Urine-washing in white-faced capuchins: a new look at an old puzzle

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Abstract
Some primates regularly wash their hands and feet with urine. This behaviour is outwardly similar to scent marking in other mammals, but it differs in that the urine is applied to the bare skin of the hands and feet rather than rubbed into the fur or applied directly onto an object in the environment. Empirical evidence for the functional significance of urine-washing remains inconsistent. We used rigorous statistical methods to examine environmental and social influences on urine-washing behaviour, using 4380 observation hours on five groups of wild white-faced capuchins (Cebus capucinus) in Costa Rica. Urine-washing frequencies were most strongly affected by environmental dryness, both within and between seasons, with markedly less urine-washing during humid conditions. Increased individual activity levels also promoted urine-washing. Among females, urine-washing was less frequent during lactation than during pregnancy and other reproductive states. Among males, urine-washing frequencies were greater in alpha males, who also exhibited a ‘vigorous’ form of urine-washing that may be functionally distinct. During the dry season, 3/5 groups exhibited more urine-washing than expected near fruit trees, but across groups there were no consistent spatial patterns for urine-washing with respect to water resources, home range overlap zones, core areas, inter-group encounter zones, and the home-range periphery. Urine-washing appears to differ fundamentally from common forms of mammalian scent marking. We suggest that its function is primarily mechanical, perhaps to apply a sticky residue to the hands and feet to improve grip on dry, arboreal substrates. Lesser signalling functions may include sexual signalling and resource labelling.

Keywords
communication, scent-marking, thermoregulation, primates, territoriality, home range, sexual attraction, seasonality.
1. Introduction

Urine is a common, almost universal olfactory medium for transmitting olfactory signals among mammals (Johnson, 1973). Some non-human primates regularly engage in urine-washing in which the animal urinates onto one limb and rubs the urine against the surfaces of the hands and feet (Hill, 1938). Urine-washing shares some superficial similarities with scent-marking behaviour, but it differs from most forms of mammalian scent-marking in that the putative scent-containing substance (urine) is applied directly to a body part rather than to an object in the environment. This distinction is important because it highlights the possibility that the ultimate goal of urine-washing might be to alter some physical or somatosensory property of the hands and feet rather than to deposit a scent (Harcourt, 1981). Some mammals, including many deer and some antelopes, also practice ‘self-anointing’ with urine, although in these cases the urine is typically sprayed onto the pelage or specialized scent glands rather than onto bare skin (reviewed by Gosling, 1982). Among primates, urine-washing has a curious taxonomic distribution that suggests its occurrence is determined in part by phylogeny. It is wholly absent from several major primate radiations, including all catarrhines and tarsiiformes, but is taxonomically widespread among both platyrrhines and lorisiformes, and it also occurs in mouse lemurs of the genus *Microcebus* (see references in Appendix 1 of Campos, 2008).

Numerous hypotheses have been offered to explain urine-washing by primates, including non-signalling functions such as thermoregulation (Schmidt & Seitz, 1967; Schwartz & Rosenblum, 1985; Roeder & Anderson, 1991; Heymann, 1995), moistening hands to improve grip (Castell & Maurus, 1967; Welker, 1973; Harcourt, 1981), stress-induced displacement behaviour (DuMond, 1968; Andrew & Klopman, 1974), and scent-trails for orientation (Eibl-Eibesfeldt, 1953; Milton, 1975); and signalling functions such as marking the home range (Charles-Dominique, 1977), asserting dominance (Perry, 1998; Campos et al., 2007), and communicating reproductive status (Hennessy et al., 1978; Milton, 1985; Boinski, 1992; Miller et al., 2008). None of these studies has examined the question using rigorous statistical methods that control for confounding variables (but see Schino et al., 2011).

In this paper, we re-examine many of these explanations of urine-washing behaviour using data from a wild population of *Cebus capucinus* (an arboreal Neotropical monkey), rigorous statistical methods, and a large dataset collected in natural conditions over an entire annual cycle. We intend to
determine whether urine-washing has the ‘design features’ (sensu Palagi & Dapporto, 2006 for urine marking in Lemur catta) that would be expected for an olfactory signal, and to evaluate which of the many possible functions can explain its occurrence with greatest parsimony. Studies that have examined urine-washing in wild capuchins (*Cebus* spp.) have produced no conclusive answers regarding its function, though they agree that the behaviour is likely multifunctional and exhibits strong seasonal fluctuations (Robinson, 1979; Perry, 1998; Carnegie et al., 2005; Campos et al., 2007). Some research on captive *C. apella* suggests that urine-washing may be used for thermoregulation (Roeder & Anderson, 1991), but two recent studies oppose this claim (Miller et al., 2008; Schino et al., 2011). There is some qualitative evidence that urine-washing in *C. apella* is associated with receiving aggression and that alpha males urine-wash in response to sexual solicitations from females (Miller et al., 2008), but these findings could not be replicated in a subsequent study (Schino et al., 2011). Miller et al. (2008) also report an inverse relationship between urine-washing rates and serum cortisol, which suggests that urine-washing may be used to relieve stress. Two studies have found that urine-washing among females is significantly reduced during the ovulatory phase and/or oestrus compared to other times (Carnegie et al., 2005; Schino et al., 2011), but to our knowledge urine-washing frequencies during other female reproductive states have not been compared analytically. We specifically set out to answer the following questions:

1. Is the temporal occurrence of urine-washing independent of environmental conditions such as temperature and relative humidity? Does urine-washing appear to be a thermoregulatory behaviour?

2. Do individual urine-washing frequencies vary predictably with sex, social dominance, or reproductive state?

3. Is the spatial occurrence of urine-washing random? If not, then what determines its spatial pattern? Does urine-washing appear to be a form of territorial scent marking?

2. Methods

2.1. Study site and subjects

This study was carried out in the Sector Santa Rosa (SSR) of the Área de Conservación Guanacaste Unesco World Heritage Site in north-western
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Costa Rica. SSR encompasses 10 800 ha of mosaic landscape dominated by deciduous forest (Fedigan & Jack, 2012). Although the study site receives an average of 1710 mm of rain each year (Fedigan et al., 2008), the region is highly seasonal, with virtually no rainfall during a six month period from mid-November to mid-May. We observed five social groups of *C. capucinus* (BH, CP, EX, GN and LV) from January–December 2011. We carried out behavioural observations on all sexually mature individuals (≥6 years; *N* = 46).

2.2. Data collection

With the aid of several field assistants, we followed a single group or two groups simultaneously from sunrise to sunset (approximately 5:00 to 18:00 h). Shorter observation days occurred occasionally when we lost contact with the focal group. We used two basic data collection procedures: focal animal sampling (Altmann, 1974) and half-hourly recording of temperature, relative humidity and location.

2.2.1. Focal animal samples

We carried out focal animal sampling in which one or two trained observers followed a single animal for 15 minutes with continuous recording of individual behaviour. In addition to collecting data himself, the first author trained six field assistants to collect data independently. Each assistant’s training period lasted 3–5 weeks, ending when the assistant and the first author achieved 100% concurrence on individual identification and 90% concurrence on all recorded behaviours. The set of behaviours that are relevant to this study (urine-washes and activity states) are easily recognizable, so for the purposes of this study, it is likely that inter-observer reliability considerably exceeded 90%. During focal samples, we maintained a continuous record of the focal animal’s visibility status, which enabled us to calculate the total duration of good-visibility time for each focal sample. This calculation was important to control for systematic variability in visibility conditions, which can be markedly worse during the wet season due to dense foliage, frequent rain, and overcast skies. We also recorded continuously all changes in the following activity states: foraging (ingesting food or actively searching for food); socializing (interacting affiliatively or agonistically with one or more conspecific animals); traveling (moving quickly while not in a food-related or social context); resting (sitting or lying while sleeping, self-grooming, or not exhibiting any other behaviour); vigilance
(scanning intently at long range); and other (any activity state that was unclear or did not fit within the above categories). Whenever we observed the focal animal urine-wash, we obtained the location using a handheld GPS receiver (Garmin GPSMAP 62s). We assigned a temperature and humidity to each urine-wash from the nearest half-hourly weather record (see below).

2.2.2. Temperature, humidity and location

We recorded ambient temperature, relative humidity, and the focal group’s current location every 30 minutes on the half-hour. We used handheld GPS receivers (Garmin GPSMAP 62s) to record the location data. Initially, we attempted to take the point near the group’s geometric centre, but visibility conditions and/or large group spread often prevented us from confidently identify the group’s centre. We, therefore, relaxed this requirement, retaining all points for which at least some group members were visible directly overhead. The temperature and relative humidity data were recorded automatically by a stationary Kestrel weather meter located in a shaded location near the centre of the study area.

2.3. Data analysis

2.3.1. Environmental factors

Based on abrupt annual fluctuations in ambient temperature, relative humidity, and precipitation (Figure 1), we defined two equal-length seasons: a dry season from 16 November–15 May and a wet season from 16 May–15 November. We aimed to determine if variation in urine-washing rates could be explained by the presumed need to regulate body temperature. Because temperatures at SSR are generally warm throughout the year (99.9% of temperature records are >20°C), we reasoned that, all else being equal, the need to thermoregulate at SSR would be greater at higher temperatures. However, C. capucinus at SSR engage in behavioural thermoregulation, in that they reduce activity levels in hot conditions (Campos & Fedigan, 2009). Furthermore, the effectiveness of evaporative cooling by urine from the hands and feet would be reduced in more humid conditions. Thus, our model needed to consider the effects of all of these variables as well as their interactions.

We calculated, for each focal sample, temperature and humidity as the half-hourly measurement nearest to the midpoint of the sample. We standardized the temperature and humidity values using Z-scores to make them directly comparable. Finally, we calculated for each focal sample the proportion of time that the focal animal spent in each of five activity states (defined
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(a) 35
   Dry
   Wet
   Dry

Temperature (°C)

Day

Relative Humidity

(b)

Dry
   Wet
   Dry

Rain (mm)

Day
above) based on the exact timing of state changes. We combined traveling with foraging because *C. capucinus* are very active foragers, regularly moving while searching or consuming for food items, and it was often impossible to make a confident distinction between these activity states. Thus, the observed activity budget for each focal sample consisted of proportions that were constrained to sum to unity, and this type of compositional data was unsuitable for our statistical modelling approach. As we were interested in using these data to account for a possible temperature–activeness interaction, we applied a principal components analysis on the activity budgets to reduce the dimensionality of the data. The first component explained 78.4% of the variance and had strongest variable loadings with forage/travel (0.786) and resting (−0.596). Therefore, a reasonable biological interpretation for the first component is that it represents the overall intensity of activity for each focal sample. We refer to this component hereafter as ‘activeness’.

Using the focal sample as the unit of analysis, we used a generalized linear mixed effects model (GLMM) to analyse urine-washing frequencies. We discarded any focal samples with fewer than 10 min of aggregate good-visibility time; the duration of good-visibility time, therefore, ranged from 10 to 15 min. We used the number of urine-washes by the focal animal as the response variable, for which we specified Poisson errors and a log link function. The fixed effects in our model included sex, temperature, relative humidity and activeness. We also included the duration of good-visibility time during the focal sample as an offset variable to account for the fact that urine-washing counts are expected to increase in proportion to this duration. To account for repeated measurements on the same individuals, we used focal animal as a random effect. Because we had good theoretical grounds for considering interactions between the fixed effects (particularly the temperature-by-activeness interaction), we included all two-way interaction terms among fixed effects in the GLMM. We implemented the analysis in the lme4 (Bates & Maechler, 2009) package for R version 2.15.2. We

**Figure 1.** Seasonal and day-to-day variation during 2011 in (a) temperature and relative humidity, and (b) daily aggregate rainfall. The temperature and relative humidity data consist of 48 measurements per day taken at regular 30-min intervals on each day of the year (17 520 total measurements). For each point in the upper plot (a), calendar date is indicated by the horizontal axis, temperature by the vertical axis, and relative humidity by the hue. This figure is published in colour in the online edition of this journal, which can be accessed via [http://booksandjournals.brillonline.com/content/1568539x](http://booksandjournals.brillonline.com/content/1568539x).
followed an information-theoretic model selection process based on AIC values in which we iteratively removed non-significant interaction terms followed by non-significant fixed effects to arrive at minimal adequate models, in which each term retained in the model was significant or removal of the term resulted in an increase in the AIC value.

2.3.2. Individual and social factors
Using known or estimated dates of parturition from 2010–2012, we divided the data for each female into categories based on her presumed reproductive status: pregnant, lactating, ‘other’ (defined below), and unknown. Female *C. capucinus* do not exhibit behavioural oestrus and we lacked the hormonal data to delimit the ovulatory state confidently, but the effects of ovulation/oestrus on female urine-washing in *Cebus* have been examined previously (Carnegie et al., 2005; Schino et al., 2011). Carnegie et al. (2011b) found that gestation in this population averages 158 ± 8 days. We, therefore, estimated the date of conception as the 158th day counting backwards from the date of parturition. We considered females to be pregnant from the estimated date of conception to the date of parturition. Following Carnegie et al. (2011b), we defined the lactation period as lasting 12 months after the date of parturition (or until the infant’s death within the first year). Because we could not confidently determine when females resumed cycling following lactation, we categorized females during all other periods as ‘other’. Thus, our ‘other’ category applies to both cycling and non-cycling females that are neither pregnant nor lactating. We used an Elo-rating script for R (Neumann et al., 2011) to arrange the females of each group into an initial linear dominance hierarchy based on dyadic interactions in which one female supplanted another. Due to generally low numbers of decided interactions, we supplemented this data with observations from 2008–2010 (Bergstrom & Fedigan, 2010; unpublished data). We standardized female dominance ranks by group size such that the highest-ranking female received a dominance score of 1 and the lowest-ranking female received a score of 0, with intermediate ranks evenly spaced between these scores. Male dominance relationships in this species appear to be more fluid than those of females (Perry, 1998), although the alpha male is always unmistakably detectable. We, therefore, used only two dominance categories to classify the males of each group: alpha and subordinate.

To evaluate the influence of these social factors on urine-washing, we built upon the GLMM for environmental variables described above (i.e.,
temperature, humidity and activeness). We fit separate models to males and females for this analysis because the new variables of interest — dominance and reproductive period — could affect each sex differently. Due to the very low frequency of urine-washing in the wet season (see Results) we excluded wet season data in these analyses of social variables. We began with full models that included all two-way interaction terms among environmental variables. For males we also included dominance without any interaction terms, and for females we included dominance and female reproductive state with an interaction term between these variables.

2.3.3. Spatial factors
To analyse the spatial pattern of urine-washing, we began by constructing a null model that assumes the spatiotemporal distribution of urine-washing is unpredictable and has no trend or association with spatial factors. If such a null model could adequately describe the patterns we observed, then further speculation on possible spatial functions for urine-washing (e.g., territorial scent marking) would not be justified. *C. capucinus* make differential use of space; thus, if urine-washes occur randomly throughout the day, a null model should expect the intensity of urine-washing to depend solely on the intensity of space utilization. For this analysis, we used dry season data only because urine-washing during the wet season was very infrequent (see Results). We constructed the null models in three steps. First, for each group/dry season combination, we defined a bounding polygon as the 100% isopleth given by applying the adaptive local convex hull method (Getz & Wilmers, 2004; Getz et al., 2007) to all systematically-collected ranging points. We implemented the method using the LoCoH R script (available online at http://locoh.cnr.berkeley.edu). We chose the ‘sphere of influence’ parameter, $a$, following recommendations by Getz et al. (2007), and we used ESRI ArcGIS 9.3 to manually remove small lacunae and repair the polygons’ geometries where appropriate (Figure A1 in the Appendix). Second, we used kernel density estimation to calculate the intensity function of space utilization for each group within these bounding polygons. A critical issue with kernel analysis is selecting a suitable smoothing parameter, as this value can strongly affect the size and shape of home range contours (Worton, 1989). Although there are data-based methods for selecting the smoothing parameter (reviewed by Gitzen et al., 2006), there is no a priori best method, and a subjective approach based on biological knowledge and data exploration may be a sensible alternative (Silverman, 1986; Kie et al., 2010). We used
a smoothing parameter of 50 m for the kernel analysis based on a visual exploration of a range of candidate smoothing parameters and because this distance corresponds roughly to the typical group spread for *C. capucinus*. Finally, we fit a model of urine-washing as an inhomogeneous Poisson process whose intensity function co-varies in direct proportion with the intensity function that describes space utilization (see Figure A2 in the Appendix). Although representing urine-washes as a Poisson point process violates a key assumption that the points should not be spatiotemporally autocorrelated, we believe that our model is justifiable because we are simply examining the difference between the intensity of urine-washing and the intensity of a background process (space utilization) that exhibits an identical autocorrelation structure.

We used spatial Kolmogorov–Smirnov tests to confirm that the observed spatial patterns of urine-washing did indeed depend on space utilisation. This test compares predicted (assuming complete spatial randomness) and observed distributions of the values of space utilization evaluated at urine-washing locations. We tested for interaction among urine-washing points (i.e., clustering or regularity) by generating simulated realizations of the null hypotheses, calculating the variance stabilized Ripley’s K function (i.e., L-function) for each realization, and comparing the resulting critical envelopes to the observed L-functions for urine-washing points. We used 39 simulations for each group to generate the critical envelopes, which corresponds to a test of size $\alpha = 0.05$ that the observed L-function value lies outside the envelope. After fitting the models, we assessed their fit using spatial Kolmogorov–Smirnov tests, as before, except that the predicted values of space utilization were given by assuming the null model rather than assuming complete spatial randomness. In cases where the null model was inadequate, we were interested in identifying areas of the home range in which the null model failed. We, therefore, used Monte Carlo tests to compare the observed count of urine-washes located within particular areas with counts from 1000 simulated realizations of the null model (see Figure A2). The home range zones examined included: overlap, core (50% kernel isopleth), periphery (a 100-m-wide strip at the outer boundary of the home range), fruit-containing (50-m buffer around all fruit trees in which an animal from the group was observed eating), intergroup encounter (50-m buffer around locations of intergroup encounters) and surface water (50-m buffer around locations of surface water in which an animal from the group was
observed drinking). We used a 50-m buffer distance for definite locations such as trees, water, and intergroup encounter zones to correspond with the smoothing parameter used for the kernel analysis. We used a larger 100-m buffer distance for the periphery zone because whereas the other buffers are symmetrical around the points (100-m diameter), the periphery buffer is asymmetrical around the home range boundary, extending 100-m inward toward the home range centre, because presumably the animals will not have used areas outside the home range. We used the R packages SPATSTAT (Baddeley & Turner, 2005), ADEHABITATHR (Calenge, 2006) and SP (Bivand et al., 2008) for the analyses, and we used $\alpha = 0.05$ for all tests except where noted.

3. Results

3.1. Data summary and qualitative observations

During 4380 total observation hours, we collected 3255 focal animal samples that were deemed of sufficient length and quality to be included in our analyses (individual mean total duration $\pm$ SE = 17.46 $\pm$ 1.20 h). We observed 875 total urine-washes by focal animals during these samples. We systematically collected 17 520 measurements of temperature and relative humidity, and we collected 8259 GPS points representing the focal groups’ locations. Table 1 provides a complete summary of the data set for each group and season.

Qualitatively, urine-washing appeared to exhibit two of the ‘design features’ that Palagi & Dapporto (2006) interpreted as evidence of communication: economy of urine production and multimodality of the washing behaviour. First, the volume of urine released during most instances of urine-washing was clearly lower than the volume typically released during urination without washing. Although we were unable to quantify this observation, it is worth noting that in a concurrent study that involved urine sample collection, few usable urine samples could be collected from animals that had washed with the urine due in part to the markedly lower volume of urine typically released during urine-washing (M. Bergstrom, unpublished data). Second, in accordance with Perry’s (1998) observations, urine-washing behaviour had two clearly different forms. The great majority of urine-washes by all individuals in the dry season had the following characteristics: (1) small volume of urine released, often only a few drops;
<table>
<thead>
<tr>
<th>Season</th>
<th>Focal group</th>
<th>Adult males</th>
<th>Adult females</th>
<th>Immatures</th>
<th>Contact hours</th>
<th>Focal hours</th>
<th>Location points</th>
<th>Urine-washes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>BH</td>
<td>3–4</td>
<td>5</td>
<td>9–11</td>
<td>343.2</td>
<td>69.7</td>
<td>671</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>6</td>
<td>10</td>
<td>18–21</td>
<td>339.9</td>
<td>106.8</td>
<td>639</td>
<td>187</td>
</tr>
<tr>
<td></td>
<td>EX</td>
<td>1</td>
<td>3</td>
<td>4–6</td>
<td>283.4</td>
<td>71.7</td>
<td>529</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>GN</td>
<td>6–8</td>
<td>10</td>
<td>14–19</td>
<td>459.5</td>
<td>117.5</td>
<td>870</td>
<td>212</td>
</tr>
<tr>
<td></td>
<td>LV</td>
<td>1</td>
<td>5</td>
<td>15–16</td>
<td>415.7</td>
<td>114.1</td>
<td>787</td>
<td>177</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1841.7</td>
<td>479.8</td>
<td>3496</td>
<td></td>
<td></td>
<td>846</td>
<td></td>
</tr>
<tr>
<td>Wet</td>
<td>BH</td>
<td>4</td>
<td>5</td>
<td>10–11</td>
<td>444.9</td>
<td>44</td>
<td>842</td>
<td>7</td>
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<tr>
<td></td>
<td>CP</td>
<td>6</td>
<td>10</td>
<td>19–21</td>
<td>555.1</td>
<td>81.6</td>
<td>1054</td>
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<tr>
<td></td>
<td>GN</td>
<td>5–6</td>
<td>10</td>
<td>18–21</td>
<td>549.4</td>
<td>69</td>
<td>1058</td>
<td>7</td>
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<tr>
<td></td>
<td>LV</td>
<td>1</td>
<td>5–6</td>
<td>13–16</td>
<td>443.3</td>
<td>59.4</td>
<td>832</td>
<td>1</td>
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<tr>
<td>Total</td>
<td></td>
<td>2538.3</td>
<td>323.6</td>
<td>4763</td>
<td></td>
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<td>29</td>
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</tr>
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</table>
few languid, unfocused washing motions; and (3) no clear social context. The urine appeared to be carefully applied to the hands and feet with little or no urine splashed onto the body or other substrates. By contrast, almost all urine-washes in the wet season and a small number of urine-washes in the dry season were characterized by (1) greater volume of urine released, which was often splashed onto the body and tail; (2) vigorous and rapid washing motions repeated several times; (3) piloerection and other signs of emotional arousal; and occasionally (4) ‘intense gargle’ vocalizations by the washing animal, which typically occur during sexual and appeasement contexts (see also descriptions in Perry, 1998; Gros-Louis et al., 2008). These vigorous urine-washes were performed almost exclusively by alpha males, often in close proximity to juveniles or other subordinate individuals who followed the male while emitting repeated ‘gargle’ vocalizations (Gros-Louis et al., 2008). Subordinate animals often sniffed the deposited urine. We did not code urine-washes as vigorous/regular during data collection, so we cannot offer a quantitative analysis of these apparently different forms.

### 3.2. Seasonal and environmental influences on urine-washing

Urine-washing was almost entirely confined to the dry season. Individual mean urine-washing rates reflect this striking and consistent seasonal pattern (Figure 2). During the dry season, washing appeared to become a routine part of urination (dry season: 86.6% urination with washing, 13.4% urination without washing; wet season: 8.6% urination with washing, 91.4% urination without washing). Across subjects, focal samples in which urine-washing was observed were characterized by a greater proportion of time spent traveling/foraging and lower proportion of time spent resting and socializing (Figure 3). The linear mixed-effects model that evaluated the influence of sex, temperature, relative humidity, and activeness on urine-washing frequencies indicated a significant positive association with activeness and significant negative associations with relative humidity and temperature (Table 2). The two-way interaction terms involving activeness and weather variables were also significantly negative, suggesting that the positive effect of activeness on urine-washing is reduced both with increasing temperature and increasing relative humidity (Figure 4). In addition, males exhibited significantly increased frequencies of urine-washing relative to females, although the estimated effect of sex was small. One interaction terms involving sex was also significant: the tendency for males to urine-wash more frequently was greater with increasing relative humidity.
Figure 2. Influence of season on individual mean urine-washing rates for 46 animals in 5 groups. Boxplots show the median (thick horizontal lines), first and third quartiles (upper and lower hinges), 1.5 times the interquartile range (thin vertical lines), and outliers (points).

3.3. Effects of reproductive status and dominance on urine-washing

The best model for female urine-washing (Table 2) included the environmental main effects temperature, relative humidity, and activeness (with both two-way interactions involving activeness). Although the estimated negative effect of temperature on urine-washing frequency only approached significance, its inclusion resulted in a lower AIC value. The model also retained the main effect female reproductive state: females in the ‘other’ and pregnant reproductive states urine-washed at greater frequencies compared to lactating females. Including standardized female dominance rank did not improve the model. For males, the best model (Table 2) retained the main effects temperature, relative humidity, and dominance status. Alpha males urine-washed at significantly elevated frequencies compared to subordinate males. As in the model for females, the estimated negative effect of temperature only approached significance. The interactions between activeness and both
relative humidity and temperature were also retained in the model, although activeness as a main effect was discarded.

3.4. Spatial distribution of urine-washing

The dry season home range boundaries are shown relative to each other in Figure A1. In all groups, the dry season spatial patterns of urine-washing depended strongly on space utilization (Kolmogorov–Smirnov tests, \( p \) values for all groups \( \approx 0 \)). The L-function simulation envelopes showed some evidence that urine-washes were spatially clustered (Figure A3). Kolmogorov–Smirnov tests of goodness-of-fit on the fitted models indicated that the null models could be rejected for some groups (\( D_{CP} = 0.131, \ p = 0.004; \)
Table 2.
Minimum adequate generalized linear mixed effects models for the factors influencing the frequency of urine-washing for all adult individuals in both wet and dry seasons, for adult females during the dry season, and for adult males during the dry season.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Fixed effect</th>
<th>Estimate ± SE</th>
<th>Z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of urine-washes (all, wet + dry seasons)</td>
<td>Intercept</td>
<td>−8.80 ± 0.09</td>
<td>-102.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Sex (Female vs. Male)</td>
<td>0.37 ± 0.13</td>
<td>2.82</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>-0.52 ± 0.06</td>
<td>-9.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Relative humidity</td>
<td>-1.54 ± 0.08</td>
<td>-19.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Activeness</td>
<td>0.80 ± 0.14</td>
<td>5.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Relative humidity × Sex (Relative humidity × Female vs. Male)</td>
<td>0.32 ± 0.08</td>
<td>3.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature × Activeness</td>
<td>-0.48 ± 0.14</td>
<td>-3.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Relative humidity × Activeness</td>
<td>-0.42 ± 0.17</td>
<td>-2.38</td>
<td>0.017</td>
</tr>
<tr>
<td>Number of urine-washes (adult females, dry season)</td>
<td>Intercept</td>
<td>-8.42 ± 0.12</td>
<td>-68.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>-0.14 ± 0.08</td>
<td>-1.75</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>Relative humidity</td>
<td>-0.81 ± 0.12</td>
<td>-6.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Activeness</td>
<td>0.61 ± 0.21</td>
<td>2.89</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Reproductive state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactating vs. Other</td>
<td>0.31 ± 0.13</td>
<td>2.51</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>Lactating vs. Pregnant</td>
<td>0.24 ± 0.12</td>
<td>1.98</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>Other vs. Pregnant</td>
<td>-0.07 ± 0.12</td>
<td>-0.60</td>
<td>0.548</td>
</tr>
<tr>
<td></td>
<td>Temperature × Activeness</td>
<td>-0.66 ± 0.19</td>
<td>-3.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Relative humidity × Activeness</td>
<td>-0.70 ± 0.28</td>
<td>-2.51</td>
<td>0.012</td>
</tr>
<tr>
<td>Number of urine-washes (adult males, dry season)</td>
<td>Intercept</td>
<td>-8.08 ± 0.15</td>
<td>-52.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>-0.19 ± 0.11</td>
<td>-1.65</td>
<td>0.099</td>
</tr>
<tr>
<td></td>
<td>Relative humidity (Subordinate vs. Alpha)</td>
<td>-0.55 ± 0.16</td>
<td>-3.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Dominance</td>
<td>0.47 ± 0.20</td>
<td>2.38</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Temperature × Activeness</td>
<td>-0.77 ± 0.24</td>
<td>-3.24</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Relative humidity × Activeness</td>
<td>-1.29 ± 0.26</td>
<td>-5.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Individual identity fitted as a random effect in all models.
Figure 4. Linear models of urine-washing (UW) frequency as a function of individual activeness at varying temperature (horizontal facets) and relative humidity (vertical facets). Temperature and relative humidity have been divided into quintiles to illustrate the effects of holding each variable approximately constant while varying the other. For a given temperature, the positive relationship between activeness and UW frequency is intensified under dryer conditions. For a given relative humidity, the positive relationship between activeness and UW frequency is reduced as temperature increases. Empty plots represent environmental conditions that did not occur naturally.

\[ D_{EX} = 0.141, \ p = 0.007; \ D_{GN} = 0.096, \ p = 0.040 \] but not for others \( (D_{BH} = 0.064, \ p = 0.675; \ D_{LV} = 0.049, \ p = 0.848) \). Visual examination of home range zones and smoothed residual plots (Figure A3) showed areas of greater or lesser than expected urine-washing intensity, but did not reveal a clear pattern across groups for the locations of such areas. The Monte Carlo
procedure to examine urine-washing in different home range zones revealed a few clear trends that were evident across groups (Table 3). First, no group showed more or less urine-washing than expected in the periphery zone or the inter-group encounter zones, and only one group showed significantly non-random urine-washing in the core zone. Three of five groups exhibited significantly more urine-washing than expected within 50 m of fruit trees, but only one of these results was significant at a more conservative $\alpha = 0.01$ level. Only 1 of 5 groups exhibited significantly more urine-washing than expected within 50 m of water sources. Urine-washing patterns in overlap zones showed an inconsistent pattern, with two groups showing elevated frequencies, one group showing reduced frequency, and two groups showing frequencies that did not differ from a random process.

4. Discussion

4.1. What is behind the seasonal difference?

The dramatic seasonal difference that we observed in urine-washing behaviour is consistent with previous findings for this genus (Robinson, 1979; Perry, 1998; Campos et al., 2007). What variables could be driving this effect? An obvious candidate is the most salient environmental characteristic of the dry season at SSR: the persistently dry conditions, typified by low relative humidity and the scarcity of surface water. Indeed, we found that, even within the dry season, urine-washing frequency varied inversely with relative humidity (Table 2). This scenario is consistent with the hypothesis that urine-washing may be used to condition the hands for improved grip (Harcourt, 1981). A concurrent study by Bergstrom & Fedigan (data not shown) that measured urinary creatinine found that the urine of capuchins at this site is significantly more concentrated during the dry season (dry season: median concentration = 0.525 mg/ml, $N = 192$; wet season: median concentration = 0.283 mg/ml, $N = 600$; Mann–Whitney $U = 82141$, $p < 0.001$).

We posit that, in dry conditions, the urine evaporates quickly from the skin surface, leaving a sticky residue of salts and other minerals that could improve grip on dry arboreal substrates. Improved grip would be particularly useful for animals engaged in foraging and traveling — precisely the contexts with increased urine-washing frequencies in this study — during which they regularly utilize thin terminal branches and adopt precarious hanging or
### Table 3.
Results of the Monte Carlo tests comparing the observed number of urine-washes in different home range zones with confidence intervals based on simulated realizations of the null models.

<table>
<thead>
<tr>
<th>Group</th>
<th>Zone</th>
<th>No. UW observed</th>
<th>Lower 99% C.I.</th>
<th>Lower 95% C.I.</th>
<th>Upper 95% C.I.</th>
<th>Upper 99% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH</td>
<td>Overlap</td>
<td>85</td>
<td>45</td>
<td>49</td>
<td>70</td>
<td>74</td>
</tr>
<tr>
<td>BH</td>
<td>Core</td>
<td>66</td>
<td>54</td>
<td>56</td>
<td>79</td>
<td>83</td>
</tr>
<tr>
<td>BH</td>
<td>Periphery</td>
<td>28</td>
<td>17</td>
<td>20</td>
<td>39</td>
<td>42</td>
</tr>
<tr>
<td>BH</td>
<td>Fruit</td>
<td>98</td>
<td>79</td>
<td>84</td>
<td>103</td>
<td>106</td>
</tr>
<tr>
<td>BH</td>
<td>IGE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>BH</td>
<td>Water</td>
<td>11</td>
<td>2</td>
<td>4</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>CP</td>
<td>Overlap</td>
<td>79</td>
<td>43</td>
<td>48</td>
<td>74</td>
<td>77</td>
</tr>
<tr>
<td>CP</td>
<td>Core</td>
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<td>75</td>
<td>81</td>
<td>107</td>
<td>111</td>
</tr>
<tr>
<td>CP</td>
<td>Periphery</td>
<td>58</td>
<td>45</td>
<td>47</td>
<td>73</td>
<td>77</td>
</tr>
<tr>
<td>CP</td>
<td>Fruit</td>
<td>132</td>
<td>102</td>
<td>105</td>
<td>130</td>
<td>135</td>
</tr>
<tr>
<td>CP</td>
<td>IGE</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>CP</td>
<td>Water</td>
<td>30</td>
<td>8</td>
<td>10</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>EX</td>
<td>Overlap</td>
<td>131</td>
<td>120</td>
<td>123</td>
<td>136</td>
<td>137</td>
</tr>
<tr>
<td>EX</td>
<td>Core</td>
<td>86</td>
<td>58</td>
<td>61</td>
<td>84</td>
<td>87</td>
</tr>
<tr>
<td>EX</td>
<td>Periphery</td>
<td>50</td>
<td>40</td>
<td>44</td>
<td>66</td>
<td>71</td>
</tr>
<tr>
<td>EX</td>
<td>Fruit</td>
<td>136</td>
<td>105</td>
<td>109</td>
<td>127</td>
<td>130</td>
</tr>
<tr>
<td>EX</td>
<td>IGE</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>EX</td>
<td>Water</td>
<td>16</td>
<td>3</td>
<td>4</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>GN</td>
<td>Overlap</td>
<td>36</td>
<td>36</td>
<td>42</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>GN</td>
<td>Core</td>
<td>116</td>
<td>86</td>
<td>91</td>
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<td>124</td>
</tr>
<tr>
<td>GN</td>
<td>Periphery</td>
<td>26</td>
<td>15</td>
<td>18</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>GN</td>
<td>Fruit</td>
<td>190</td>
<td>160</td>
<td>164</td>
<td>186</td>
<td>190</td>
</tr>
<tr>
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<td>IGE</td>
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<td>0</td>
<td>0</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>GN</td>
<td>Water</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>LV</td>
<td>Overlap</td>
<td>103</td>
<td>78</td>
<td>82</td>
<td>105</td>
<td>111</td>
</tr>
<tr>
<td>LV</td>
<td>Core</td>
<td>81</td>
<td>65</td>
<td>69</td>
<td>93</td>
<td>97</td>
</tr>
<tr>
<td>LV</td>
<td>Periphery</td>
<td>23</td>
<td>17</td>
<td>20</td>
<td>39</td>
<td>43</td>
</tr>
<tr>
<td>LV</td>
<td>Fruit</td>
<td>113</td>
<td>94</td>
<td>98</td>
<td>121</td>
<td>124</td>
</tr>
<tr>
<td>LV</td>
<td>IGE</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>LV</td>
<td>Water</td>
<td>27</td>
<td>10</td>
<td>13</td>
<td>29</td>
<td>32</td>
</tr>
</tbody>
</table>

The analysis includes dry season data only because urine-washing during the wet season was very infrequent. Zones with significantly greater or fewer urine-washes than expected are shown in boldface, and the confidence interval that was exceeded is underlined. Due to the large number of comparisons, we also present the more conservative 99% confidence intervals.
Urine-washing in white-faced capuchins

clinging postures. This action would be less useful in a wet environment because the urine would not evaporate quickly and the urine of well-hydrated animals would be more dilute. Below, we consider an alternative explanation that the seasonal difference is driven by reproductive seasonality.

4.2. Urine-washing is not a thermoregulatory behaviour

Because heat stress can be greater in the dry season (Campos & Fedigan, 2009), it is tempting to conclude that the large seasonal difference that we observed supports a thermoregulatory explanation for urine-washing. However, our multivariate analysis demonstrates that the seasonal difference alone is not sufficient to support this explanation. If the increase in heat stress that the animals experience during the hot hours of a typical dry season day were enough to cause the large seasonal difference (as predicted by the thermoregulation hypothesis), then one would expect urine-washing frequency to track temperature closely, with more urine-washes occurring at relatively high temperatures. Moreover, a positive temperature-by-activeness interaction effect would be strong evidence of a thermoregulatory function. We found that, after controlling for other possible influences on heat stress including relative humidity and activeness, the likelihood of urine-washing was reduced under conditions of both high temperature and high activeness, despite the overall positive relationship between activeness and urine-washing frequencies (Figure 4). Water conservation and the animals’ hydration status could possibly explain this interaction effect. The late dry season is the hottest time of year, and water availability is at its minimum (Campos & Fedigan, 2009). During especially hot periods, the animals may conserve water by producing less urine, which may prevent them from using urine-washing in its typical contexts. The significant humidity-by-activeness interaction is consistent with the idea that dryness and activeness work in concert to promote urine-washing except during certain periods, usually characterized by high temperatures, when the need for water conservation may take over. Taken together, our results suggest that ambient temperature is not likely to be the primary driver of variation in urine-washing frequencies, as predicted by the thermoregulation hypothesis.

These results are not entirely consistent with some recent studies on captive animals. Schino et al. (2011) found that urine-washing was positively affected by temperature in captive C. apella housed in Italy, but this effect was only evident at relatively cold temperatures (5–20°C) that are rarely encountered by the animals in natural habitats. Miller et al. (2008) found no
relationship between urine-washing and temperature in captive *C. apella*, but humidity did not vary in their study, and the abovementioned confounding variables may not have had an impact on captive animals. Nonetheless, neither of these studies supports the thermoregulation hypothesis. Past studies that claim support for the thermoregulation hypothesis for urine-washing in *Cebus* have based their support either on broad seasonal differences (Robinson, 1979) or on simple linear correlations between urine-washing and temperature without controlling for covariates such as relative humidity and activity levels (Roeder & Anderson, 1991). Our results provide the strongest evidence to date that the thermoregulation hypothesis for urine-washing in wild capuchins is not substantiated and should probably be abandoned.

4.3. *Is urine-washing a sexual signal?*

Our results provide mixed support for a sexual signalling function for urine-washing. The fact that the majority of conceptions in this species occur during the dry season (10/11 conceptions in this study; see also Carnegie et al., 2011a), when urine-washing is most common, offers some support for the sexual-signalling hypothesis. A similar pattern in the scent marking behaviour of other mammalian species has often been interpreted as evidence of inter-sexual signalling (Di Fiore et al., 2006; Palagi & Dapporto, 2006; Jordan et al., 2007). However, if this were a genuinely causal relationship among our study animals, we would expect seasonality in urine-washing and conceiveptive mating behaviour to be strongly associated. But whereas urine-washing behaviour is tightly linked with dry conditions, conceptions in this population occur throughout the year, even though they peak during the wet-to-dry season transition, (44% during the wet season and 66% during the dry season based on 100 conceptions over a period of 25 years: Carnegie et al., 2011a). Furthermore, sexual-signalling does not explain why sexually immature animals, including infants less than one year old, also urine-wash regularly during the dry season (Campos, 2008).

More notably, female urine-washing in our study varied with female reproductive status, with elevated urine-washing frequencies in the pregnant state and especially the ‘other’ state compared to the lactating state. Our findings complement recent studies showing that female capuchins do not urine-wash more around the time of ovulation (Carnegie et al., 2005; Schino et al., 2011), but taken together, the relevance of these temporal patterns in female urine-washing with respect to the sexual signalling hypothesis is
unclear. More urine-washing might be expected among pregnant and ‘other’ females to attract potential mates and confuse paternity, respectively, but this scenario would not explain why others have found that urine-washing frequencies are lower around the time of ovulation than during other reproductive states. Our observation that alpha males urine-wash more frequently than subordinates could also be taken as support for the sexual signalling hypothesis because alpha males engage in more conceptive mating, siring from 70–90% of the infants in multi-male groups (Jack & Fedigan, 2006). Although this might explain why alpha males urine-wash at elevated frequencies compared to subordinate males, it is not a satisfactory explanation for the other seasonal, environmental, and inter-individual variation in urine-washing behaviour that we observed. Moreover, very few urine-washes in this population occur in sexual contexts (10/787 = 1.27% in a previous study, Campos, 2008).

Intriguingly, glucocorticoid levels in this population follow a similar pattern to urine-washing, with significantly higher cortisol levels among females in the dry season compared to the wet season (Carnegie et al., 2011b), and higher cortisol levels among alpha males than subordinate males (Schoof & Jack, 2013). We lack hormonal data during our study period to examine this relationship analytically. The possibility that urine-washing frequencies track stress or general arousal levels warrants further study.

4.4. Urine-washing is not obviously a form of territorial scent marking

Our spatial analysis did not reveal strong evidence of a strategic spatiotemporal pattern in the locations of urine-washing. All of the null models that assumed urine-washing was a completely random process were inadequate to varying degrees. Although the spatial Kolmogorov–Smirnov tests did not reject the null hypothesis for BH and LV groups, this test is only useful for assessing whether the trend in the data was correctly identified — that is, whether the intensity of urine-washing was directly proportional to space utilization. Another critical assumption of the model is that the points were independent of each other, and here, all of the null models failed (except perhaps for the BH group) because urine-washing was spatially clustered. In light of the results discussed above, this finding is not surprising. Because urine-washing is significantly affected by other factors that vary continuously — including temperature, humidity, activeness and social context — we should expect urine-washing frequencies to vary accordingly with these factors. Spatially, this would be manifested as clustering.
Regarding the hypothesis that a major function of urine-washing in *C. capucinus* is to advertise occupancy of a territory or ownership of resources, the spatial analysis of urine-washing in this study was equivocal. Opposing sides in a recent debate (Heymann, 2000, 2006; Roberts & Gosling, 2001; Lledo-Ferrer et al., 2011, 2012; Roberts, 2012) about the optimal spatial pattern for scent marks that advertise occupancy of a territory or ownership of a resource share one fundamental idea: after accounting for other factors, territorial scent marks should show *some* non-random spatial pattern that is influenced by the locations of resources, competitors, or some combination of the two. Several common predictions, including the ideas that territorial scent marks should establish exclusive areas, prevent intrusions, ensure access to feeding areas, or enable avoidance of rival groups, may not be theoretically sound because animals may choose to ignore scent marks that they encounter if the benefits of doing so outweigh the potential costs (see Roberts, 2012, for a detailed discussion). In support of the territoriality hypothesis, urine-washing in three of five groups was spatially clustered near fruit trees, which suggests that urine-washing could be used to communicate use of valuable trees to rival groups. This interpretation would be consistent with theoretical models of optimal scent marking strategies in mammals (Gosling, 1981; Gorman & Mills, 1984; Gosling & Roberts, 2001; Roberts & Gosling, 2001; Roberts, 2012) in cases where home ranges are not economically defensible or the costs of producing or depositing the marking substance are high. These conditions are likely to be true in this population of *C. capucinus*: they have extensively overlapping home ranges, with up to three groups competing for access to the same food tree; and they are water-limited during the dry season (Campos, 2008), suggesting that urine could indeed be in scarce supply. However, this line of reasoning is weakened by the facts that in two groups there was no tendency for urine-washing to occur disproportionately near fruit trees, in two other groups the association was not significant at a more stringent $\alpha = 0.01$ confidence level, and in only 1/5 groups was there a significant association between the locations of urine-washing and water sources. Furthermore, the territory-marking hypothesis does not neatly explain some other observed characteristics of urine-washing. For one, why should urine-washing virtually disappear for the entire duration of the wet season? *C. capucinus* at this site continue to eat fruit during the wet season, and in fact the late wet season represents the nadir of fruit abundance at SSR
(Carnegie et al., 2011a), making the fruiting trees during this period all the more valuable. An alternative explanation is that other conditions that promote urine-washing, such as generally increased activeness, are intensified when the animals are near fruit trees.

4.5. Converging on a parsimonious hypothesis

Below, we build on the widely-held idea that urine-washing is a multifunctional behaviour, but we emphasize that our data suggest that the primary function of urine-washing is non-social. If lesser social functions for urine-washing exist for wild *C. capucinus*, they appear to be largely masked in the dry season by a non-social function that becomes overwhelmingly primary in very dry conditions. Wet, humid conditions appeared to practically eliminate the need or usefulness of urine-washing for most animals in our study. Urine-washing patterns do show some signalling characteristics, such as economy of production, some variation with social context, and a non-random spatiotemporal distribution. Specifically, a lesser volume of urine is typically used during washing than during urination, the timing of most conceptions coincides very roughly with the period of peak urine-washing, alpha-males engage in more urine-washing than subordinates, and urine-washing may be spatially clustered around fruit trees. However, the lesser volume of urine merely suggests that urine-washing has some function; it does not necessarily indicate that the function is communication. Moreover, the links to reproductive seasonality, dominance, and territoriality do not adequately explain one characteristic that distinguishes urine-washing from typical scent-marking behaviour: that the urine is rubbed against the bare skin of the hands and feet. If the goal were to transmit the signal to an audience of conspecific animals, why not apply the urine onto a prominent physical object in the environment, as nearly all other mammals do when they scent mark? If the goal were to mark oneself with the urine’s odour, why not focus on rubbing the urine into the fur? Although some animals, especially agitated adult males, do rub the tail or abdomen during vigorous urine-washing (Perry, 1998), most instances of urine-washing that we observed involved only the hands and feet. Applying the urine to the animal’s own hands and feet, which are in frequent contact with aromatic food objects and abrasive substrates, would seem an inefficient way of transmitting an olfactory signal.
Perry (1998) was the first to identify that urine-washing by *Cebus* may take two different forms. Perry suggested that the non-vigorous urine-wash might be a surreptitious act by subordinate males, who perhaps restrain themselves from washing vigorously to escape detection by dominant males. Our observations are more consistent with the idea that the non-vigorous urine-wash is the ‘typical’ form, practiced by all group members in dry conditions, and that the vigorous urine-wash performed chiefly by agitated alpha males may have additional display or signalling functions. This may explain two of the three significant interaction effects in our statistical model. Under conditions in which the ‘primary’ mechanical function for urine-washing is reduced (higher relative humidity), the tendency for males to urine-wash more frequently than females increased, and urine-washing frequencies more-closely tracked activeness levels. Thus, there may be a male-specific signalling function for urine-washing that, based on the frequently accompanying vocalizations, may be related to sexual or appeasement contexts (Gros-Louis et al., 2008).

In light of our results and past research on this topic, we offer the following hypotheses for the functional significance of urine-washing in *C. capucinus*. We believe that the behaviour is primarily stimulated by the need to condition the hands and feet during locomotion and foraging in dry conditions, perhaps to improve grip on arboreal substrates. This effect is intensified by activeness and general arousal level. The behaviour appears to become habitual during persistently dry conditions such that it becomes a routine part of urination. Thus, not all urine-washes in the dry season occur in an obviously purposeful context. Because capuchins are capable of gleaning social information from olfactory inspections of urine (Phillips et al., 2011), urine-washing may be exploited for various signalling purposes that may include sexual signalling or advertising ownership of resources. The way in which urine-washing would function as a sexual signal remains unresolved, as female urine-washing varies with reproductive state, but not in a way that conforms to sexual-signalling predictions. Finally, agitated dominant males may perform a vigorous form of urine-washing that involves splashing urine onto the body, piloerection, and vocalizations. Future studies that aim to assess the possible uses of urine-washing for communication should distinguish between the regular and vigorous forms of urine-washing, as their functions may differ.
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References


Appendix

Figure A1. Bounding polygons for the home ranges of the five study groups during the 2011 dry season.

Figure A2. Visual representations of the spatial analyses for each group (a, BH group; b, CP group; c, EX group; d, GN; e, LV group), based on the null model that urine-washing is a random process. The top left panel (“Home range usage”) shows the boundary polygon (thin black line) and the intensity function of space utilization (coloured shading). The top centre panel (Smoothed raw residuals) shows the observed locations of urine-washing (points) over the residuals of the fitted model. Red areas indicate greater than expected intensity of urine-washing, while blue areas indicate lesser than expected intensity of urine-washing. The top right panel (Simulation) shows an example of one realization of the null model (points), which we used for the Monte Carlo procedure. Note that none of the real, observed urine-washes are depicted, and that the spatial pattern of the simulated points reflects the intensity of space utilization. The six panels in the centre and bottom rows show the smoothed residuals of the fitted model in relation to the home range zones (thick black lines) that we examined with the Monte Carlo procedure. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/1568539x.
Figure A2. (Continued.)
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Urine-washing in white-faced capuchins

**Figure A3.** Multi-distance spatial cluster analysis based on the L-function. Plots show simulation envelopes comparing the observed pattern of urine-washing to 39 simulated realizations of the null hypothesis — that urine-washing is a random process whose intensity covaries in direct proportion with space utilization. Plots show the observed value (solid black line), the sample mean from simulations (dashed red line), and the critical envelope (grey shaded area). The probability that the observed value lies outside the envelope is 0.05. Observed values above the critical envelope indicate significant clustering, whereas observed values below the critical envelope indicate significant regularity. The spatial pattern of urine-washing showed clear evidence of clustering in all groups, especially at spatial scales smaller than 200 m, except for the BH group. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/1568539x.