Using fecal profiles of bile acids to assess habitat use by threatened carnivores in the Maulino forest of central Chile

Empleo del perfil de ácidos biliares fecales para evaluar uso del hábitat por carnívoros amenazados en el bosque maulino de Chile central

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ABSTRACT

The distribution and habitat use by carnivores can be assessed by studying their tracks or feces. If these methods are to be used confidently, they should not only unequivocally discriminate among species, but should also render the same patterns of spatial distributions. We assessed the fulfillment of these requirements with five carnivores inhabiting the Maulino forest of central Chile: *Galictis cuja*, *Oncifelis guigna*, *Pseudalopex culpaeus*, *Pseudalopex griseus*, and *Puma concolor*. Fecal bile acid thin layer chromatographic profiles were assessed, and shown to be species-specific, invariant within samples of a given individual and among individuals of a given species, but consistently different across species. The spatial distribution of feces in mixed stands of native forests and exotic pine plantations in the coastal Maule region of central Chile was compared with the expected distribution according to habitat offer, and also with the expected distribution based on earlier track records. The results revealed that *Pseudalopex culpaeus* makes extensive use of pine plantations; *Oncifelis guigna* prefers native forests, and *Pseudalopex griseus* thrives in pine plantations, native forests, and patches of native forest, in proportion to habitat availability. Results from scat distribution were similar to those obtained by tracks records. Feces and tracks were thus useful indicators of habitat use by carnivores, and could be used complementarily to study species with conservation problems.

Key words: fecal bile acids, *Galictis,* habitat use, *Oncifelis*, *Pseudalopex*, *Puma*, thin layer chromatography.

RESUMEN

El uso del hábitat por carnívoros puede ser examinado a partir de la distribución de sus huellas y fecas. Para que ambas aproximaciones sean usadas en forma segura y complementaria, ellas deberían no solamente discriminar inequívocamente entre especies, sino que también deberían entregar la misma información sobre los patrones de distribución espacial. Analizamos el cumplimiento de estos requisitos con cinco especies de carnívoros en el bosque maulino de Chile central: *Galictis cuja*, *Oncifelis guigna*, *Pseudalopex culpaeus*, *Pseudalopex griseus* y *Puma concolor*. Los perfiles de ácidos biliares fecales, establecidos mediante cromatografía de capa fina, resultaron ser especie-específicos, invariantes tanto dentro de muestras de un mismo individuo como entre muestras de diferentes individuos de una misma especie, pero son consistentemente diferentes entre especies. La distribución especial de fecas en áreas de bosque nativo y plantaciones de pino en el bosque costero del Maule se comparó con la distribución esperada en base a estudios previos basados en registro de huellas. *Pseudalopex culpaeus* usa extensamente plantaciones de pino; *Oncifelis guigna* prefiere bosque nativo y *Pseudalopex griseus* usa bosque nativo y plantaciones de pino en proporción a su disponibilidad. Los resultados de la distribución de fecas son similares a los obtenidos por registro de huellas.

Palabras clave: ácidos biliares fecales, cromatografía en capa fina, *Galictis*, *Oncifelis, Pseudalopex, Puma*, uso de hábitat.

INTRODUCTION

Chilean carnivores, particularly those inhabiting central Chile are of conservation concern. Habitat changes and illegal hunting have decimated populations, threatening their survival (Miller et al. 1983). Sound conservation practices require to properly

assess habitat use by target species. This is particularly true for carnivores inhabiting the Maulino forest, a severely reduced and shrinking forest (Grez et al. 1997). Here, *Oncifelis guigna*, the smallest known cat, endemic to *Nothofagus* forests, prefers habitats with dense bush cover and is almost exclusively restricted to native forests, while *Pseudalopex culpaeus* uses open habitats, close to roads, including Monterrrey pine plantations (*Pinus radiata*; Acosta-Jamett & Simonetti 2004). This pattern of habitat use is based on a handful of track records despite a significant sampling effort devoted to assess habitat use (Acosta & Simonetti 1999, Acosta-Jamett & Simonetti 2004). Therefore, another indicator of their presence is required to confirm the pattern of habitat use by these and other threatened carnivores of the Maulino forest, including *Galictis cuja*, *Pseudalopex griseus*, and *Puma concolor*, all of conservation concern (Miller et al. 1983).

Besides tracks, carnivores can be recognized through their profiles of fecal bile acids, which are species-specific (Haslewood 1967). The technique has been used with varying degrees of success. Major et al. (1980) and Johnson et al. (1984) used it to assess the distribution and abundance of carnivores in different habitats in North America, while Capurro et al. (1997), Fernández et al. (1997), and Cazón & Suhring (1998) used it to identify several Neotropical carnivore species. However, Jiménez et al. (1996a) were unable to distinguish feces from *P. culpaeus* and *P. griseus* in Chile, two species that were nevertheless distinguished by Capurro et al. (1997). Here, our aim is two-fold. First, we explore if carnivores inhabiting the Maulino forest can be unequivocally recognized by their bile acid patterns. If species can be recognized, each feces can be used as an indicator of an animal presence in a given habitat, enabling the use of such record to reinforce assessment based by other means, such as track records. Second, we test if the pattern of habitat use as depicted by the spatial distribution of feces is similar to the pattern assessed based on track records. If patterns depicted by these two different types of evidence are similar, they can reinforce each other, providing more robust estimates of habitat use.

Chemical analysis

The analytical method, developed using feces from animals in captivity followed Major et al. (1980). Powdered dry feces (3 g) were extracted with dichloromethane: methanol (1:1; 18 mL). The organic extract was filtered, concentrated to 3 mL, and redissolved in ethyl acetate (30 mL) before being subjected to alkaline extraction (3 x 20 mL of 4 N NaOH) in order to purify the bile acids from other lipophilic steroidal compounds. The alkaline extract was neutralized and extracted with ethyl acetate (3 x 20 mL). This new organic extract was washed with saturated $NaHCO_3$ (3 x 20 mL), and water (3 x 20 mL), and dried over anhydrous $MgSO_4$. The resulting solution was spotted (50 µL) on thin-layer chromatographic plates (Merck, Silica Gel 60 WF254, 10 x 10 cm) which were eluted with a mixture of hexane: methyl-ethyl ketone: acetic acid (56:36:8). The plates were developed by spraying on them a mixture consisting of 80 mL glacial acetic acid, 16 mL water, 4 mL sulfuric acid and 0.5 3,4-dimethoxybenzaldehyde, and heating to 100 °C for 3 min. Bile acids were identified by comparison of their R_f values and color with those of commercially available standards of cholic, dehydrocholic, deoxycholic, hyodeoxycholic, lithocholic, chenodeoxycholic, and ursocholic acids, and cholesterol (Sigma Chemical Co.). The Rf value is the ratio of the distance traveled by a compound relative to the distance traveled by the solvent front in the chromatographic plate.

Samples from captive animals

Feces were obtained from captive animals at Santiago's Metropolitan Zoo, Temuco´s Zoo and, and Cajón del Maipo-CODEFF Rehabilitation Center. Six feces were analyzed from each of the following species: *P. concolor*, *P. culpaeus* and *P. griseus,* and two feces were analyzed from each of the following species: *G. cuja* and *O. guigna*. Feces can not be assigned to any given individual as two or more specimens shared the same exhibit cage, excepting *O. guigna* who was the single captive individual in all Chilean zoos. Thus, each scat could not be associated to the sex of the producer. However,

sex has been shown to infuence concentration but not composition of bile acids in feces (e.g., Hellou et al. 1988). On the other hand, only fiber-rich diets could decrease the incidence and concentration of fecal bile acids (Quinn & Jackman 1994). This was not the case in either captive or in wild animals at the study area: captive carnivores were fed with livestock remains while wild carnivores largely prey upon small mammals and invertebrates, with a low consumption of plant items, mostly seeds (J.A. Simonetti personal observations, Correa & Roa 2005). Each scat was divided into three sub-samples, which were extracted and analyzed independently.

Collection of field samples

Sixty-three unknown feces were collected from three different habitats in Tregualemu, coastal central Chile: continuous native forest (Los Queules National Reserve and adjoining forests of private lands), patches of native forest, and commercial pine (*P. radiata*) plantations (Acosta-Jamett & Simonetti 2004). The study area covered 680 ha, 230 of which (34 %) were continuous forest, 120 ha (18 %) were fragments of native forests scattered in the landscape, usually surrounded by *Pinus radiata* plantations. Isolated remnants of native forests ranged from 1 to 10 ha. Plantations covered 330 ha (48 %) of the study site, stands ranging from 33 to < 150 ha (Acosta-Jamett & Simonetti 2004).

Feces were collected from September 1998 to January 2002, while surveying each type of habitat. Feces were placed in paper bags and air-dried, and stored in paper bags until used. Fecal bile acids are stable to environmental conditions and to long storage periods enabling to confidently use the available sample (Lin et al. 1978, Fernández et al. 1997). Sampling effort (hour/person/day) devoted to collect feces was proportional to habitat availability (Acosta-Jamett & Simonetti 2004). Such sampling allows deriving a "no-preference" expectation assuming animals do not prefer any recognized habitat type. If such were the case, the relative abundance of feces should be in proportion to the abundance of each habitat type (e.g., Price & Kramer 1984).

The assessment of habitat use by carnivores based on tracks was performed in the same area where feces were collected. Habitat use by carnivores was assessed by Acosta-Jamett & Simonetti (2004) through the scent-station method (Roughton & Sweeny 1982). The relative use of continuous forest, forest fragments and pine plantations was estimated as the number of stations with animal tracks over a total of 32 scent-stations installed in accordance with the relative abundance of the different habitat types across the landscape. A total sampling effort of 620 sampling stations night^{-1} rendered 58 tracks, of which 77 % of *O. guigna* tracks were recorded in continuous forest, 15 % in forest remnants and 8 % in pine plantations. Regarding foxes, 21 % of tracks of *P. culpaeus* were recorded in continuous forest, 4 % in forest remnants and 75 % in pine plantations, respectively (Acosta-Jamett & Simonetti 2004).

Statistical analysis

Similarity in bile acid composition was assessed through the Jaccard index (Krebs 1989). Comparisons between sub-samples and between individuals of the same species should give a value of 1.0 (complete similarity), and comparison between different species should give values smaller than 1.0 (partial similarity).

The observed location of scats in different habitats (native forest, patches of native forest, and pine plantations) was compared in relation to (a) the use predicted on the basis of the availability of the different habitats, using a G-test. If animals use habitats randomly, feces distribution should be proportional to habitat availability; otherwise, it should suggest habitat selection. (b) The pattern of habitat use assessed also was compared with the assessment based on track records (see Acosta-Jamett & Simonetti 2004), using a G-test. If both types of evidences convey similar results, no statistically difference ought to be observed.

RESULTS

The five carnivore species produced unique fecal bile acid patterns (Table 1). Six of the seven bile acid standards used were detected in some or all of the samples. Ursocholic acid was not detected in any species. Some acids, like cholic acid, were detected in all species while others were

recorded in only one species, like dehydrocholic acid in *G. cuja*. Three unidentified spots, presumably bile acids on the basis of extraction and purification procedures, R_f values, and color of spots, were detected in some of the samples. Cholesterol was present in all the samples as a purple spot with R_f value of 0.8.

Pseudalopex culpaeus differed from *P. griseus* by the presence in the former of deoxycholic and chenodeoxycholic acids. *Puma concolor* differed from *O. guigna* by the presence of hyodeoxycholic acid only in *P. concolor*. Only *G. cuja* showed dehydrocholic acid and the unknown compound X1 (Table 1). Only the felids exhibited unknown compound X2 (Table 1).

Bile acid profiles of sub-samples from any given individual were always identical (Jaccard index of 1.0 in all cases). Likewise, the composition of extracts from different individuals of the same species was invariable (Jaccard index of 1.0 in all cases). When comparing different species, Jaccard indexes were always smaller than 1.0. Jaccard indexes for intraspecific comparisons were significantly larger than for interspecific comparisons (U = 3.06, P = 0.002), demonstrating that bile acid profiles are characteristic of each carnivore species.

The Jaccard index for comparisons between the different species of carnivores studied varied between 0.44 and 0.86 (Table 2). Relative similarity in the profile of bile acids did not follow phylogenetic relatedness among species. Thus, the bile acid profile for *P. culpaeus* was more similar to that of *P. concolor* or *O. guigna* than to that of congeneric *P. griseus*. On the other hand, the profile of *P. griseus* was more similar to that of *P. culpaeus* that to any of the other species, and the felids (*P. concolor* and *O. guigna*) showed the greatest degree of similarity.

All but three of the sixty-three field collected fecal samples could be unequivocally assigned to one of the known species. The profiles of the three odd samples did not match exactly with those of any of the species studied, and might belong to other carnivore, such as a domestic species. These samples were excluded from the analysis of habitat use (Table 1).

Twenty feces were assigned to *O. guigna*, 26 to *P. culpaeus*, 14 to *P. griseus* and 3 to *G. cuja*; no scat was assigned to *P. concolor*. Most feces from *O. guigna* were found in the native forest. This distribution was significantly different from that expected on the basis of relative occurrence of each habitat in the area sampled; hence, *O. guigna* prefers continuous native forests over forest fragments or pine plantations (Table 3). Feces from *P. culpaeus* were found in pine plantations in numbers

TABLE 1

Bile acids present in feces extracts of different carnivores: *Galictis cuja*, *Pseudalopex culpaeus*, *Pseusalopex griseus*, *Oncifelis guigna* y *Puma concolor*, according to thin-layer chromatography technique, color and R_f value of spots. Presence or absence are indicated by "+" and "-", respectively. Abbreviations for bile acids are as follows: Coi (cholic), Deh (dehydrocholic), Hyd (hyodeoxycholic), Deo (deoxycholic), Che (chenodeoxycholic), Lit (lithocholic), X1, X2 and X3 (unknown components)

Ácidos biliares presentes en fecas de diferentes carnívoros: *Galictis cuja*, *Pseudalopex culpaeus*, *Pseusalopex griseus*, *Oncifelis guigna* y *Puma concolor*, de acuerdo a la técnica de cromatografía en capa fina, color y R_f de las manchas. Presencia o ausencia de un ácido se indican como "+" y "-", respectivamente. Acronismos para los ácidos biliares son: Coi (cólico), Deh (dehidro cólico), Hyd (hiodeoxi cólico), Deo (deoxi cólico), Che (quenodeoxi cólico), Lit (lito cólico), X1, X2 y X3 (compuestos desconocidos)

Bile acid R_f Color	Coi 0.17 Brown	Hyd 0.30 Violet	X1 0.34 Orange	Che 0.46 Blue	Deh 0.50 Orange	Deo 0.55 Brown	X2 0.57 Brown	X3 0.61 Yellow	Lit 0.71 Violet
P. culpaeus	+	+	-	+	-	+	-	+	+
P. griseus	+	-	-	-	-	+	-	+	+
P. concolor	+	+	-	+	-	+	+	+	+
O. guigna	+	-	-	+	-	+	+	+	+
G. cuja	+	+	+	-	+	+	-	+	+

TABLE 2

Jaccard index for comparisons of fecal bile acid composition between *Galictis cuja*, *Pseudalopex culpaeus*, *Pseusalopex griseus*, *Oncifelis guigna* y *Puma concolor*

Índice de Jaccard para comparaciones en la composición de ácidos biliares entre *Galictis cuja*, *Pseudalopex culpaeus*, *Pseudalopex griseus*, *Oncifelis guigna* y *Puma concolor*

	P. culpaeus	*P. griseus*	*P. concolor*	*O. guigna*	*G. cuja*
P. culpaeus	1.00	0.67	0.86	0.71	0.62
P. griseus		1.00	0.57	0.57	0.57
P. concolor			1.00	0.86	0.56
O. guigna				1.00	0.44
G. cuja					1.00

higher than expected on the basis of habitat offer; hence *P. culpaeus* shows a preference for pine plantations. Most feces of *P. griseus* were found in pine plantations; however, the numbers were not significantly different from the habitat offer, suggesting that *P. griseus* does not have particular preferences for a given habitat (Table 3). The low number of feces collected from *G. cuja* precluded a study of its habitat use.

No significant difference was detected between the spatial use of *O. guigna* and *P. culpaeus* based on the distribution of their feces and that recorded based on the distribution of their tracks –obtained by Acosta-Jamett & Simonetti (2004)- (Table 4). That is, the two types of evidence convey the same pattern of habitat use by these two carnivores.

DISCUSSION

The analysis by thin layer chromatography of fecal bile acids proves to be an effective technique to study habitat use by Chilean carnivores. Species can be unequivocally assigned to the species level. Thus, contrary to the study of Jiménez et al. (1996a), the method described herein may be used to distinguish bile profiles from *P. griseus* and *P. culpaeus*, most likely on account of the pre-purification of bile acids by extracting them with an alkaline solvent. In fact, our analysis provides the first evidence of the presence of *P. griseus* in the coastal Maulino forest of central Chile and its

sympatry with *P. culpaeus*. A previous study based on the observation of tracks (Acosta-Jamett & Simonetti 2004) unraveled only the presence of *P. culpaeus*. A lower abundance of *P. griseus* might have precluded its detection through tracks. Sympatry of these foxes though, seems more common than previously thought. Usually, *P. culpaeus* and *P. griseus* were regarded as allopatric, but cases of sympatry and parapatry have been frequently discovered, suggesting that this phenomenon might be more common than hitherto detected (e.g., Johnson & Franklin 1994, Jiménez et al. 1996b).

The pattern of habitat use obtained with feces from *O. guigna* and *P. culpaeus* is similar to the spatial distribution of their tracks (Acosta-Jamett & Simonetti 2004). Thus, both indicators (feces and tracks) render similar information regarding habitat use by these carnivores, strengthening current assessments of habitat distribution. The comparison of feces distribution with habitat surface available in the area studied showed that *O. guigna* preferred native forest over pine plantations and patches of native forest. This is consistent with its preference for habitats with dense bush cover, far from roads, and close to large patches of forest as assessed by tracks (Acosta-Jamett & Simonetti 2004). Regarding *P. culpaeus*, it apparently preferred pine plantations, possible due to increased abundance of its main prey, small mammals (Saavedra & Simonetti in press). Therefore, everything else being equal, *O. guigna* would be more severely affected than foxes by ongoing landscape changes.

TABLE 3

Number of feces found (obs) compared with number expected on the basis of habitat resource (exp)

Número de fecas encontradas (obs) comparadas con el número de fecas esperado en función de la disponibilidad de hábitat (exp)

Species	Habitat			Total	G-value	P-value
	Pine plantations (obs/exp)	Native forest (obs/exp)	Patches of native forest (obs/exp)			
O. guigna	4/9.6	12/6.8	4/3.6	20	7.50	0.025
P. culpaeus	20/12	5/9	1/5	26	11.34	0.005
P. griseus	10/6.7	3/4.8	1/2.5	14	3.41	0.250

TABLE 4

Number of feces found (obs) compared with number expected (exp) on the basis of tracks registered by Acosta-Jamett & Simonetti (2004)

Número de fecas encontradas (obs) comparadas con el número esperado (exp) en función del número de huellas registradas por Acosta-Jamett & Simonetti (2004)

Species	Habitat			Total	G-value	P-value
	Pine plantations (obs/exp)	Native forest (obs/exp)	Patches of native forest (obs/exp)			
O. guigna	4/2	12/15	4/3	20	2.50	0.50
P. culpaeus	20/20	5/5	1/1	26	0.00	1.00

The conservation of endangered carnivores such as *O. guigna* in the coastal Maulino forest requires to be based in solid empirical evidence (Acosta-Jamett et al. 2003, Simonetti et al. 2002). The validity of bile acids in feces to differentiate closely related species and hence to assess habitat use and the congruence in data obtained from feces and tracks, offers more robust data upon which to elaborate a conservation strategy of the carnivores of coastal central Chile.

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