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Morphometrics of the Tracks of *Puma concolor*: Is It Possible to Differentiate the Sexes Using Measurements from Captive Animals?

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Karla P. García, Juan Carlos Ortiz, Marcela Vidal, and Jaime R. Rau (2010) Morphometrics of the tracks of *Puma concolor*: Is it possible to differentiate the sexes using measurements from captive animals? *Zoological Studies* **49**(4): 577-582. Studies of felines have attempted to identify individuals by their tracks, since this ability would allow obtaining data such as the presence/absence and abundance of these species, which are difficult to obtain because of their secretive habits. Distinguishing between the sexes is important, because it permits estimation of the sex ratio and the relation of this to population dynamics; however, few studies have attempted to determine the sex of *Puma concolor* using tracks. This study uses linear, angular, area, and geometric morphometrics to correctly classify individuals by sex using tracks from captive animals. The results indicated that males had greater mean values for many variables, except for the area of internal toe, the area of exterior toe, the length of 3rd toe and the length of the 2nd toe. In the geometric analysis, the shape of male tracks was narrower in the middle than that of females. Although no other methods are available that allow identification of individuals by their tracks, this study demonstrates that the use of different tools may provide favorable results. Finally, it will be necessary to increase the sample size of pumas in the wild, which will allow the inference of the population dynamics of this species in Chile and elsewhere. http://zoolstud.sinica.edu.tw/Journals/49.4/577.pdf

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The characterization of tracks was suggested as an alternative way to infer the distribution and population dynamics of mammals which are hard to observe and/or capture (Beier and Cunningham 1996, Simonetti and Huareco 1999, Miller 2001). This method is used as an indirect measure to determine the presence, relative abundance and predator impact of these mammals on their prey (Rau et al. 1992, Muñoz-Pedreros et al. 1995, Acosta and Simonetti 1999, Muñoz-Pedreros and Rau 2005).

A detailed analysis of tracks may provide additional information such as distinction of individuals, age, sex, method of locomotion, and foraging strategy (Currier et al. 1977, Miththapala et al. 1989, Karanth 1995, Smallwood and Fitzhugh 1995, Beier and Cunningham 1996). However, tracks may vary as a function of substrate type and the velocity of the animal, which may produce a complete or partial track of the foot or toes in the substrate (Simonetti and Huareco 1999). Tracks may also vary between individuals of a species as a function of age, weight, and size (Smallwood and Fitzhugh 1993); thus the analysis of the tracks of large felines requires large sample sizes which are representative of the study population

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(Simonetti and Huareco 1999). The analysis of puma tracks has concentrated on establishing individual identification (Smallwood and Fitzhugh 1993, Grigione et al. 1999, Lewison et al. 2001). Because distinguishing between male and female felids is not always as easy as gender determination in other mammals, few studies have examined variations between sexes (e.g., Shaw et al. 2007). This kind of information is relevant to establishing protection plans for this and other species, since among other things, it would allow estimating the proportions of the sexes and to relate this to population dynamics and home ranges (Zalewski 1999).

This contribution includes an approach not previously used to discriminate tracks of pumas. We analyzed the morphometrics of the tracks of adult pumas of known sexes by means of 2 morphometric approximations: (a) linear, angular, and area measurements (Smallwood and Fitzhugh 1993, Grigione et al. 1999, Lewison et al. 2001) and (b) geometric morphometrics, to study variations attributable to shape (Zelditch et al. 2004). If both methods are good predictors at the individual level, we can expect a correct assignation of individuals to sex.

MATERIALS AND METHODS

Puma tracks

Data were obtained from 8 adult pumas, 4 males and 4 females, of “Parque Zoológico de Quilpué” near Valparaíso (Chile) and “Parque Zoológico de Concepción”, Concepción (Chile). All individuals were more than 3 yr old according to records of the zoos. None of the individuals were related, because they come from separate localities according to records upon their entry to the zoos. Only fore tracks were considered for measurement because Shaw et al. (2007) showed that a size difference exists between them. Tracks were obtained by leaving trays with clay in the floor of cage. Each track was photographed with a Sony Cyber-shot DSC-600 digital camera.

Morphometric analysis

We used 5 linear measures, 1 angular measure (Smallwood and Fitzhugh 1993, Lewison et al. 2001), and 5 area measures (Fig. 1) for each toe and for the surface of the heel pad of each track (Grigione et al. 1999). Additionally, we measured the total length (TL) and total width (TW) of the track (Muñoz-Pedreros et al. 1995). The angular, linear, and area measures were calculated

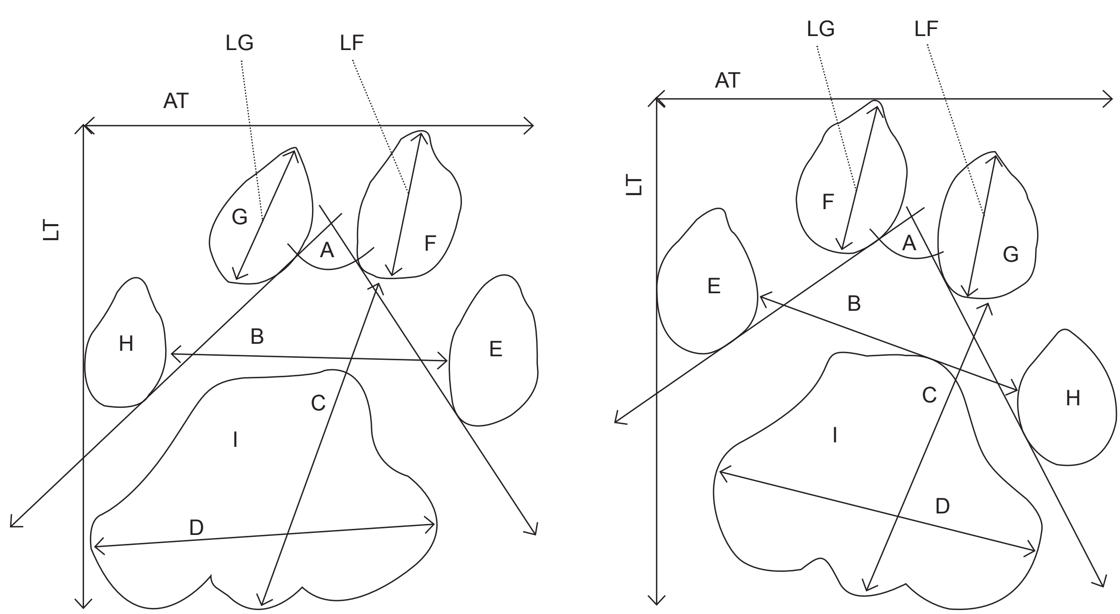

Fig. 1. Measures of left and right puma tracks. (A) Angle between inner toes; (B) outer toe spread (the line is the midpoint of each toe); (C) heel to lead toe length (line from the midpoint of the central lobe of the heel pad to the midpoint of the 3rd toe); (D) heel pad width (line of maximum width); (E) area of inner toe; (F) area of the 2nd toe; (G) area of 3rd toe; (H): area of outer toe; (I) area of heel pad. LG, length of the 3rd toe; LF, length of the 2nd toe; TL, total length; TW, total width.

with the program IMAGE J 1.37 (Rasband 2006). Measurements were made on digital pictures. Left and right tracks were analyzed in combination, since there were no differences between them (Grigione et al. 1999).

To estimate the variation attributable exclusively to the shape of the heel pad, we used a geometric morphometric shape analysis. The shapes of each photograph were drawn using the software program tpsDig (Rohlf 1999). We used an elliptical Fourier transformation (which consists of expressing the shape in periodic signals), in which each signal is adjusted by the sum of trigonometric (or harmonic) functions with different amplitudes and phases (Renaud 1999). This method is based on separating the Fourier decompositions of the increment of changes in and Y coordinates as a function of the accumulated length around the shape (Kuhl and Giardina 1982). Each harmonic is decomposed into 4 coefficients: A_n and B_n for X, and C_n and D_n for Y, which define an ellipse in the XY plane. Coefficients of the 1st harmonic describe the best fit of the ellipse to the shape, which are used to standardize the size and orientation of the object (Renaud and Michaux 2003). Thus, these coefficients correspond to the residuals after standardization (Crampton 1995). This method also limits the influence of measurement errors by filtering out the noise that occurs on details of the outline (Renaud and Millien 2001). Based on the Fourier coefficients, the shape was reconstructed by an inverse method (Crampton 1995), which allows the visualization of the changes of the form involved, which are directly developed in the program Morpheus et al. (Slice 1998). For each shape, 32 coefficients were obtained from 8 harmonics. Since the 1st harmonics showed no variation, only 29 coefficients or variables were considered in the statistical analyses. A characteristic of the Fourier harmonics is that the higher the rank of the harmonic, the more details of the outline which are described. This property can be used to filter out measurement noise, which increases with harmonic rank (Renaud 1999).

Statistical analysis

We first used a one-way multivariate analysis of variance (MANOVA) with sex as the factor, to determine if there were differences among sexes for linear, angular and area morphometrics as well as for the coefficients obtained from the harmonics in the geometric shape analysis. Then for both types of morphometric approximations, a discriminant function (DF) analysis (DFA) was used to determine: i) the morphological variables that best discriminate among sexes and ii) the assignment percentage for each group. All data were analyzed using STATISTICA 5.1 (STATSOFT 1998), after being evaluated for normality using the Kolmogorov-Smirnov test and for homogeneity of variances using Levene's test (Sokal and Rohlf 1995).

RESULTS

We obtained 38 puma tracks (17 of males and 21 of females) from the 2 zoological parks (Table 1). MANOVA indicated that for all linear, angular, and area variables, males had larger values than females (Wilk's test statistic = 0.281, $p < 0.001$), except for E, H, LG, and LF, which did not show differences between the sexes. The DFA showed significant differences between sexes (Wilk's test statistic= 0.0392, $p \leq 0.001$) and produced only 1 DF (Fig. 2A). In this analysis, the variables with the greatest discriminatory power between the sexes were the heel pad width (1.334), the area of the internal toe (1.382), the area of the 2nd toe (-1.432), and the area of the heel pad (-1.051).

The shape analysis showed significant differences among sexes (Wilk's test statistic = 0.0142, $p \leq 0.001$), and generated only 1 DF (Fig. 2B). The harmonics which best explained the form were 2, 3, 4, 5, 6, and 7, which are equivalent to coefficients 4, 5 10, 13, 16, 18, and 22. The percentages of correct assignment were 88% for males and 100% for females.

DISCUSSION

A number of studies tried to identify individual felines from their tracks (Smallwood and Fitzhugh 1993, Grigione et al. 1999, Lewison et al. 2001), but few have tried to identify the sex of individuals (Karanth 1995). The sizes of tracks recorded in this study (8.7 cm in mean length and 7.9 cm in mean width) are larger than those reported by Muñoz-Pedreros et al. (1995) (6.7 cm long and 6.8 cm wide) for Chilean pumas. Even though those authors included juveniles and used a larger sample size, they could not discriminate between the sexes. The variables which best discriminated between sexes were the heel pad width, the area of the inner toe, the area of the 2nd toe,

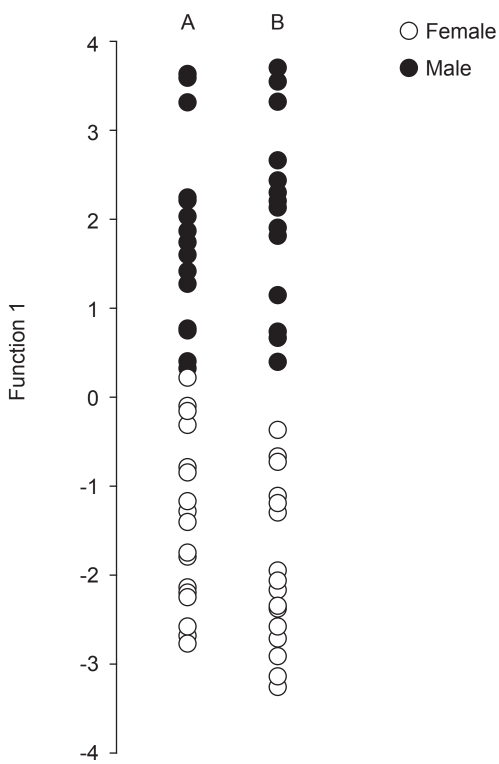

Fig. 2. Discriminant functions registered for (A) linear, angular, and area variables, and (B) shape variables of the heel pad. Solid circles are males, and open circles are females.

and the area of the heel pad. These differences between sexes are consistent with the sexual size dimorphism of this species; males are larger and heavier than females (Eisenberg 1989, Franklin et al. 1999).

According to Smallwood and Fitzhugh (1993), the measures that best discriminate among individuals are the outer toe spread and heel pad width, which coincide with our results. However, Grigione et al. (1999) indicated that area measures (excluding the area of the heel pad) are the variables that best described variation among individuals, in contrast with the width and area of the heel pad, which showed less variation. Thus, the predictive value of the measures considered by Smallwood and Fitzhugh (1993) may have been lower. More recently, Lewison et al. (2001) indicated that the variables with greater predictive value were the angle between the toes and the length of the heel pad, and they incorporated the depth of the track as a covariate. These authors reported that although the depth of the heel pad was significant for some variables (such as the angle between the toes and the length of the heel pad), its total effect in the combination of measures was not significant.

The morphometric geometric method allows the analysis of differences in form without the influence of size variables (Rohlf et al. 1996). This approximation offers a geometric vision of a given structure (Rohlf and Slice 1990), which permits information to be obtained about the form with a

Table 1. Morphometric measures (mean (cm) ± standard deviation (in parentheses)) of the tracks of *Puma concolor*

Variable	Male		Female	
n	17		21	
Angle between the external toes	81.46	(6.67)	76.85	(5.50)
Outer toes spread	5.59	(0.74)	4.37	(0.72)
Heel to lead toe length	5.98	(0.81)	5.16	(0.43)
Heel pad width	5.92	(0.72)	5.00	(0.51)
Area of internal toe	3.14	(1.51)	2.67	(0.54)
Area of 2nd toe	3.74	(1.08)	3.14	(0.67)
Area of 3rd toe	3.14	(0.85)	2.57	(0.48)
Area of outer toe	2.58	(0.80)	2.18	(0.59)
Area of heel pad	18.41	(4.44)	14.14	(2.55)
Length of 3rd toe	2.58	(0.35)	2.49	(0.22)
Length of 2nd toe	2.87	(0.39)	2.72	(0.26)
Total length	9.18	(1.07)	8.13	(0.66)
Total width	8.54	(2.11)	7.36	(0.81)

n, number of tracks.

more-complete biological interpretation (Rohlf and Marcus 1993). In the present case, the shape analysis showed a clear separation between the sexes, which is consistent with the results obtained from linear, angular, and area measures. The shape of the heel pad in males can be described as relatively narrow in the middle, while that of females is more extended in this zone. Since this is the 1st use of this method in this type of study, it is difficult to indicate what the implications of form differences among sexes may be.

Certainly, an extensive field study may in the future allow the discrimination of individuals and sexes by their tracks alone, and thus permit a more-adequate inference of population dynamics of those species which are hard to observe (Grigione et al. 1999, Lewison et al. 2001). However, some field variables are important to consider (Grigione et al. 1999): for example, fine soils (such as clay and those covered with dust) highlight the borders of the tracks allowing easy measurement, while coarser soils (such as sand) do not provide a clear definition of the edges of the tracks, which may lead to less-precise measurements (Rau 2000). Also, the time of day at which the photographs are taken may produce errors due to changes in the direction of the light (Grigione et al. 1999).

Finally, robust methodological approximations which permit the identification of individuals by their tracks have not been developed (Lewison et al. 2001). Moreover, this type of methodological approach has not been widely applied and further evaluation of it is needed. This study suggests that the identification of sexes may be possible by means of the techniques utilized.

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