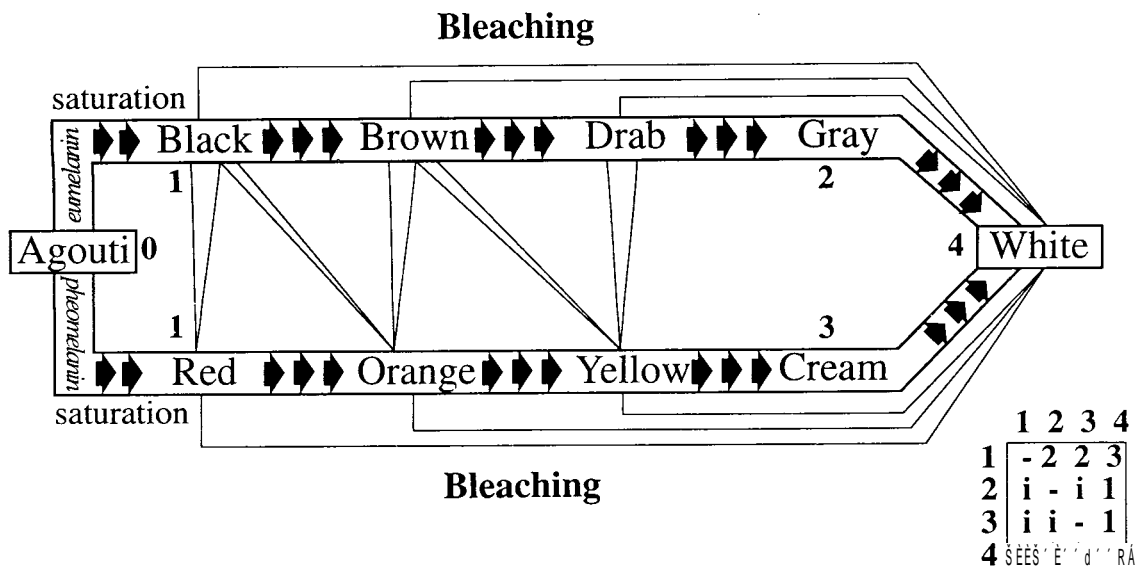


Fig. 2: Saturation and bleaching of hair through eumelanin and pheomelanin pathways. The stepmatrix defines the number of steps between states, with "i" = infinite, which represents the improbability of reverting back to a previous state or switching pathways late in the bleaching process. Figure modified from Hershkovitz, 1967.

Saturación y blanqueamiento de pelo por las vías de eumelanina y feomelanina. La matriz define el número de pasos entre estados, con "i" = infinito, representando la improbabilidad de revertir hasta un estado previo, o saltando vías posteriores en el proceso de blanqueamiento. Figura modificada de Hershkovitz, 1967.

bootstrap resampling method, each iteration randomly samples data points from the original data with replacement until a new data set containing the original number of observations is obtained (Swofford & Olson 1990). The bootstrap values at each node of the tree represent the percentage of trees in the analysis which contained that particular group of taxa. A hypothetical outgroup was used to root the inferred groups within the *Pseudalopex*. This outgroup was created by assigning a character state of 1 to all 10 chromogenetic fields. Since agouti hairs were not scored, character state 1 represents the first identifiable stage of bleaching in this data set.

RESULTS

Analysis of the ten chromogenetic fields in the seven individuals representing *P. griseus* and *P. fulvipes* resulted in two most parsimonious trees of 18 steps with a CI of 0.944. The majority-rule consensus tree suggests that Darwin's fox retains more ancestral characters in terms of its pigmentation than subspecies of *P. griseus* (Fig. 3). This con-

clusion is possible since the tree was rooted with a hypothetical outgroup, and the character states are ordered (Fig. 2). These results also support the observations that mainland subspecies of *P. griseus* are generally lighter in color than Darwin's fox (Osgood 1943, Medel et al. 1990). Bootstrap analysis for the inferred monophyletic groups provides strong support for subspecies of *P. griseus* (94 percent of all trees) although one individual of *P. g. maullinicus* falls outside this clade (Fig. 3). In fact, the two specimens of *P. g. maullinicus* appeared to be considerably different, although their collection localities were not far apart (Fig. 1). Possible explanations for this discrepancy include the relative age of the museum specimens and the natural color variation within this subspecies. The g_1 statistic for the eight taxa in Fig. 3 was -1.08 suggesting the presence of phylogenetic signal in this data set ($p < 0.001$). Furthermore, the CI suggests that there was very little homoplasy in this data set (CI = 0.944).

Analysis of the ten chromogenetic fields for all thirteen individuals scored resulted in three most parsimonious trees of 27 steps

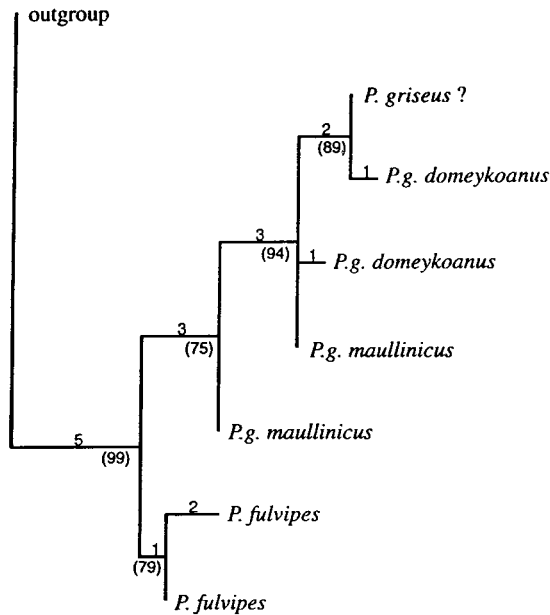


Fig. 3: MP Majority-rule consensus tree obtained from analysis of 10 chromogenetic fields obtained from museum study skins. This tree required 19 steps with a CI of 0.944. Apomorphies assigned by MP are shown above each branch. Bootstrap percentages of clades (1 000 iterations) are indicated in parentheses below internal nodes.

Arbol de consenso por regla de mayoría MP obtenido por análisis de 10 campos cromogenéticos usando pieles de museo. Esta topología exigía 19 pasos con un CI de 0,944. Apomorfías asignadas por MP son indicadas arriba de las ramas. Porcentajes de clados por "bootstrap" (1.000 iteraciones) son indicados en paréntesis abajo de los nodos internos.

with a CI of 0.704 (Fig. 4). Darwin's fox and *P. sechurae* appear to be outgroups for the remaining *Pseudalopex*, although bootstrap support for most of the inferred monophyletic groups is low (Fig. 4d). When all taxa are considered, a g_1 statistic of -0.679 is produced. Although the amount of skew appears to be reduced, it is nonetheless highly significant ($p < 0.001$), suggesting the presence of phylogenetic signal in this data set. Finally, the relatively low CI value (CI = 0.704) for this data set suggests an increase in homoplasy. However, this low CI value may be due in part to the negative relationship between CI and the size of the data set (Archie 1989).

DISCUSSION

Wilfred Osgood (1943) noted morphological differences between Darwin's fox and other

members of *Pseudalopex griseus*. Its Araucanian name "Paineguru", which means "Blue fox" (Gay 1847; p. 59), suggests a markedly different color for this fox than the lighter-colored members of *P. griseus*. As demonstrated by the metachromatic analysis, Darwin's fox most closely resembles the Sechura fox (*P. sechurae*) in its color pattern and pigmentation. However, this relationship is suggestive, and is poorly supported in the bootstrap analysis, with Darwin's fox and the Sechurae fox forming a monophyletic group in only 31.5 percent of the trees. Although it is possible that this analysis is not suitable for comparisons among higher taxa (i.e. species versus subspecies), it may be useful for generating general phylogenetic hypotheses. For example, two general patterns are corroborated by mtDNA sequences (D-loop) obtained from these taxa (Yahnke et al. in press). First, Darwin's fox and *P. sechurae* are outgroups to the clade consisting of other members of the genus (except *P. vetulus*), and second, *P. culpaeus* and *P. griseus* are paraphyletic (Fig. 4). However, the presence of one *P. g. maullinicus* outside the *P. gymnocercus* clade is questionable and the relationships within *P. griseus* are difficult to discern. Although variation within subspecies may introduce anomalies in the cladogram, other less obvious factors may contribute. For example, one individual of *P. griseus* scored was reportedly collected in Tierra del Fuego in 1919 and donated by the Argentine Commission. However, Jaksic and Yañez (1983) report the absence of *P. griseus* in Tierra del Fuego prior to the 1950s when they were introduced to control rabbit populations. Thus the origin of this specimen is questionable, and therefore so is the subspecific designation. Unfortunately, properly identified skins of the southern-most subspecies, *griseus*, were not available for comparison.

Medel et al. (1990) suggest two explanations for the disjunct geographical distribution of Darwin's fox. First, the population of Darwin's fox in Nahuelbuta resulted from the successful establishment of captive individuals. Second, Darwin's fox has a relict distribution, with intervening populations having become extinct. The apparent disparity between the topologies in Fig. 4

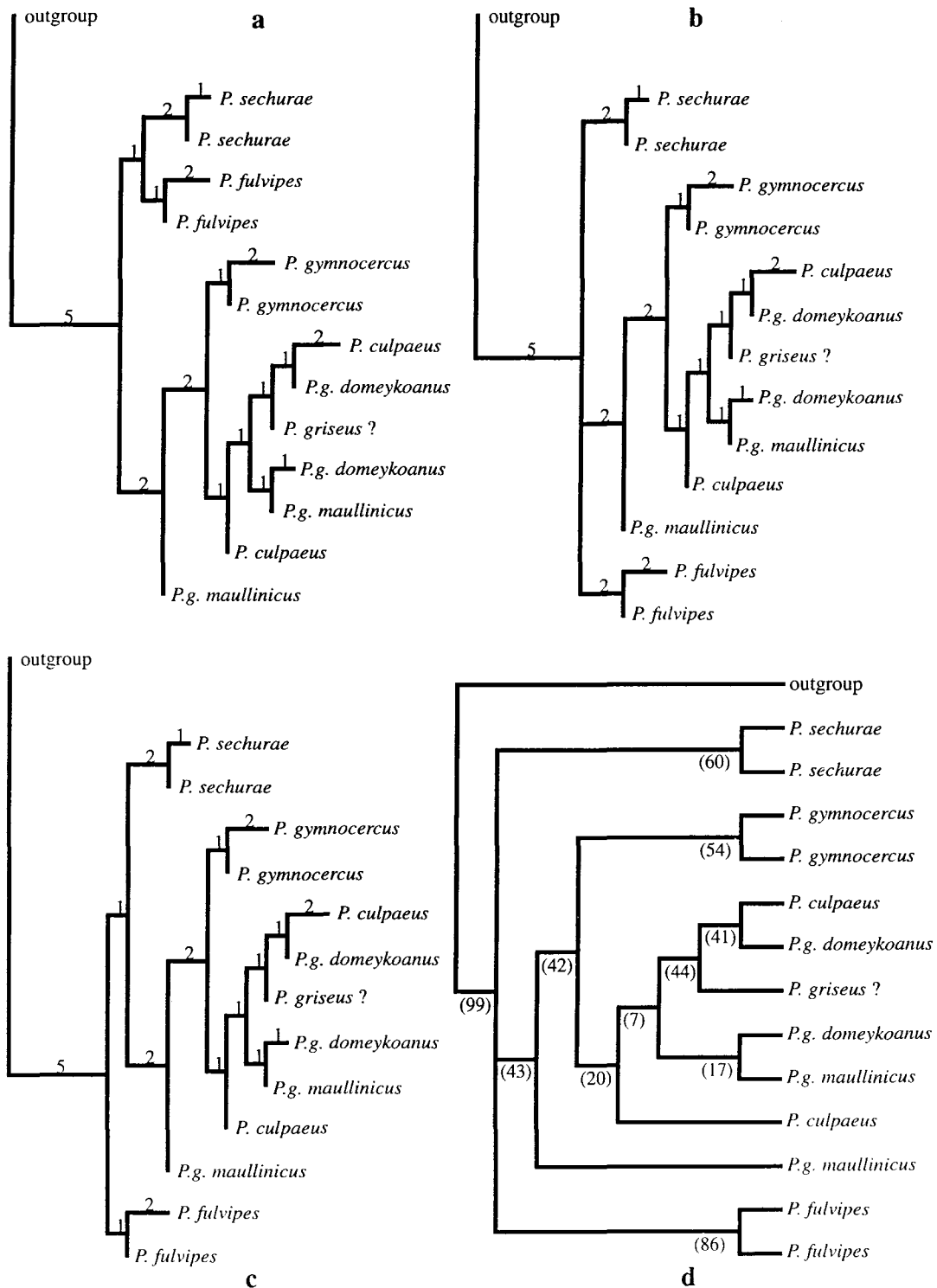


Fig. 4: MP trees obtained from analysis of 10 chromogenetic fields using museum skins. Trees a-c represent three equally parsimonious trees of 27 steps with a CI of 0.704. Apomorphies assigned by MP are shown above each branch. Tree d is a strict consensus of trees a-c. Bootstrap percentages of clades (1,000 iterations) are indicated in parentheses below internal nodes.

Arboles MP obtenidos por análisis de 10 campos cromogenéticos usando pieles de museo. Arboles a-c representan tres topologías igualmente parsimoniosas de 27 pasos con un CI de 0,704. Apomorfías asignadas por MP son indicadas arriba de las ramas. Topología d representa un consenso estricto de las topologías a-c. Porcentajes de clados por "bootstrap" (1.000 iteraciones) son indicados en paréntesis abajo de los nodos internos.

also suggests the following intriguing possibility: Darwin's fox and the Sechura fox represent a true polytomy, in which a vicariant event preceding the speciation of these two taxa may have led to the isolation and divergence of multiple populations of an ancestral species at approximately the same time. Although the frequency of polytomous speciation is unknown, it is reasonable to expect that the range of a once widespread species would become fragmented when the species is in decline, with polytomous speciation occurring when several isolates survive in relic habitats and become differentiated (Hoelzer & Melnick 1994). Although both Darwin's fox and the Sechura fox exploit beach habitats, Darwin's fox is primarily a forest fox (see Jiménez et al. 1991) and the Sechura fox is primarily a desert fox (Huey 1969). The idea of polytomous speciation is consistent with the hypothesis by Medel et al. (1990) that Darwin's fox demonstrates a relict distribution, with intervening populations having become extinct. However, differentiation between island and mainland populations of Darwin's fox is less striking since both populations exploit structurally similar forest habitat (Jiménez et al. 1991).

The metachromatic evidence presented here is consistent with a former connection of *P. fulvipes* with *P. sechurae* rather than with *P. griseus*. Although Osgood (1943) did go on to say that *P. fulvipes* may have been an offshoot of the *P. griseus* group, this statement was based primarily on the known distribution of Darwin's fox in 1943. The existence of the insular population of Darwin's fox on Chiloé Island was most readily explained by migration and subsequent differentiation from an adjacent mainland fox population, with the two populations possibly becoming isolated at the end of the last glaciation. The most likely candidate for the founding mainland population was the extant *P. griseus maullinicus*, whose range included the neighboring Valdivian forest region of south-central Chile, mainly in the provinces of Cautín, Valdivia, and Llanquihue (Osgood 1943).

Although taxonomic rank may carry conservation implications, since conservation priority could be given to protecting rare en-

demically species over rare subspecies, it also carries with it certain biogeographic and evolutionary implications. Darwin's fox is currently considered to be a subspecies of the South American grey fox (*Pseudalopex griseus*). This taxonomic hypothesis presumes that Darwin's fox differentiated from its closest mainland congener *P. griseus*, possibly at the end of the last glaciation when Chiloé became isolated from the mainland. In addition to the observations on cranial morphology (Osgood 1943), the metachromatic evidence suggests that Darwin's fox may not have *P. griseus* as its sister group, but rather *P. sechurae*. This taxonomic hypothesis suggests that ancestors of Darwin's fox had a broader range on the mainland and subsequently dispersed to Chiloé Island.

Metachromatic analysis can resolve general phylogenetic relationships among taxa, however bootstrap percentages of inferred monophyletic groups may decrease, and homoplasy increase as higher level taxa are added to the tree. The original studies using metachromism (Hershkovitz 1967, 1977) described subspecies of saddleback tamarins (*Saguinus fuscicollis*) in lowland rainforests of South America. Species-level studies using metachromism have not been published. Therefore, these data should be treated as only generating very general phylogenetic hypotheses which need to be corroborated by independent data sets (i.e. DNA sequences, morphometric analyses). For example, the phylogenetic hypothesis generated using this data set reflects patterns generated from DNA sequence analysis (Yahnke et al. in press). Since metachromatic analysis is relatively inexpensive, non-destructive, and accessible, it makes sense to test its potential usefulness in inferring phylogenetic relationships.

ACKNOWLEDGMENTS

I would like to thank B.D. Patterson and B. Stanley for access to materials at the Field Museum of Natural History. I also thank P.L. Meserve, C.N. von Ende, and B.D. Patterson for helpful comments on early drafts of this manuscript, and two anonymous reviewers

for much needed improvements. Finally, I would like to thank P. Hershkovitz for helping me understand metachromism, F.M. Jaksic for introducing me to Darwin's fox as an interesting taxonomic question, and B.A. Lohr for moral support.

LITERATURE CITED

- ARCHIE JW (1989) Homoplasy excess ratios: New indices for measuring levels of homoplasy in phylogenetic systematics and a critique of the consistency index. *Systematic Zoology* 38: 253-269.
- CABRERA A (1958) Catálogo de los mamíferos de América del Sur. *Revista del Museo Argentino de Ciencias Naturales "Bernadino Rivadavia"* 4: 1-307.
- CLUTTON-BROCK J, GB CORBET & M HILLS (1976) A review of the family Canidae, with a classification by numerical methods. *Bulletin, British Museum (Natural History), Zoology* 29: 117-199.
- DARWIN C (1839) *Journal of researches into the geology and natural history of the various countries visited by H.M.S. Beagle, under the command of Captain Fitzroy, R.N. from 1832-1836.* Henry Colburn, London. xi + 432 pp.
- GAY C (1847) *Historia física y política de Chile según documentos adquiridos en esta república durante doce años de residencia en ella.* Museo de Historia Natural de Santiago, Chile. 471 pp.
- HERSHKOVITZ P (1967) Metachromism or the principle of evolutionary change in mammalian tegumentary colors. *Evolution* 22: 556-575.
- HERSHKOVITZ P (1970) Metachromism like it is. *Evolution* 24: 644-648.
- HERSHKOVITZ P (1977). *Living new world monkeys (Platyrrhini).* Volume 1. University of Chicago Press, Chicago. xiv + 1117 pp.
- HOELZER GA & DJ MELNICK (1994) Patterns of speciation and limits to phylogenetic resolution. *Trends in Ecology and Evolution* 9: 104-107.
- HONACKI JH., KE KINMAN & JW KOEPL, eds (1982) *Mammal species of the world: A taxonomic and geographic reference.* Allan Press, Inc. and Association of Systematics Collections, Lawrence, Kansas. ix + 694 pp.
- HILLIS DM (1991) Discriminating between phylogenetic signal and random noise in DNA sequences. In: Miyamoto MM & J Cracraft (eds) *Phylogenetic analysis of DNA sequences*: 278-294. Oxford University Press, Oxford.
- HUEY RB (1969) Winter diet of the Peruvian desert fox. *Ecology* 50: 1089-1091
- JAKSIC FM & JL YANEZ (1983) Rabbit and fox introductions in Tierra del Fuego: History and assessment of the attempts at biological control of rabbit infestation. *Biological Conservation* 26: 367-374.
- JAKSIC FM, JE JIMENEZ, RG MEDEL & PA MARQUET (1990) Habitat and diet of Darwin's fox (*Pseudalopex fulvipes*) on the Chilean mainland. *Journal of Mammalogy* 71: 246-248.
- JIMENEZ JE, PA MARQUET, RG MEDEL & FM JAKSIC (1991) Comparative ecology of Darwin's fox (*Pseudalopex fulvipes*) in mainland and island settings of southern Chile. *Revista Chilena de Historia Natural* 63: 177-186.
- LAWLOR TE (1969) The principle of metachromism: A critique. *Evolution* 23:509-512.
- LANGGUTH A (1969) Die südamerikanischen Canidae unter besonderer berücksichtigung des mahnen wolfe *Chrysocyon brachyurus* Illiger. *Zeitschrift Wissenschaftliche Zoologie* 179: 1-88.
- MEDEL RG, JE JIMENEZ & FM JAKSIC (1990) Discovery of a continental population of the rare Darwin's fox, *Dusicyon fulvipes* (Martin, 1837) in Chile. *Biological Conservation* 51: 71-77.
- OSGOOD WH (1943) The mammals of Chile. *Field Museum of Natural History, Zoological Series* 30: 1-268.
- PHILLIPI RA (1869) Ueber einige thiere von Mendoza. *Archiv fur Naturgeschichte* 35: 38-51.
- SWOFFORD DL (1993) PAUP: Phylogenetic analysis using parsimony, version 3.1. Smithsonian Institution, Washington, D.C.
- SWOFFORD DL & OLSON (1990) Phylogeny reconstruction. In: Hillis DM & C Moritz (eds) *Molecular systematics*: 411-501. Sinauer, Sunderland, Massachusetts.
- WILEY EO, D SIEGEL-CAUSEY, DR BROOKS & VA FUNK, eds (1991) *The compleat cladist: A primer of phylogenetic procedures.* Special Publication Number 19, The University of Kansas Museum of Natural History, Lawrence, Kansas. x + 158 pp.
- WOZENCRAFT WC (1993) Order Carnivora. In: Wilson DE & DM Reeder (eds) *Mammal species of the world: A taxonomic and geographic reference*: 279-348. Smithsonian Institution Press, Washington, D. C.
- YAHNKE CJ, WE JOHNSON, E GEFFEN, D SMITH, F HERTEL, MS ROY, CF BONACIC, TK FULLER, B VAN VALKENBURGH & RK WAYNE (in press) Darwin's fox rediscovered: A distinct endangered species from a vanishing habitat. *Conservation Biology*.