Objective 3: Assess if chemical signatures encode group membership

3.1 Introduction. Objectives 1 and 2 document how the Ngogo community fragmented and why the split became violent, but they do not explain how individuals *perceive* who belongs to which group, particularly when boundaries are shifting. The *goal* of this objective is to determine whether chemical signatures encode group membership and whether sniffing behavior is positioned to detect these differences. Our *working hypothesis* is that Western and Central chimpanzees have distinguishable urinary volatilome profiles; and that sniffing behavior clusters at territorial boundaries where such information would be most valuable. This objective is *justified* by three considerations. First, chimpanzees have long been observed sniffing the ground during territorial patrols (Goodall 1986), and experiments from captivity suggest that chimpanzees distinguish urine from in-group and out-group members (Henkel and Setchell 2018), yet the olfactory abilities of chimpanzees have received little theoretical attention. Second, work on social mammals demonstrates that odors can encode social information including group membership (Burgener et al. 2009; Theis et al. 2013; Weiß et al. 2025). Our *approach* combines high-sensitivity analysis of urinary volatile organic compounds with spatial analysis of sniffing behavior. The *expected outcome* is a mechanistic account of how group membership may be perceived through olfaction, connecting behavioral observations of sniffing to chemical ecology.

3.2 Research Design. We propose to achieve these goals through the following studies:

3.2.1 Characterize urinary volatilome and test for group differences. If chemical signatures encode group membership, Western and Central chimpanzees should have distinguishable urinary volatilome profiles. Testing this requires identifying volatile organic compounds (VOCs) in chimpanzee urine, assessing individual and sex-based variation, and determining whether group membership predicts chemical composition above these other sources of variation. We will collect urine samples non-invasively when chimpanzees urinate onto vegetation or the ground, store samples at -20°C in the field, and ship them to UT Austin for analysis at −80°C. We will analyze samples using a Vocus 2R proton transfer reaction time-of-flight mass spectrometer (Vocus PTR-TOF-MS) in collaboration with Senior Personnel Dr. Pawel Misztal (UT Austin). PTR-TOF-MS offers several advantages over traditional gas chromatography-mass spectrometry for this application: higher sensitivity for trace VOCs, softer ionization that preserves molecular structure, and rapid analysis time that enables high sample throughput. We will sample at least 40 individuals (20 Western, 20 Central), with 3-5 samples per individual across different seasons to assess within-individual stability and seasonal variation. We will use Partial Least Squares Discriminant Analysis to classify samples by group and assess classification accuracy using leave-one-out cross-validation. We will identify compounds driving group separation. We will use Permutational Multivariate ANOVA to test for group differences while controlling for sex, age class, and individual identity. We will consider groups distinguishable if classification accuracy significantly exceeds chance.

3.2.2 Determine timing of chemical divergence using archived samples. A key question is whether chemical divergence preceded, paralleled, or followed behavioral divergence. If chemical signatures diverged *before* behavioral separation, this would suggest chemical identity may have contributed to fission. If divergence paralleled or followed behavioral separation, this

would suggest chemical identity is a consequence of social and ecological separation. We will analyze archived urine samples collected in 2014-2015 from individuals who later became Western or Central group members. These samples are stored at -80°C at the PI's lab at University of Texas at Austin. We will apply the same protocols used for current samples. Because we are comparing *within-period* differences (Western-affiliated vs. Central-affiliated individuals in 2014-2015), any storage-related degradation should affect both groups equally and not bias our comparison. We will compare the magnitude of group differences in 2014-2015 samples versus current samples. We predict that 2014-2015 samples will show less differentiation between proto-Western and proto-Central individuals than current samples show between established Western and Central groups.

3.2.3 Identify biological and ecological correlates of volatilome variation. Our preliminary analysis identified compounds driving group differences, including fatty acid derivatives, plant-derived aromatics, and fruit-related compounds. These suggest that diet, microbiome, and physiological differences may all contribute to group-level chemical signatures. Understanding which factors drive VOC variation will help distinguish "group markers" (compounds that could signal group membership) from ecological confounds (compounds that differ simply because groups occupy different territories). We will link volatilome data to three sources of individual-level and ecological data. (1) *Feeding behavior.* We will extract dietary data from long-term behavioral records, quantifying the proportion of feeding time spent on different food types for each individual in the months preceding sample collection. (2) *Territory-specific food availability.* A Ugandan MSc student has collected ecological plot data (2025) on food tree distribution across Western and Central territories. We will assess whether food availability differences predict VOC differences. (3) *Physiological state.* We will record health status, reproductive state (for females), and recent social interactions (e.g., involvement in aggression) that might affect stress-related compounds. We will use variance partitioning to assess the relative contributions of group membership, diet, and individual identity to VOC variation. We will identify candidate "group marker" compounds as those that (a) strongly differentiate groups (high VIP scores), (b) remain significant after controlling for dietary variation, and (c) are plausibly informative given primate physiology (e.g., compounds related to hormonal state, skin secretions, or microbiome).

3.2.4 Test whether sniffing behavior clusters at territorial boundaries. If chemical signatures encode group membership, sniffing behavior should be concentrated at territorial boundaries where such information is most valuable. We have 200 GPS-tagged sniffing observations from 2014-2021, spanning the period of major network change (2015), gradual separation, and final split (2018). This allows us to test whether sniffing is spatially concentrated at boundaries and whether this pattern changed as boundaries emerged and stabilized. We define a sniffing event as an instance when a chimpanzee puts its nose to the ground (excluding feeding contexts). Each observation is tagged with GPS coordinates. We will overlay sniffing locations on territory maps derived from GPS ranging data, classifying locations as "boundary zone" (areas of home range overlap between groups) or "interior" (areas used exclusively by one group). We will compare sniffing density in boundary zones versus interior zones using spatial point pattern analysis. We predict that sniffing is disproportionately concentrated in boundary zones. We will also test whether this pattern strengthened over time: we predict that sniffing at

boundary locations increased as the territorial border emerged and became contested (2015-2018) and may have decreased as the border stabilized (post-2018).

3.3. Expected Outcomes. Accomplishment of this objective is expected to yield knowledge of the compounds present in chimpanzee urine that may play a role in their psychophysiology and social behavior. We expect to confirm that Western and Central chimpanzees have distinguishable volatilome profiles and to identify a subset of compounds that reliably differentiate groups. We expect chemical divergence to be a consequence of social separation. Some group differences may reflect ecological variation, while other compounds represent candidate group markers that may encode social or physiological information independent of diet. We expect sniffing behavior to cluster at territorial boundaries, consistent with olfactory assessment of group membership and territorial status. Changes in sniffing distribution over time would suggest that chimpanzees adjusted their olfactory sampling as social geography changed.

3.4 Potential problems. Our preliminary analysis of 47 urine samples from 18 individuals (10 Western, 8 Central) demonstrates that Western and Central chimpanzees cluster separately in PCA and PLS-DA analyses. However, sample sizes are modest, and we have limited replication within individuals. Although the possibility is considered remote given the strength of our preliminary findings, our working hypothesis of group differences could find weak support after expanding the sample size. However, results will still be valuable for identifying volatile compounds in urine, and identifying possible olfactory cues used by chimpanzees. Another problem arises from analyzing archived samples prior to the permanent fission. Such samples may show significant degradation that differentially affects compound classes, we may not be able to directly compare effect sizes across time periods. However, we can still assess whether proto-Western and proto-Central individuals were distinguishable *within* the 2014-2015 period.