Pan African Programme
The cultured chimpanzee

Guidelines for research and data collection

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Notes on this version: Changes from the June 2012 version of the protocol are highlighted and/or footnoted in this July 2014 version. Addendums to the original protocol are listed in the last chapter of this document.

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[1] Added in this (2014) version of protocol

1 Pan African overview

Chimpanzees are disappearing from many regions in Africa at an alarming rate, while our understanding of chimpanzee biology, social life, tool use and culture is still strongly limited by our narrow sampling of their population diversity. The aim of this program is to answer specific hypotheses about the evolutionary-ecological drivers that have generated the behavioural variability that we find in chimpanzees across Africa. Questions that we address include but are not limited to the following:

- Under which socio-demographic and ecological conditions is meat consumption likely to increase in frequency?
- Under which conditions does diversification of behaviour emerge?
- Under which conditions does tool use complexity increase?

The specific project aim is to collect systematic ecological, social, demographic and behavioural data on 35 to 40 chimpanzee populations spread out over their whole natural range. This will include both 'temporary research sites' (TRS) with totally unhabituated chimpanzees as well as established 'long-term research sites' (LRS) with well-studied chimpanzees.

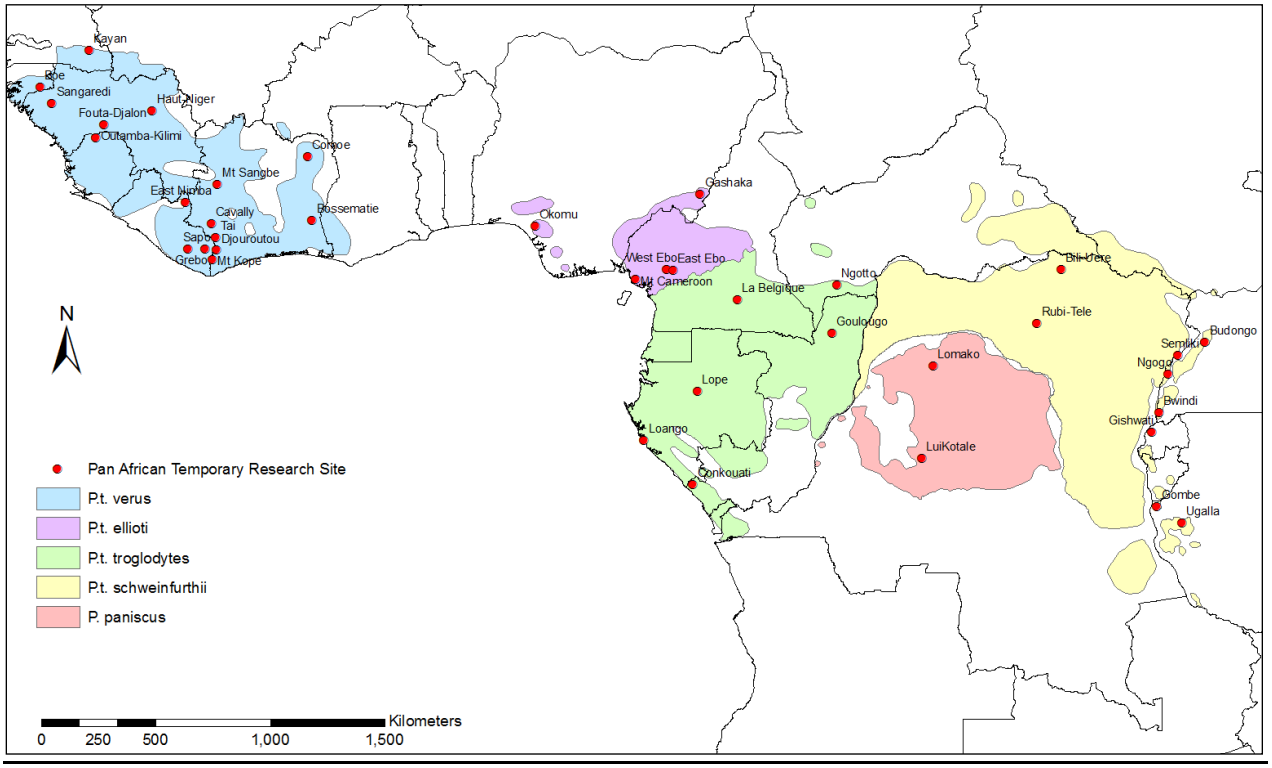

Figure 1 Locations of temporary research sites across the chimpanzee range

Over the course of at least 12 continuous months we gather at each site detailed data on the ecology, demographic and social structure and tool use behaviour of ideally one social unit of great apes. By systematically collecting data with one same protocol and using the latest methodologies for example with stable isotopes, automated video analysis, remote sensing data and complex modelling techniques, we aim at making great progress towards understanding chimpanzees, and test hypotheses that have been forwarded for the evolution of humans.

2 Site Selection

Purpose: Identify chimpanzee core area for grid placement, which will be used for all data collection

IMPORTANT

- All data collection will only take place within a 'data collection zone' defined as the area within a 20 to 100 km^2 grid with cell size of 1x1 km
- If no chimp community is known at the site, firstly conduct a recce survey to identify hotspots of chimpanzee activity
- Once identified place the grid with 1x1 km cell size and centre it on a hotspot of chimpanzee activity
- Ask if unsure about the grid placement as this will be the basis of the TRS for the next 12 months
- Use data entry sheet 'Recces' for the recces (see annex III)

2.1 Setting up a temporary research site

If the chimpanzee home range limits or core area are already known at your site, place a grid with 1x1 km cell size on a map of the area that completely covers the chimpanzee home range. However, in most cases the boundaries or core area of a chimpanzee community will not be known. A recce survey is needed to determine the hotspots of chimpanzee activities, remembering that chimpanzee territory use is seasonal and the recce survey reveals only the activities for the last months. An area of a minimum of 50 km² (about 7x7 km) is covered with recces evenly spaced by 500 m in a closed rainforest (Figure 2-1) and an area of about 100 km^2 (10 x 10 km) in a woodland savannah.

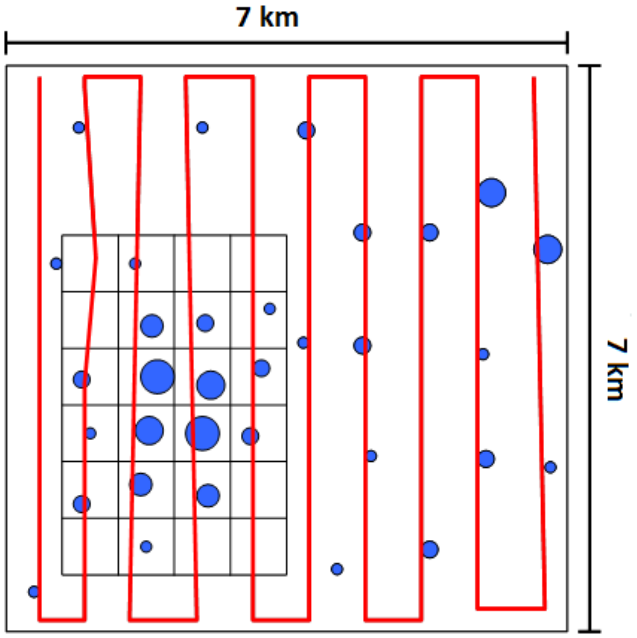

Figure 2-1 Example for the establishment of a temporary research site, with recce walk (red line), 1x1km grid (black) and cluster of chimpanzee signs (blue circles).

A recce (or reconnaissance) is a path of least resistance through an area following a compass bearing (e.g. north-south, southeast-northwest, east-west). During the recce walk all signs of chimpanzees are recorded (sightings, vocalization, feeding signs, footprints, nests, dung and carcasses) and a GPS coordinate taken. Remember to activate the tracklog on the GPS. Recorded signs are pooled per every 500 m of recce and plotted in a GIS map. Different signs (nest, faeces, feeding remains, tool use sites, traces, and vocalisations) are separately pooled and plotted. When plotted, the signs will form one or several clusters which indicate areas of different intensity of use by chimpanzees. A grid with 1x1 km cell size will be laid on the centre of one such cluster. The grid is extended at minimum 2-3 km into all directions to cover a minimum of 20-50 km² in rainforest and 50-100 km² in woodland savannah. This is a function of resource and animal density so if you have a site with very low density you should try to have a grid in the upper range. Ideally this grid includes one complete chimpanzee community home range. All data collection takes place only within the gridded area and is named the 'data collection zone'. If this procedure does not deliver a convincing site selection result, please contact MPI to find a solution.

2.2 Data collection and sampling design

The grid covering the data collection zone and divided up into 1x1 km cells is labelled for better referencing during communication as followed: Columns are labelled with numbers starting with '1' for the Western most cell and letters for rows, starting with 'A' in the Northern most cell (Figure 2-2).

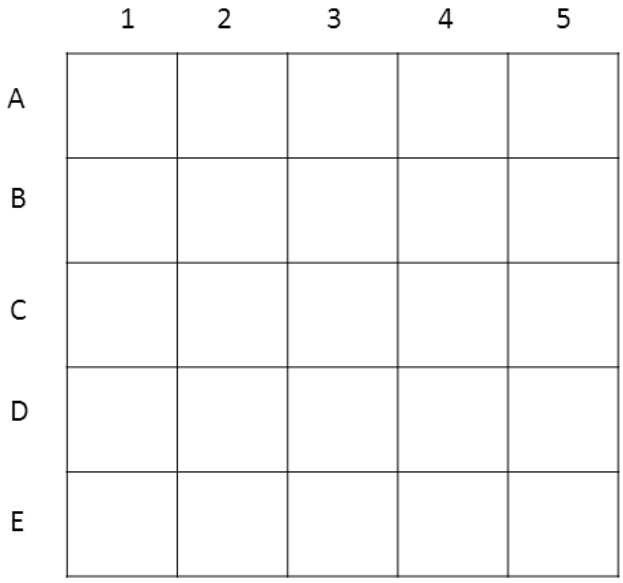

Figure 2-2 Example of a grid and its labelling methodology

The grid is used to distribute sampling units, such as camera traps (see chapter 8 for details) or line and strip transects (see chapter 13 for details) systematically across the study area. This will ensure a balanced spatial coverage, which is essential to draw reliable conclusions from collected data. The collection of organic material such as hair or faecal samples is opportunistic and not bound to particular cells within the grid.

2.3Definitions

It is important to distinguish between the following terms (Figure 2-3):

- *Grid*: divided up into 1 x 1 km cells that is placed over a cluster of chimpanzee signs recorded during recces
- *Data collection zone*: the cells that contain chimpanzee signs and are used for the study

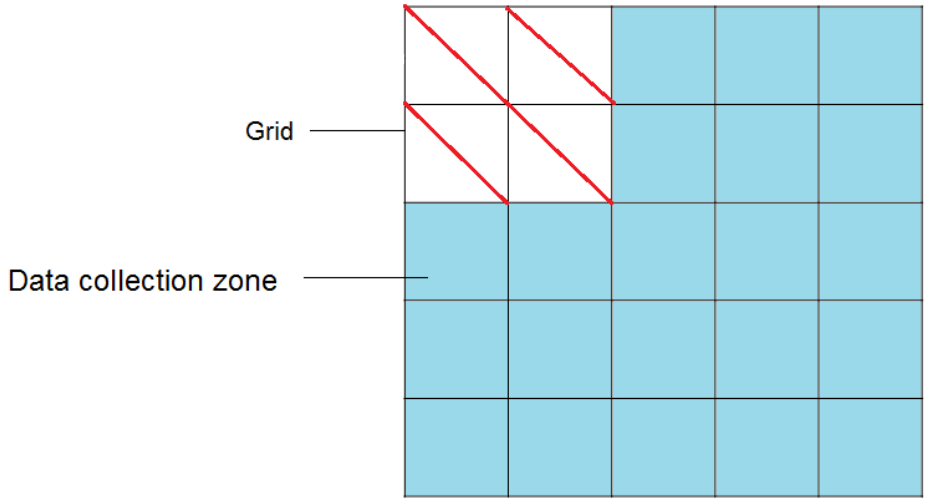

Figure 2-3 Illustration of the different terms. Cells with red diagonal lines indicate cells that are either inaccessible (e.g. lake, river etc.) or totally degraded (e.g. field), where data collection will not take place. The grid therefore would include the non-sampling area, whereas the data collection zone (blue cells) excludes these inaccessible/degraded zones.

2.4References

White, L. and Edwards, A. (2000) Conservation research in the African rain forests – a technical handbook. Wildlife Conservation Society.

3 Data and Samples

In order to draw valid conclusions from collected grid and to make them comparable across the chimpanzee range a minimum sample size is required per TRS (Table 3-1). Contact MPI if you have issues with the collection of any of these data/samples or if you anticipate that you will not be able to reach required minimum sample sizes.

More details will be found in the relevant section about the quality and amount of each sample needed for the different category and species to be sampled.

Table 3-1 Minimum amount of data required for each sample type.

Class	Type of sample	Category	Sample type	Minimum sample no.		Frequency of collection
				No. species per category/ sample type	No. samples per category / species	
Cameras	**Camera traps**	Chimpanzees	All videos	-	200	continuous
			Tool videos	-	As many as possible	
		Other species	All videos	-	200	continuous
Transects	**Strip**	-	-	>20 km		once
	Line	-	-	>20 km per survey (i.e. across 12 months surveyed every 4 months = min. 60 km		every 4 months
	Habitat plots	-	-	> 200 plots (i.e. 200 *20x20m plots = min. 80 km^2)		once
	THV plots	-	-	> 200 plots (i.e. 200*1x2m plots = min. 400 m^2)		once
Phenology	-	-	-	10 individual trees for each of 15-20 important tree species		12 continuous months
Organic samples	**Genetic samples**	Chimpanzees & Gorillas[2]	Faeces	-	200	continuous
	Pathogen samples	Chimpanzees & Gorillas	Faeces	-	50	continuous
	Diet samples	Chimpanzees	Faeces	-	100	continuous
		Chimpanzee	Urine	-	10	continuous
	Isotope samples	Chimpanzees & Gorillas[2]	Hair	-	50 nests	Continuous
		Chimpanzees	Bones	-	3	continuous
		Plants: Important species	Herbs	15	3 per species per habitat	2-3 times across the study period
			Leaves			
			Seeds			
			Nuts: *Coula, Detarium, Elaeis, Panda, Parinari, Sacoglottis*	1-6	3 per species per habitat	

[2] Although collected at other field sites, previous versions of the protocol omitted gorillas in this table

			Fruits	15-20	3 per species per habitat	
		Insects: Important species	Termites	3	3 per species per habitat	along strip transects
			Army ants	5	3 per species per habitat	2-3 times across the study period
			Bees: *Apis*, *Meliponae*, *Xylocopa*	3	3 per species per habitat	2-3 times across the study period
		Herbivores	Shells, bones, hair, feathers, scales,	-	10	continuous
		Omnivores		-	10	continuous
		Insectivores		-	10	continuous
		Carnivore		-	5	continuous
		Insects		-	5	continuous
		Water	Rivers/ streams	-	2-3 per habitat type	continuous
	Geology reference samples	Snail shells	-	Same samples as for isotope (see above)		
		Nuts	-			
		Termite soil	-	1-3	3	continuous
Traps	Stingless bees			4 traps per habitat type per height per season, evenly distributed across the grid.		once for each height in dry & wet season (i.e. four times)
	Blow flies			every ~1km along line transects in different habitats. Max. 20 samples at each sampling spot		once in dry & wet season (i.e. twice)

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4 Habitat Structure

Purpose: To identify tree and herb species present, and their densities in the data collection zone.

IMPORTANT

- 20 m x 20 m habitat plots are placed every 100 m along the transect crossing the grid cells
- Measure all trees with DBH ≥ 10 cm
- DBH measured at 1.30 m height
- Between the plots, record all nut tree species within a 10 m belt along the transect
- Sample one 2 m^2 THV plot consistently in one of the corners within each habitat plot
- THV = count separately the number of herbs and number of saplings <2m height within the plot
- Conduct in parallel with camera trap installation
- Use data entry sheet 'Habitat Structure' (see annex III)

4.1 Habitat plots

Vegetation abundance and distribution will be determined to allow comparison of forest productivity and food availability across different sites. Habitat plots are sampled as one of the first activities once the TRS is established and the data collection zone is defined. Habitat plots are also the basis for selecting trees for the phenology study.

Habitat structure plot data will be collected every 100 m within a 20 m x 20 m plot centred on the transect (Figure 4-1). For details on transects, see chapter 13. Within these plots, all trees with a Diameter at Breast Height (DBH) of ≥ 10 cm will be measured. DBH must be identified and measured at a height of 1.30 m on the up-hill side of the tree. Wrap the tape measure around the tree trunk where possible and record the circumference to calculate the diameter:

Diameter = circumference / π where π = 3.14

Woody climbers that are associated with these trees are not to be recorded. In the case of buttress trees, the tape measure is pushed above the buttresses to take the measurement. If the buttress is too high for you to reach, estimate the diameter with the help of a stick held horizontally. Where there is a swelling in the tree, measure the girth below the swelling. A tree is considered to be in the plot when the centre of the tree is inside the plot.

In between the 20 m x 20 m plots, data on tree species, whose nuts are cracked by chimpanzees, are recorded continuously within a 10 m belt, i.e. 5 m on each side of the transect. Data recorded are the same for all other species in the quadrat plots.

In woodland savannahs where the habitat is heterogeneous, conduct habitat plots across gallery forests (Figure 4-2). The interval of the transects, along which habitat plots will be placed, and the distance between the habitat plots will be adapted to the site so that at least 200 plots can be surveyed. The size of each plot may be smaller and will depend on the particular site. The transects will be placed such that it extends across different habitat types.

Ideally trees are identified to species level on the spot but if not, collect samples and take pictures of the bark and branches with leaves, and if possible fruits, flowers or seeds for later identification at camp. When taking a picture always put a ruler or some other size reference next to the plants. If samples need to be stored for more than one or two days they have to be pressed in a plant press between newspapers (Figure 4-3) and kept for identification by an expert.

4.2 THV

To measure terrestrial herbaceous vegetation (THV), count the number of non-woody monocotyledon plants and small woody saplings in a 1 x 2 m plots (2 m²) once in each habitat plot. The monocotyledons and woody saplings are recorded separately.

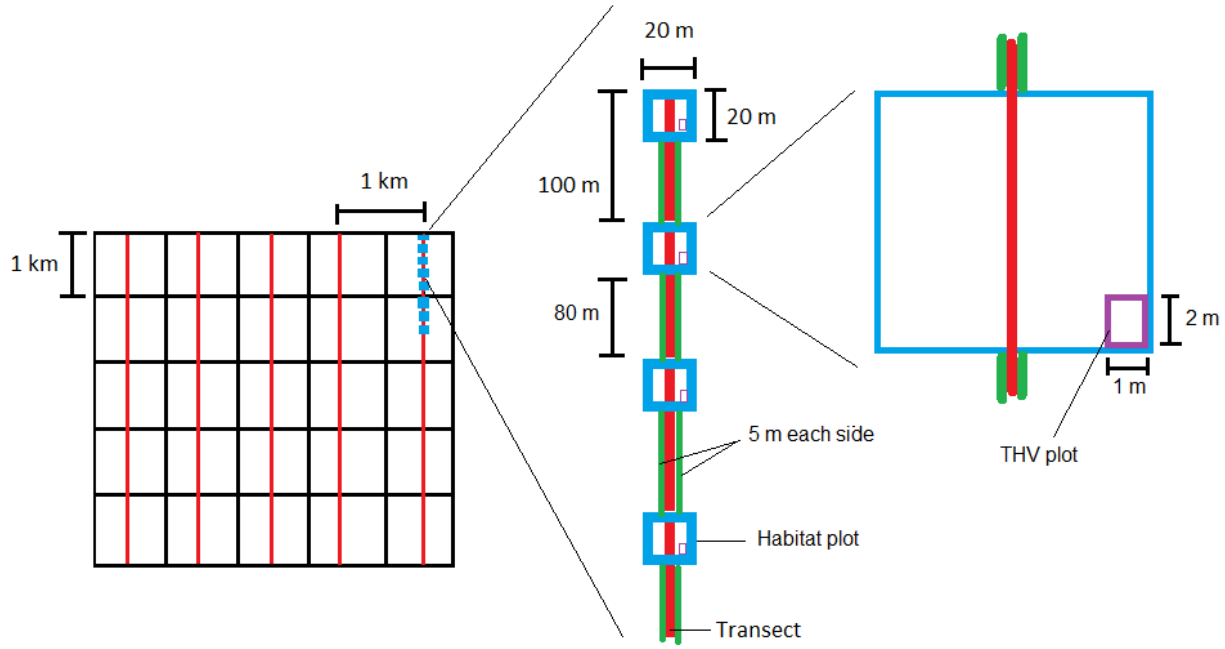

Figure 4-1 Grid of for example of 5 x5 km (black lines) with 5 transects (red lines) running through the centre of the grid cells. The 20m x 20m habitat plots (blue) are placed all the way along the entire length of the 5 km transect at an interval of 100 m. In between the habitat plots, only the tree species, whose nuts are cracked by chimpanzees, are recorded in a 10 m belt transect, i.e. 5 m on each side of the transect (green lines). All trees with DBH ≥ 10 cm within the habitat plots will be recorded. Within each habitat plot, consistently placed in one of the corners, is a 2 m^2 THV plot (purple), in which the number of herbs < 2 m is counted.

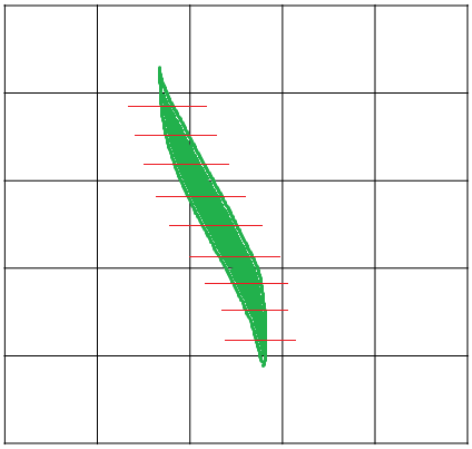

Figure 4-2 An example of transect placements for habitat plot surveys in a savannah woodland. Place the transects (red lines) across gallery forests (green) at an interval to create a minimum of 200 habitat plots.

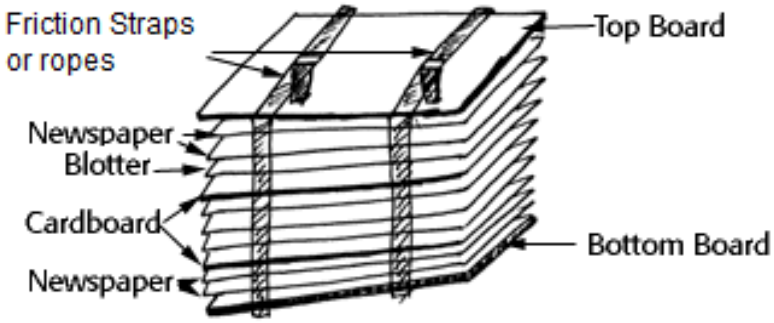

Figure 4-3 Illustration of a plant press. The plant samples will be kept in between the newspapers (Illustration: The University of Arizona)

4.3Definitions

- *Transect*: a linear line that extends across the grid through all of the cells in the data collection zone, where habitat structure, THV, line and strip transects are conducted
- *DBH*: diameter of a tree measured at 1.30 m above ground
- *Grid cell*: 1x1 km cell within the grid
- *Habitat plot*: 20x20 m quadrate that is placed centrally on the transect every 100 m, where all tree species with a DBH ≥ 10cm are measured and identified.
- *THV plot*: 1x2 m plots placed consistently in one of the corners of the habitat plot, in which the number of herbs (monocotyledons) and the number of woody saplings are separately counted.

4.4References

Ganas, J., Nkurunungi, J.B. and Robbins, M.M. (2008) A preliminary study of the temporal and spatial biomass patterns of herbaceous vegetation consumed by Mountain gorillas in an Afromontane rain forest. *Biotropica*, doi: 10.1111/j.1744-7429.2008.00455.x.

Nkurunungi, J.B., Ganas, J., Robbins, M.M. & Stanford, C.B. (2004) A comparison of two mountain gorilla habitats in Bwindi Impenetrable National Park, Uganda. *African Journal of Ecology* **42**, 289 – 297.

5 Phenology

Purpose: To identify the annual cycle and productivity of chimpanzee feeding trees species at the site

IMPORTANT

- Identify a minimum of 15-20 tree species that are important chimpanzee food, select 10 individuals per species using the list of important tree species and through plant parts identified in faecal samples
- Visit each tree once a month to assess its phenological status
- Conduct in parallel with camera maintenance in the monthly visits
- Conduct phenology for 12 continuous months
- Use data entry sheet 'Phenology–Initial observations' and 'Phenology–Monthly observations' (see annex III)

5.1 Sample size

A phenological study will be carried out over a period of at least 12 months to allow estimation of seasonal variation in forest productivity and food availability for chimpanzees. Ideally this study involves a large number of plant species that are consumed by the study population. However, as it is not possible, for reasons of time constraint, to first determine the diet of the study population and then collect phenology data, we will base the selection of food plant species for the phenological study on published data from chimpanzee populations living in comparable habitats in the same geographic area. A list of key fruit tree species is provided in annex I.

Ten individuals for each of 15 to 20 tree species will be included in the phenological study. Data will be collected once a month. The different individual trees will be picked from the trees inventoried when sampling the habitat plots. If habitat plots did not provide enough trees, new individuals may be added to reach 10 trees per species. This will be particularly important for large tree species, which are low in density. If possible, the different individuals from one species should be selected so as to be evenly spread across the grid, i.e. territory or home range and are not clumped in a single grid cell. The selected tree should have a DBH $\geq$ 10 cm and must be measured as described in section 4.1.

5.2 Tree species

A list of candidate fruit tree species is available in the annex and the 'tree guide' contains pictures of most of the trees.

5.3 Scoring

Ideally, the phenology scores should be given by estimating the proportion of the tree crown that contains flowers, fruits and leaves applying the below scoring system. However, in the forest or forest galleries it is often impossible to have a good view of the whole of the tree crown. Therefore, the convention is to select three branches of a tree crown, of which at least a 2 m long section is visible. Along these braches the proportion of flower, fruit and leaf will be estimated. It is essential to choose a branch part that is known to carry either flowers/fruits/leaves for this species. In most cases this is the tip of young branches, but exceptions are some fig species which bear fruits on old branches.

A tree bearing no flower/fruit/leaf or only a couple on the entire tree receives 0 for each category; 1 if up to one quarter of the visible sections bear flowers/fruits/leaves, and so on (Table 5-1). Within these three categories, percentages will also be given to opened flowers, ripe fruits, and mature leaves. In addition, the amount of fruits on the ground is noted. These are classified as 0 = none; 1 = little; 2 = some; 3 = many.

Table 5-1 Scoring system for tree parts along 2 m of visible sections of 3 branches.

	Score		
% crown cover	**Flowers**	**Fruits**	**Leaves**
0 - 1%	0	0	0
1 - 25%	1	1	1
26 - 50 %	2	2	2
51 - 75 %	3	3	3
76 - 100%	4	4	4

5.4References

Chapman, C.A., Chapman, L.J., Wrangham, R., Hunt, K., Gebo, D. and Gardner, L. 1992. Estimators of fruit abundance of tropical trees. *Biotropica*, 24, 527-531.

Chapman, C.A., Wrangham, R. and Chapman, L.J. 1994. Indices of habitat-wide fruit abundance in tropical forests. *Biotropica*, 26, 160-171.

6 Climate

Purpose: To record the annual change in climatic variable at the site

IMPORTANT

- Record everyday around the same time in the morning
- Use data entry sheet 'Climate' (see annex III)

Daily rainfall (mm), daily minimum and maximum temperature (^{o}C) and daily maximum and minimum humidity (%) are important variables to make cross-site comparisons. They will be recorded every day at camp using a rain gauge and a thermo-hygrometer. The rain gauge has to be placed in a very open spot with no tree branches within a radius of 5 m, and the thermo-hygrometer has to be placed at 1.5m above the ground in a constantly shaded spot with no sun arriving within 5 m during the day. Ideally recordings will be made in the morning and every day around the same time.

7 Chimpanzee tools

Purpose: The most obvious and convincing case for chimpanzee intelligence and culture are their **tools**. Therefore tool use is a key component of this project, not only to document distribution of tool use but also to document new tool techniques and uncover new tool types.

IMPORTANT

- A **tool** is any natural object that presents signs of intentional modification(s) in the raw material to change its shape, and/or length (e.g. cut to correct length, side branches removes, bark pealed, extremities narrowed or sharpened with teeth).
- The principal exception to this definition is a hammer that can be unmodified stones and wooden branches, but the second criteria for **used tool** need to be satisfied.
- A **used tool** is a tool that shows clear signs of use such as traces of hitting, wear from use, and remains of sand, honey, or termites, etc.
- When touching or handling tools always wear gloves or disinfect your hands before and after in order to avoid disease transmission
- Look out for signs of **UNNATURAL MODIFICATIONS or UNKNOWN TOOLS** in the environment
- All tools found should be photographed and given a unique ID code
- Any interesting or unusual tools collected, must be clean and dry for shipment to Leipzig, and be given a unique ID code
- Use data entry sheet 'Recces'[3]
- Refer to Annex IV-3 for an update on tool collection[4]
- Refer to Annex IV-12 & IV-13 for the Tree-drumming and Algae fishing protocols[4]

Tools will be collected along line transects (section 13.1.8), during habitat and phenology surveys, and also opportunistically (section 15), i.e. any time you are walking in the forest. However you should in addition conduct targeted sampling. On days that are not specifically allocated to other data collection, walk around the grid, particularly areas not covered by other data collection, in order to try and find new tools and tool use sites, and record these data.

7.1 Photograph

Tools such as wood or stone hammers, anvils, and stick tools that are encountered will be measured and 5 pictures will be taken: one from above and one photo each from four different angles from the side. Always put a ruler or tape measure next to the object and place tools on a white plastic sheet for the photograph. Interesting stick tools may be collected, stored dry and clean, and shipped to Germany if feasible. Every tool that is recorded gets a unique ID code and record the GPS location.

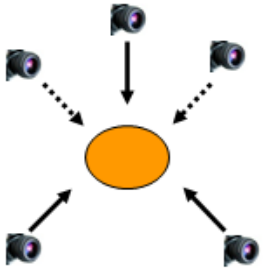

Figure 7-1 Photographs of detected objects (e.g. a tool indicated in orange) are taken at four different angles and from above whenever possible.

[3] An error in previous versions stated data should be collected in the Organic samples worksheet
[4] Addendum to original protocol: Annex IV

7.2Hammers

Hard and heavy tools used to pound open hard-shelled fruits. They have to present clear signs of wear from hitting a hard surface on at least one of the face and at least possess micro-traces on the worn surface. Not to confuse with stones that have been thrown around or clubs that have been stepped one.

7.2.1 Wooden hammers

(i) Definition

Length: min. 15 cm; max. 300 cm
Diameter: min. 3 cm; max. 15 cm

(ii) Hardness test

Conduct the following test and those that do not break are measured:
1. Step on it – if already rotten it will break
2. Pick up potential hammer and hit it twice on tree trunk

(iii) Measurement

Measure length, width (diameter at widest part) (Figure 7-2) and the weight.
Wear signs: Measure length and width

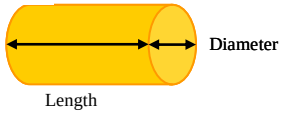

Figure 7-2 Wood hammers are measured by recording the radius of the circular face and the length of the hammer.

7.2.2 Stone hammers

(i) Definition

Weight: min. 100 grams; max. 20 kg
Stone material: lateritic, granite, quartz

(ii) Measurement

Measure the maximum width of the rock hammer, i.e. width at the widest part (Figure 7-3). Also weigh each rock hammer. Important is to count the number of surface with traces of wear and estimate the size of each of them (Figure 7-4)

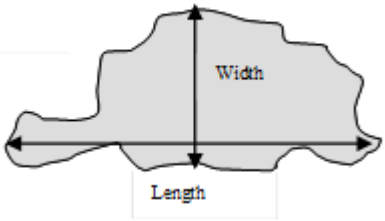

Figure 7-3 Measure the maximum width and length of rock hammers.

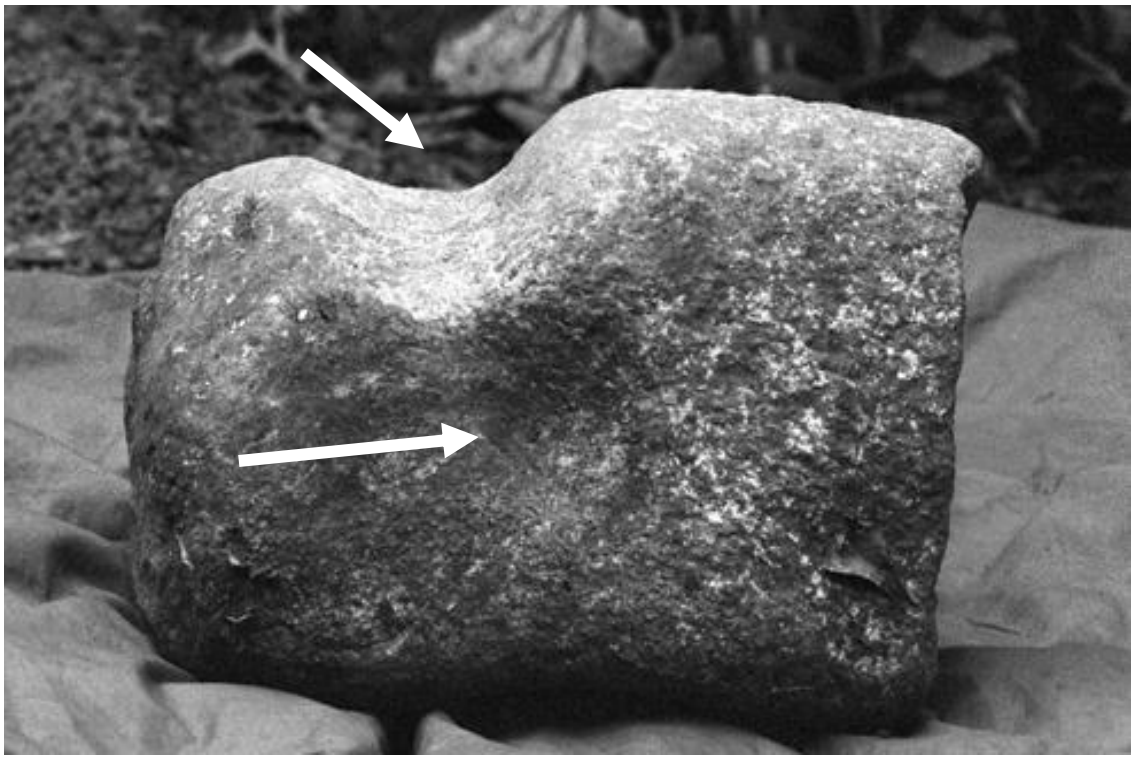

Figure 7-4 Granite stone hammer with two important wears on two different surfaces resulting from intensive Panda nut cracking (indicated by the two arrows). The size of the wear reflects the size of the panda nuts (photo: Christophe Boesch).

7.3 Anvils

Anvils are roots (Figure 7-5 a), rocks (Figure 7-5 b, c) and the base of tree trunks (minimum width of 5cm) (Figure 7-5 b) that have, **per definition**, to show clear indents or marks (a scar) where the nuts are positioned on the anvil when cracked with the hammer (red circle in Figure 7-5 b, yellow circle in Figure 7-5 d), otherwise they cannot be considered as an anvil even if many nuts are found around them. Anvils can be surrounded in some cases by hammers, but this does not have to be as chimpanzee transport often hammers. **Beware**, as chimpanzees can bang fruits, as *Treculia* or large *Landolphia*, as well as termite mounds, like *Thoracotermes*, against roots directly with their hands and in that case you will never find a hammer associated with them.

The following information has to be taken of anvils:

(i) Type of anvil
- Root
- Rock
- Base of tree trunk

(ii) Tree species

(iii) Measurements
- Root: maximum width and length
- Wear: maximum width and length of the upper surface of the root where the bark has been removed by the pounding (Figure 7-6).
- Rock: maximum width and the length. When possible weigh the rock anvil
- Base of tree trunks: maximum width (minimum of 5cm)
- Number of pits created by the nut cracking (Figure 7-6)

Figure 7-5 a) Rock hammer used by chimpanzees to crack nuts on a root anvil; b) rock hammer and root anvil where the place where the nut was cracked left an imprint on the root anvil (encircled in red); c) rock used as an anvil and a thick branch used as a hammer to crack nuts; d) rock hammer (encircled in yellow) used to crack nuts, here the base of the tree trunk was used as an anvil (Photos: Lydia Luncz).

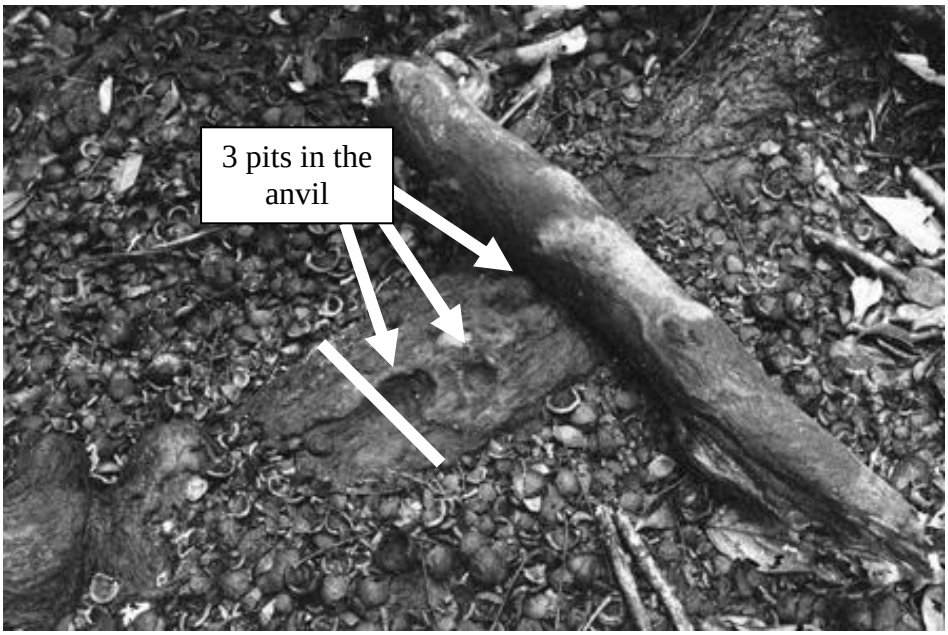

Figure 7-6 Root anvil with wooden hammer to crack *Coula* nuts. The wear on the wooden hammer is clearly visible, as are the three pits the chimpanzees have made to place the nuts. When a pit is too deep, the chimpanzees will normally make a new one nearby. You should measure the width of the of the anvil, illustrated by the white line (Photo: Christophe Boesch).

7.4Stick tools

Sticks found near termite mounds, ant nests or bee hives, can be considered as tools only if they present both signs of modifications and wear. Their thickness and length have to be measured. Also record in association with which chimpanzee food source they were found (e.g. termite mound, bee hive). Tools collected will be found in association with obvious signs of chimpanzee activity next to or under a bee nest (Figure 7-7), and covered by honey if fresh (or smelling strongly of honey), above-ground termite mounds, ant or termite underground nests or nut cracking sites. Sticks that were used as tools often show signs of manufacturing: broken ends, stripped or brush ends (Figure 7-8), leaves removed etc. Only tools associated with such signs will be collected. Upon encountering sticks used as tools, the following information needs to be recorded:

(i) Measure length and diameter
(ii) Modification types and number (cut one or two ends, remove side branches, peel bark)
(iii) Wear types (brush end, frayed or blunt end, food remains)
(iv) Tree species
(v) Associated food source

Figure 7-7 Branches used for honey dipping (circled) with side leaves removed, lying next to a bee nest (arrow) (Photo: Christophe Boesch)

Figure 7-8 Sticks used by chimpanzees to fish for honey from a large stingless bee hive. Note the pounder on the upper left that is almost 4 cm thick, and some smaller honey collectors that present different types of wear (with brushy ends), and different modifications with the bark more or less peeled away (Photo: Christophe Boesch).

7.5New behaviours and tools[5]

One of the important goals of the project is to uncover new chimpanzee behaviours as they are known to show much behavioural flexibility as they adapt to new habitat types. The camera traps are a potentially very powerful approach to uncover new behaviour. **LOOK OUT FOR SIGNS OF UNNATURAL MODIFICATIONS IN THE ENVIRONMENT**. If you see one of those, then place a camera to understand what happened.

For example, in some regions of Guinea, an **accumulation of moveable stones within the buttresses of trees** has been seen. What could have caused this? Other animal species within this habitat are not known to move stones, or not known to be able to carry stones and nothing indicates that humans do that. So it might likely be due to chimpanzees. Only a video footage will provide the answer. Similarly, in the forest of Loango in Gabon, **fresh leaves have been seen inserted forcefully into vertical cuts of big tree trunks** (Figure 7-9). What could have made that? Neither elephants nor bushpigs seem able to do this, so chimpanzees and gorillas seem the most likely candidates. Here again a video clip will settle that issue. In large regions of northern DRC, **termite mounds** of *Thoracotermes* and other species have been found to **be broken near roots**. The pounding of *Thoracotermes* mounds has been observed in Taï chimpanzees, but until we have direct observation it remains speculative for DRC. Video clips of camera placed near mounds before they are pounded by the chimpanzees might confirm this.

Take photos of any artefacts that you are unsure of and cannot be explained by your team, and send them via email to MPI so that they can be discussed. Broken sticks that are sticking out of the ground is easily missed but should be checked as they could be tools. The current tool use behaviours known by chimpanzees are:

- Pounding
- Throwing
- Inserting

These behaviours are currently known to manipulate rocks, wooden sticks, wooden hammers and leaves.

[5] Also see Annex IV-12 & IV-13 for the Tree-drumming and Algae fishing protocols

So remain very open to **new "unnatural" looking modification** of the environment in the grid of our site and place cameras to find answer to how it come to be like that.

Figure 7-9 Fresh leaves found inserted into a cut in the tree (Photo: Christophe Boesch)

7.6 Coding system for tools

7.6.1 Coding for photographs

Apply the following coding system for photographs, as with samples:

"TRS code" _ "unique sample ID" _ "extension"

When photographs of an object which you have also collected as a sample are taken, give the photographs the same ID as the sample. Photographs are treated the way as samples, so are numbered continuously. For example, if a code Gas_003_a is given to a sample collected for isotope analysis, the corresponding photos will be given the codes Gas_003_b, Gas_003_c etc.

7.6.2 Coding for tool samples

Apply the following coding system for tools collected:

"TRS code" _ "unique sample ID"

The tool entry with measurements in the data entry spread sheet gets an ID code without an extension, e.g. Gas_004. Then the photos of that tool would receive the codes Gas_004a, Gas_004b etc.

7.7References

Boesch, C., Head, J. and Robbins, M. M. (2009) Complex tool sets for honey extraction among chimpanzees in Loango National Park, Gabon. *Journal of Human Evolution*, doi:10.1016/j.jhevol.2009.04.001.

Sanz, C. M. and Morgan, D. B. (2009) Flexible and persistent Tool-using Strategies in Honey-gathering by Wild Chimpanzees. *Int. J. Primatol.* 30, 411-427.

8 Camera trapping

Purpose: To capture video footages of chimpanzees to calculate abundance, density and demographic structure using facial recognition, and to capture tool use behaviour

IMPORTANT

- The systematic camera placement requires one camera to be placed per grid cell,
- Where there are more than 20 grid cells, cameras should still be placed systematically, e.g. in every second cell. Try to optimise capture by relocating cameras to a neighbouring cell if the habitat is heterogeneous. Hence use a systematic and target approach.
- Add extra cameras non-systematically in high-activity areas, tool use sites or in sites with potential new chimpanzee behaviour discoveries,
- Always keep the cameras on video mode
- Do NOT delete any of the video clips even if they do not contain any footages of animals
- Record the total duration a camera is placed at a particular spot and its GPS location
- Strictly follow the guidelines for video camera treatment and data storage and make backup copies
- Use the data entry sheet 'Video cameras' (see annex III)

8.1 Systematic placement

In order to ensure balanced data collection camera placement has to strictly apply the following guidelines:

1. Determine locations of high chimpanzee activity throughout the study, initially from the recce then as you find interesting ones (Figure 8-1).
2. Within each cell with chimpanzee activity, one camera is placed at a location that is frequently used by chimpanzees for increasing chances of filming them. Cameras may be moved during the course of the study within and between their respective cell (Figure 8-2)
3. If your grid is small and has only about 20 cells, place one camera per cell.
4. If the territory/home range is too large and/or not sufficient cameras are available to place one camera per cell, one camera is placed in every other cell only (Figure 8-2a)
5. If the territory/home range is very heterogeneous in its suitability as chimpanzee habitat (Figure 8-2a, blue-green area), e.g. very fragmented forest with high human impact or savannah habitat with gallery forest then a systematic grid is laid across the area and cameras are placed in the following fashion:
    a. available cameras are systematically placed across the grid1x1km cell
    b. cameras that fall into cells which do not have suitable ape habitat (Figure 8-2a) are not installed but are instead
    c. installed in cells that have suitable habitat but are not covered by the systematic design; cameras will be placed in a stepwise manner following the rule that each camera which is placed is always placed the furthest away from the next camera (Figure 8-2b)
        i. in case of doubt, cells that have a higher percentage of suitable habitat are preferred over cells with lower percentage of habitat, OR
        ii. cells with low percentage of suitable habitat but lots of chimpanzee signs are preferred over cells with higher percentage of suitable habitat but few signs of chimpanzee use

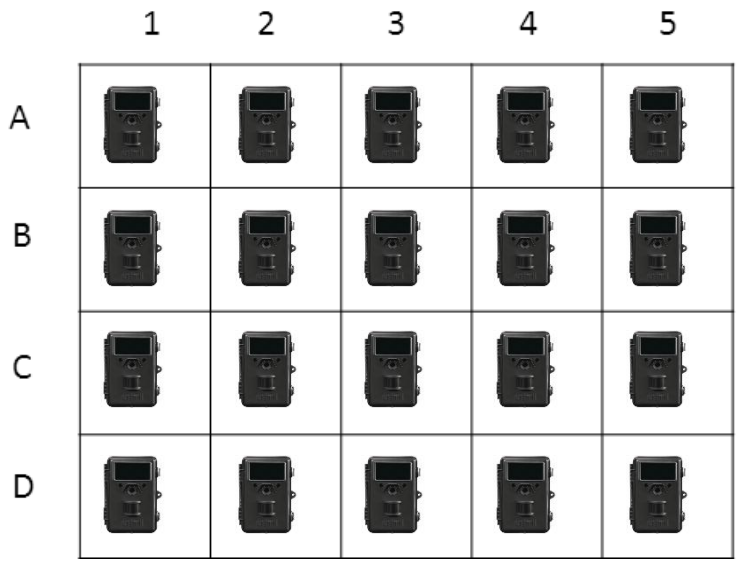

Figure 8-1 Example of a systematic placement of cameras for the study design of a grid with twenty 1x1 km cells and 20 remote video cameras in each cell. To start, base the placement from the recce information and later update the placement from the new information about chimpanzee activity (see Figure 8-2).

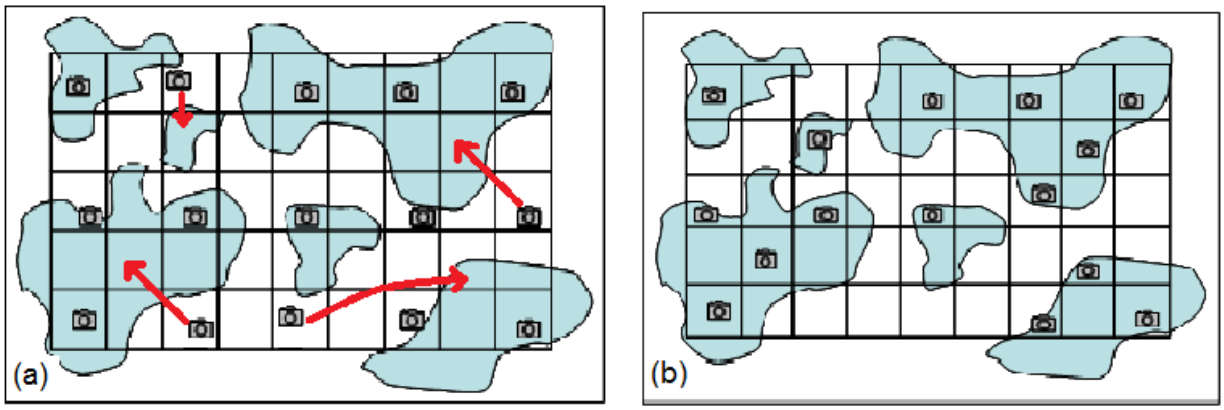

Figure 8-2 Illustration of protocol for systematic placement of video cameras for a grid that include more than 20 cells and where cameras have to be placed in preference in high chimpanzee activity areas (shaded in blue here).

8.2Non-systematic placement

In addition to the systematic design, remaining cameras are placed at promising/interesting locations such as known and potential tool use sites - areas that could reveal new behaviours as well as sites that are heavily used by chimpanzees (water holes in dry regions, bridges for water crossing, etc.). These cameras may be placed in cells that already contain a camera.

8.3Identifying suitable camera spots

Camera trap studies at different sites have revealed chimpanzee visitation rates of about 1-3 events per camera and month, depending on local chimpanzee density. Thus 20 cameras at a study site

should provide about 20 or more chimpanzee videos per month. However, only about 30% of chimpanzee footages are usable when it comes to the identification of individuals, which is roughly 6-7 videos/month. Certain analytical methods for estimating chimpanzee density require a minimum of 50-80 good videos. Consequently, great care must be taken to ensure that camera traps are set up soon after the start of the study and good locations are selected to ensure a large enough sample size at the end of the study.

Once the grid design has been developed, cameras need to be placed in each selected cell. For this we have to consider that if cameras would be placed randomly in a cell, we would only rarely get pictures or footage of chimpanzees and other species because most species do not use their habitat randomly. Instead, they have feeding spots they visit regularly, and travel paths they use to move from one place to another. We therefore have to locate these travel routes or feeding spots, by intensively searching in each grid cell in order to ensure maximum recording rate. Camera spots should be open enough to ensure that individuals can be filmed clearly without being blocked by, for example, the forest under-storey. In each selected cell, one or several of these potential camera spots should be located for the placement of cameras. For location with long-term use by animals, cameras may remain for a long time (e.g. regularly used travel paths or bridges), but for locations with only seasonal use (e.g. fruit trees), cameras can be moved to other locations as long as they remain within the same cell.

8.3.1 Travel routes

As a **general rule**, chimpanzees like to use open routes for long distance movements. In the dense forest, elephant paths are abundantly used and are therefore very good for placing cameras. In drier habitats, they tend to make their own path by repeatedly following the same ones. Look for them, as they will also make movements in the cell easier and the crossing of thickets quicker. Here again it is good for camera placement.

Well-trodden paths with feeding signs, faeces or tracks are clearly good indicators of a promising camera location. However be aware that certain animals (particularly elephants) use different paths seasonally. Therefore if a well-trodden path is not available in a particular grid cell, cameras can still be installed as activity on the path can change over the duration of the camera study.

Travel routes that have intersections are always a good choice for camera locations. The camera should be placed either at the start of the intersection or up to 10 m away from it. If the travel route is an elephant path that is very open, care should be taken not to choose a mounting tree that can easily be knocked or rubbed by an elephant.

8.3.2 Large fruit trees

Large fruit trees tend to be hotspots for chimpanzee activity and larger individuals which are rarer in abundance should be located to increase the chance of filming individuals. Trees associated with chimpanzee diet can be identified through the phenology data.

Cameras should be placed at fruiting trees just before the fruits turn ripe and removed when there are no more branches visible that contain ripe fruit. In addition, chimpanzees can have preferred fruiting trees, so it is important to determine the location of more than one tree per species within a grid cell if possible. It is therefore always better to know more than one individual tree of each species, so that if visitation rate of the tree proved to be low, you can move the camera quickly to a preferred tree.

Beware that in order to achieve successful camera placement, it is extremely important to assess how the apes will access the fruiting tree, i.e. which tree(s) they use to climb to reach the trunk or branches of the large fruit tree. This, however, requires some good feeling about how chimpanzee climb trees and you need to be careful here. Be aware that some trees will have more than one access point and others will have an access point located up to 20 m away from the tree itself. These camera locations

should be monitored more frequently during the start of fruiting in order to determine whether or not the camera is facing the correct access point.

8.3.3 Natural bridges

Chimpanzees also have preferred bridges (fallen trees and branches) over water sources in the forest such as swamps and rivers. Good indications of a well-used bridge include a smooth surface with either end of the bridge free from obstructions (e.g. under storey). Locations where there is more than one bridge should not be selected as this might allow the chimpanzees to deviate the camera by using an alternative crossing. Bridges can be located over temporary or permanent water sources (e.g. large rivers or permanently inundated swamps) that will not dry up throughout the dry season.

Natural bridges are an ideal location for placing two cameras facing each other at either end of the bridge (Figure 8-3) in order to identify individuals. On paths, chimpanzees can cross perpendicular to the camera, however at natural bridges the apes are obliged to walk the entire length of the bridge (providing there is a permanent water sources below it) and therefore pass both of the cameras, increasing the chance of a clear facial image.

Figure 8-3 A suitable location for camera trap installation by a "natural bridge" which may be used by chimpanzees.

8.3.4 Tool use sites

Chimpanzees can have preferred tool use sites where there is a higher activity and an indication of this can be the number of tools present at the site. Potential tool sites can be found **for cracking nuts** under or nearby nut producing trees, such as *Coula*, *Panda*, *Parinari*, *Detarium*, *Sacoglottis* and *Elaeis* trees (especially known for Cote d'Ivoire, Liberia and some regions in Sierra Leone and Cameroon); for **fishing** which can be thin sticks, herbs or bark strips **for some species of termites** building epigeal and underground large mounds (especially known in Tanzania, and Congo); to **extract honey** from bee hives of different species of bees (especially famous for central African chimpanzees but also found for honey bees in western and eastern chimpanzees; Figure 8-4). This list of tool use context is **NOT** exhaustive and it is our aim to uncover some new tool use so please keep your eyes open for new context.

Figure 8-4 a) *Meliponae* bee nest; b) *Meliponae* bee nest entrance tubes (circled); c) stick tools found in and by bee nest (Photos: Christophe Boesch)

Luckily **tools** can be recognised from the raw material being **modified** to become a tool (cut to correct length, side branches removes, bark pealed, extremities narrowed or sharpened with teeth) and being used (hitting traces, wear of use, sand, honey, or termites remains on tools, etc.). Look carefully for such tool remains near/under potential food sources.

Important and rare behaviours can be captured at tool use sites so cameras should be placed close to the tool site, **ideally 4-5 m away** (Figure 8-5).

8.3.5 Waterholes

Waterholes are important camera trap locations, especially if they are in low density. As for the tool use site, place the camera approximately 4-5 m away from the source.

8.3.6 Uncovering new chimpanzee behaviours

See section 7.5, Annex IV-12 and Annex IV-13[6]

[6] Addendum to original protocol: Annex IV

Figure 8-5 Bushnell placed next to a tool use site.

8.4Settings

8.4.1 Installation

The settings on the Bushnell can be changed on the camera itself. For details please read the instruction manual of the camera carefully. Although the Bushnell is waterproof, they should be kept in a plastic Tupperware boxes with silica and sealed with cling film at all times (Figure 8-6), even during dry seasons. The Tupperware case offers extra protection against wildlife damage and cobwebs or dust which may accumulate on the camera lens. Previous experience also shows that the high humidity and rain in the rainforest affects Bushnell functioning.

Figure 8-6 Bushnell protected against humidity and external damage by a Tupperware box containing silica with cling film.

8.4.2 Camera coverage

For each location, the optimal camera coverage must be established. Every location is completely different in terms of topography, lighting and visibility, natural obstructions and available/suitable mounting posts for the cameras. These environmental variables have a considerable effect on the detection zone around a camera. Therefore detection tests must be conducted at every camera location upon installation in order to obtain the optimal height and angle (sometimes it is necessary to place a stick between the top of the camera and the tree to adjust the camera at the right angle) that would result in the furthest trigger point for a chimpanzee.

The camera should be tested (by pushing the button onto 'set-up' mode). Once the camera is in test mode, the blinking red LED will indicate where the sensor first detects a person crawling (to mimic chimpanzee detection conditions) in front of the camera or at some distance. The tester must adopt the position/height of a chimpanzee (e.g. 50 cm height) whilst crossing perpendicular to the camera lens from left to right, gradually increasing the distance between the tester and the camera until the furthest trigger point is obtained. The distance between the furthest left and right trigger points should then be measured in metres and this is y (Figure 8-7). The perpendicular distance from the camera lens to the line of y should then be measured and this is x (Figure 8-7). The area of camera coverage is then calculated using the following formula: area (m^2) = x*y/2

Sometimes there is an area directly in front of the camera, z, which falls outside of the detection zone due to animals passing under the camera (Figure 8-7). If this is the case, this area should be determined and calculated as above and the size of the area is subtracted from the area of camera coverage.

8.4.3 Direction

Cameras can get triggered by the sun, especially during the rainy season where there will be much less cloud cover than in the dry season. In order to reduce the chance of false triggers by the sun and images that are too bright, cameras should ideally be placed with the sensors facing towards the north or south rather than to the east or west. At times this might not be possible depending on the location (e.g. bridge faces east or west), and if so the camera maintenance protocol should be modified to include more frequent checks for those locations that are more susceptible to solar triggers.

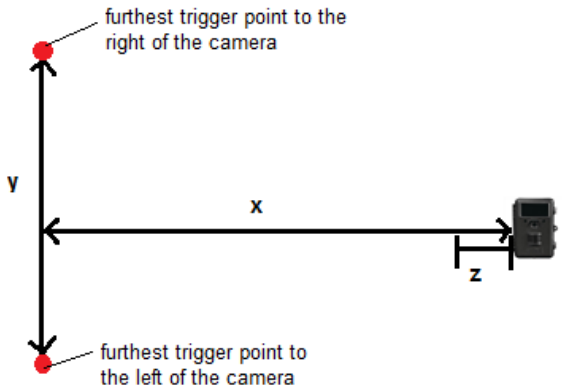

Figure 8-7 Distance between the furthest points of camera detection (y) and the perpendicular distance from the camera to this line of y are measured and noted (x). In addition, the distance of blind-spot directly in front of the camera where camera does not detect (z) is recorded.

8.4.4 Height

The camera should be mounted tightly onto a tree that has a minimum diameter at breast height of 5cm to ensure that it is sturdy enough and will not sway in the wind or be knocked down easily by wildlife. The camera should be placed ideally at a height that ranges between 0.9 and 1.1m measured from the forest floor to the middle of the camera lens. Some location, e.g. tool use site may require a different height for placing cameras. Height will also need to be adapted on a slope depending on which side of the slope.

8.4.5 Settings

The sensitivity setting on the Bushnell cameras should initially be set to 'high' and this will help to increase the detection range of the camera. If you experience a high false trigger rate (no animals) when downloading the data, you should change the setting to 'normal'. The maximum recording time of 60 seconds with an interval of 1 second is recommended in order to capture as many individuals as possible during an event. See Table 8-1 below for more details.

8.4.6 Visibility

The area in front of the camera should be free from obstructions (e.g. leaves, branches, tall under storey) to allow clear images of the chimpanzees (and other wildlife) and to prevent false triggers by foliage and branches moving in the wind. Once the camera is mounted, any obstructions to the detection zone of the camera should be removed using secateurs, never cut foliage using your hands. However, be aware that any modification may draw the attention of chimpanzees and other wildlife to deviate and avoid the cleared spot. Therefore, any removal of samplings herbs etc. should be as minimal as possible. If leaves and small branches are used to camouflage the camera (recommended) ensure that they will not fall in front of the camera lens.

Table 8-1 Recommended settings for Bushnell Trophy cameras

Option	Setting
Mode	Choose 'video' setting
Image size (for camera option)	Not applicable to video mode
Capture number (for camera option)	Not applicable to video mode leave as '1 photo'
Video size	Highest resolution
Video length	60S (seconds)
Interval	1S (second)
Sensor level	In most cases 'normal' will be best but try out 'high' first
Format	Enter 'yes' (to format a new SD card, this also deletes any data previously stored on the SD card; Make sure you have downloaded and backed up the files first)
TV Out	Select 'PAL' (video standard/format)
Time stamp	On
Set clock	Adjust to correct date and time
Field Scan	Off (deactivates timer setting)
Video Sound	On
Default Set	Do NOT press 'OK' – Just press MENU to exit settings

8.5Maintenance and re-visits

Camera traps need permanent maintenance, including exchange of storage medium (SD card), checking for wildlife damage, recharge of batteries, exchanging/reactivating silica gel etc. Previous works at other field sites suggest an approximately monthly to bi-monthly maintenance schedule. However, cameras may also work as long as two months without any maintenance. The intervals, however, are capture rate and model dependent so should be checked. Areas of low mammal density for instance can increase the inter-visit interval. Cameras may need extra maintenance during the rainy season and especially following heavy rainfall.

Camera maintenance at each location should adhere to the following:

(i) Disinfect hands with hand sanitiser before touching each device to reduce the risk of disease transmission as chimpanzees can become curious and touch the cameras
(ii) Record the battery life remaining and whether or not batteries were changed
(iii) Change the silica in each box that contains the cameras
(iv) Wipe the camera lenses and sensors with alcohol
(v) Check the positioning of the camera through viewing the images for each device

The following equipment should be carried during camera maintenance:

- Rechargeable AA batteries
- Alcohol
- Gaffa tape (for emergency waterproofing)
- Cling film
- Hand sanitiser
- Secateurs
- SD cards
- Silica gel
- Straps
- Tape measure
- Toilet paper/cloth for wiping with alcohol

EVERY time you do camera maintenance, please make a video of you standing in front of the video with a measuring tape, measuring out some fixed distance (50cm or 100cm) and walking away from the camera, while always holding up the measuring tape so that we can estimate size from the videos.[7]

8.6Troubleshooting

Common problems encountered with Bushnells include the following in Table 8-2.

Table 8-2 Troubleshooting for Bushnells

Problem	Solution
Camera triggered continuously without subject	Check the angle of the camera to ensure that it is not triggered by the sun. Check that there are no branches creating motion in front of the camera. Once these are checked and are still experiencing problems, lower the sensitivity of the camera to 'normal'.
0 kb videos taken which do not play	Contact MPI to be returned to manufacturer to be serviced
Blurry images due to rain/humidity	Ensure that silica gels are changed frequently and that the cling film is covered well

[7] Addendum to protocol added July 2014

8.7 Storage of video files

Over the course of one year usually hundreds or even thousands of video files are recorded. Excellent data storage and labelling system is therefore the key to prepare video files for subsequent analysis. Every data file needs associated metadata, including the date and location of recording.

Create one folder for each cell in the grid, consisting of:

"TRS code"_ "grid cell code"

For instance: **Loa_A7**

Create subfolders that will contain the video clips and apply the following coding system:

"TRS code"_"camera ID"_"location"_"date of installation"[8]

'location' is in UTM format (east-west_north-south)
'date of installation' is in 'yyyymmdd' format

For example a TRS at Loango with a camera trap device number 6, installed on 23rd May 2012, the code for the folder would be: **Loa_vid6_....._20120523** (Figure 8-8).

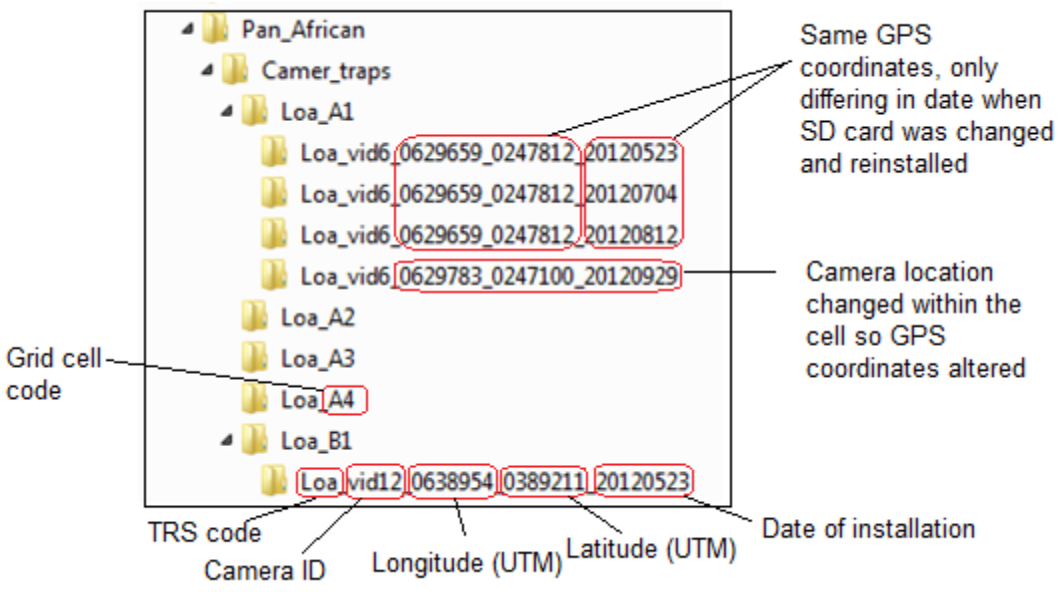

Figure 8-8 Example of a folder structure for video clip storage

Remember: Do NOT delete any of the video clips, even if they do not contain any images of animals.

[8] This is the date the SD card was installed OR changed – ie: every time you download videos you create a NEW folder for those videos.

8.8References

Rovero, F., Tobler, M. and Sanderson, J. (2010) Camera trapping for inventorying terrestrial vertebrates. In *Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories and Monitoring* (eds. J. Eymann, J. Degreef, C. Häuser, J.C. Monje, Y. Samyn and D. VandenSpiegel), pp. 100-128. The Belgian National Focal Point to the Global Taxonomy Initiative.

Silver, S.C., Ostro, L.E.T., Marsh, L.K., Maffei, L., Noss, A.J., Kelly, M.J., Wallace, R.B., Gomez, H. and Ayala, G. (2004) The use of camera traps for estimating jaguar *Panthera onca* abundance and density using capture/recapture analysis. *Oryx*, 38, 1-7.

9 Isotope samples

Purpose: Determine the diet of the chimpanzees with in particular the possibility to determine the amount of meat or protein consumed. To collect samples which correspond to trophic level, forest cover, photosynthetic pathway and other biological properties of the main dietary sources of chimpanzees

IMPORTANT

- Chimpanzee hairs and bones are uniquely important samples to do the stable isotopes analysis,
- Environmental sample of where the chimpanzee live, will allow us to make sense from the isotope data,
- Double sampling should be the rule to prevent loss of samples
- Only collect plant samples that you can identify down to species level
- Only need small amounts (4 mg) – cut a small piece of nut/fruit/snail shell; 3 or 4 ants/ termites per sample only
- NEVER touch a decomposing carcass – also refer to Annex IV-1 for more on Bone collection[9]
- Use data entry sheet 'Organic samples' (see Annex III)
- Also refer to Annex IV-7 and IV-10 for the most up to date information on isotope collection[9]

Samples for stable isotope analyses will be collected continuously throughout the field period. These include fresh leaves, and soil. Water samples are to be taken only from streams or swamps and not large rivers, because if we take samples from large rivers, the information that we get out of such a sample would only tell us something about the location of the source of the river. Store the water samples in a 15 ml tube. Collect 2-3 water samples per habitat type. Along the entire transect, survey teams will look for snail shells, army ants, termites, bones, feathers, etc. which they will collect and store when encountered. Always record a GPS position for each of the samples collected and fill in the data sheet.

On days that are not specifically allocated to other data collection, conduct targeted sampling by walking around the grid, particularly in areas that are not covered by other data collection, in order to try and collect the samples.

9.1 Ecological isotope samples

For carbon and nitrogen analyses

9.1.1 Great ape hair[10]

a) Sampling methodology

Collect hair from nests whenever you find a nest, especially a group of nests. Collection of hair samples from chimpanzees in different habitats (e.g. primary swamp-rainforests, drier rainforests, areas closer to the coast) is very important for comparison. Hairs collected from a group of nests are most important for analysis as they represent several individuals at the same point in time. Sample ALL nests from a nest group. Climb up to the nest and put the nest in a rice bag so that it can be taken to the ground for all team members to search for as many hairs with intact hair root as possible. Put all hair samples found in a single nest together in an envelope and note down an estimated age of the nest (stages 1-4; Figures 9-1 to 9-4), and the nest ID (to identify the nest group). When climbing the tree is impossible, try to shake down parts of the nest and find hairs on that may have fallen on the ground.

Where sympatric gorillas exist in the study site, remember to also collect gorilla hair samples.

[9] Addendum to original protocol: Annex IV

[10] Also refer to Annex IV-7 – the importance of temporal sampling

b) Target sample number[11]

Collect hair samples from **50 nests** (min. 20 fresh[12] nests with at least 5-15 hairs with ~5 cm length). Try to obtain hair from at least 5 nest groups during the survey.

c) Sample treatment

Hair samples collected should be put in Pergamin envelopes including a complete label on the envelope (Table 9-1). Store the sealed envelope in a Ziploc bag with some silica to maintain dryness if necessary. Note: Treat all hair samples from gorilla nests and other mammals in the same manner i.e. store in labelled envelope and then in a Ziploc bag with silica. These can then be stored at room temperature. Write nest age on the envelope AND in the organic data sheet (comment column).[13]

Table 9-1 Label for use on chimpanzee and all mammal hair samples.

Sample ID	date	sample type	UTM zone	latitude	longitude	nest age (1-4)	height (m)
		chimp hair					
		chimp hair					

Figure 9-1 Fresh nests (stage 1): all leaves in the nest are green and generally faeces or urine odours are underneath the nest (Photos: Célestin Kouakou)

Figure 9-2 Recent nests (stage 2): drying leaves of different colours, green may dominate, but no dung and no urine odour underneath the nest (Photos: Célestin Kouakou)

[11] Please see Annex IV-7 and IV-10 for updated sampling instructions for chimpanzee hair with a focus on fresh nests, nest groups and a good temporal spread

[12] In the previous versions of the protocol there was no focus on fresh nests, but this point is very important.

[13] As this war rarely done (and there is no extra column for nest age) we added this explicitly in the new version of the protocol.

Figure 9-3 Old nests (stage 3): structure still roughly intact with the majority of leaves brown (Photos: Célestin Kouakou)

Figure 9-4 Decayed nest (stage 4): nest with holes showing few or no leaves, but still identifiable by bent twigs (Photos: Célestin Kouakou)

9.1.2 Plants

a) Sampling methodology

Collect samples of plants that are common food for chimpanzees (see Annex I for list of tree species) and sample the part of the plant which is eaten: NOT the discarded part. If you find leftovers of chimpanzee plant foods, note these down and take samples for isotope analyses. Note down the plant part type (e.g. fruit, fruit pulp, herbs, leaves, nut, seed, mushroom, flower, pith or bark), name and its position in the canopy (ground, mid-height or high in canopy). Note the habitat (e.g. primary swamp forest, savannah, clearing etc.). Plant samples should be collected throughout the year including the species already collected. See Annex IV-10 for additional monthly sampling of tree leaves during phenology[14].

For each habitat type collect approximately 15 plant food samples (e.g. seeds, nuts, piths, fruit, leaves, herbs, fruit pulp). In nut cracking populations, samples of nuts the chimpanzees are known to crack and eat (e.g. *Parinari excelsa*, *Coula edulis*, *Panda oleosa*, *Detarium senegalense*, *Sacoglottis gabonensis* and *Elaeis guineensis* etc.) should also be collected. Plant samples for isotope analyses have to be identified to the species level otherwise they cannot be used. Seeds, nuts, fruits are preferable to leaves, unless chimpanzees eat the leaves. When collecting hard shelled fruits, break or cut open the fruit and collect the pulp i.e. the part that is actually eaten by chimpanzees. We only need about 4 mg per sample. In the case of big fruits there is no need to collect the entire fruit.

[14] In this new version of the protocol we added this monthly sampling of tree leaves, see Annex IV-10 for details

Only collect plant samples that you can identify down to the species level. If we do not know the species we cannot compare isotope ratios within species across different locations.

b) Target sample number

15 fruit species including different types of fruit and fruit parts (e.g. pulp, peel, seed, shell) and 15 other food items. Aim for at least two samples per species, so a total minimum number of 30 fruits and 30 other plant food samples should be collected.

c) Sample treatment

Small parts of the plant item should be taken as a sample: these can be as small as 10 g. Fruit commonly consist of 80-90% water so a 10 g sample of fruit will be equal to 1 g of dry weight. Whenever possible, firstly dry the samples in the sun before transferring to a 50 ml tube filled two-thirds of the way with silica gel to prevent rotting or moulding. The sample should not come into direct contact with the silica gel so make sure that some barrier such a piece of toilet tissue or paper is put on the silica before placing the plant sample (Figure 9-5). Leaves can be put in paper envelopes, which can then be stored in ziplock bags with silica[15].

Add a note inside the tube with the sample, with the information included in the table below (Table 9-2). Then label the tube again on the outside, and tape over it to protect the written information. For sample ID coding system see section 9.3. Reuse the saturated silica gel by reactivating for other isotope samples only.

If the colour of the silica gel has turned from orange to colourless, transfer the sample into another tube with silica gel. Remember that the samples should NOT be dried over fire unless absolutely necessary, and that smoke and burning must be avoided. Indicate that fire was used to dry on the tube. These can then be stored at room temperature.

Table 9-2 Label for use on the plant sample tubes. (For sample ID coding system see section 9.3)

Sample ID	date	sample type	UTM zone	latitude	longitude	plant part	species	habitat
		plant						
		plant						

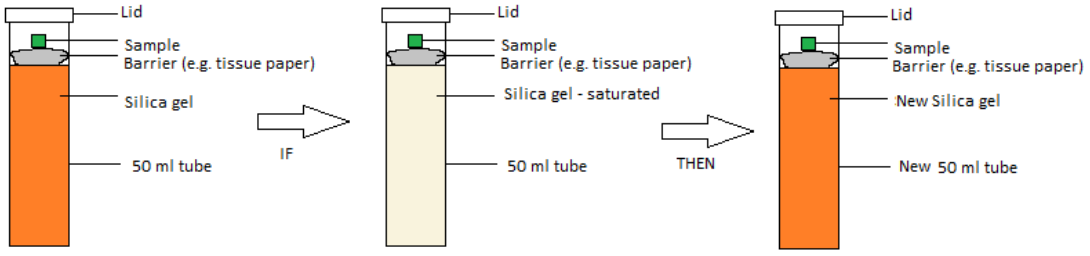

Figure 9-5 Plant sample storage method and sequence.

9.1.3 Animals

a) Sampling methodology

Collect tissue samples of animals whenever you find animals and identify the species. **Never touch a fresh decomposing carcass (*bio hazard!*) - only sample dry skeletons and attached tissues**. If possible (and only when completely dry), collect the whole dead animal. Scan the surrounding area for any other animal remains[16].

[15] Not in the previous versions of the protocol, but it saves space, silica and tubes.

[16] An error in previous versions stated "Collect 2 tubes of the same sample for duplication in case of loss during transit back to Leipzig." This has now been deemed unnecessary.

Examples of animal tissue samples that can be collected:

- Feathers: better if bird species can be identified or at least type of bird (raptor, ground dwelling)
- Hair: plucked from carcasses (stored in an envelope - see section below on handling methods[17])
- Bones: dry them if they are fresh samples to avoid moulding, wear gloves to protect against diseases (see section below on handling methods)[18]
- Insects: collect entire specimens of e.g. ants, termites, caterpillars, grasshoppers
- Scales: from fish, snakes, lizards, etc…

Collect insect samples that are relevant to chimpanzees (i.e. bees, ants, termites). Hair from mammals is generally more difficult to find. However, carnivores' faeces are normally full of their prey hairs and therefore look out for faeces from leopards, small cats, hyenas, wild dogs, lions and sample them (try to identify the hair after washing them in water. Use also the bone remains in the faeces to identify the hairs and store these bones). Sample for hair from resting spots of duikers or any remains on direct observation so that they can be identified. Find hair from leopard resting places. Sample tissues of other primates (monkeys and gorillas) including skeletal remains, whenever you find them. Tissues should include hair, feathers, scales, insect chitin. Try to estimate the age at death (from coat colour, bone epiphyses open/closed, dentition etc.) and if it is a sub-adult or an adult (this is highly relevant for isotopes). Indicate if the animal is either a herbivore, omnivore, carnivore or an insectivore, and if the animal feeds on the forest floor or high in the canopy.

Chimpanzee carcasses:

Chimpanzees may have died from diseases that can kill humans, such as Ebola virus and anthrax. Bones should therefore NEVER be touched and handled without wearing appropriate protective clothing and following the below precautions.[19]

(i) What protective clothing to wear?

- Gloves up to the long sleeved shirt
- 2 surgical face masks in front of nose and mouth
- Goggles to protect the eyes
- long trousers
- long sleeved shirt
- (rubber) boots

(ii) What equipment is necessary?

- Protective clothing
- GPS, notebook, pen
- big strong plastic bags
- smaller plastic bags
- forceps, pliers
- RNAlater tubes
- big bucket
- formalin (best buy a whole bottle of formalin (= ~37-43% formaldehyde) at the local pharmacy)
- water (~ 10 ml water)
- if available: hand- sanitizer
- paraffin + lighter (to burn contaminated gloves etc.)
- [potentially: spade or shovel to bury carcasses that are yet not decomposed]

[17] Omitted in previous version but the utmost care must be taken with any found carcasses.
[18] An error in previous versions stated "bones with tissue on them". We can however use all bones.
[19] Also refer Annex IV-1 for an updated protocol for collecting UNTREATED bones

(iii) How to safely collect and handle the bones/skeletons?

1. BEFORE closely approaching or touching the bones, put on your protective clothing
2. Take a GPS coordinate of the location where the bones were found and potentially also write down some notes (e.g. date, vegetation, any obvious signs concerning the cause of death, any other bones / carcasses found in the area ?....)
3. If you **find any maggots on the bones**, transfer a few of them into a tube with RNAlater using a pair of forceps (disinfect them overnight in 2-5% formalin). Close the tube and shake it vigorously. Store the tube overnight in a fridge where available or at room temperature. Then freeze it or store it as cold as possible.
4. Take tissue samples as described above.
5. Use forceps to transfer the bones into a strong plastic bag. If you have the choice, long bones are preferred.
6. Whenever the **skull** is available, take it with you and disinfect it as described below.
7. Then take off your protective clothing WITHOUT CONTAMINATING yourself. Dispose of gloves and masks by burning them on site or put them in a plastic bag, transport them back to camp and burn them there.
8. Before taking off the mask, place the bag with the bones into a second plastic bag. Do not touch the bag containing the bones while doing so as its outside might be contaminated. Simply pull the second bag inside out over the first bag and close it. Now the bones can be safely transported.
9. Take off your mask taking care to only touch their straps, then dispose of the mask (burn it).
10. Wash your hands and use hand-sanitiser if available
11. Disinfect the outside of your boots overnight in 2-5% formalin and wash your clothes in bleach.

(iv) How to disinfect the collected bones?

1. Disinfect the bones for several days in 5% formalin (i.e. about 500 ml of concentrated formalin (~37-43% formaldehyde) per 10 litres of water).
2. Put on gloves (formalin is quite toxic!), fill a bucket with formalin-water mix and place the plastic bag containing the bones in the bucket. The bag should be fully submerged. Now open the bag and let it fill up completely. Cover the bucket and leave the bones for several days in the formalin solution.
3. Afterwards remove the bones from the formalin bath (wear gloves while doing so!) and rinse them in water or in a bleach-water mix, Afterwards, let them air-dry.
4. Burn the plastic bags and dispose of the formalin in a safe place (do NOT pour it into streams or rivers!)

(v) What if I find a carcass that is not yet decomposed?

1. First put on protective clothing as described before
2. Take a GPS coordinate and some notes (see above)
3. If present, collect **some maggots** and place them in RNA later as described before
4. If the body is not too decomposed yet, take **a nose swab**:
   - ⇨ Insert a swab into one of the nostrils and turn it a few times to swab the inside of the nose. Then place the swab in RNAlater and store the tube as described above.
5. Afterwards bury the carcass in the ground or covered by soil, but in such a way that there are some passages for flies or ants to consume the meat.
6. Take a GPS point of the grave and maybe additionally mark it with e.g. flagging tape.
7. Burn or disinfect your protective clothes as before.
8. After a few weeks or months, depending on the stage of decomposition, come back to the carcass to retrieve some of the bones (wear protective clothing!!).
9. Disinfect the bones in 5% formalin as described above.

b) Target sample number

- 10 x herbivores
- 10 x omnivores/insectivores
- 5 x carnivores
- 5 x insects (per termite, ant and bee category)[20]

c) Sample treatment

If sample is small, store in a 15 or 50 ml tube filled two-thirds with silica gel. Insects such as ants, termites and bees can be stored in empty 15 ml tube without silica. In the case of large specimens e.g. bones, store in a Ziploc bag with silica and change the silica until completely dry (Figure 9-6). Remember to include a note with all information (Table 9-3) in the tube/bag and to also label the tube/bag on the outside, which should be secured with a tape. See section 9.3 on sample ID coding system. Store the labelled tubes/bags at room temperature.

Table 9-3 Label for use for the storage of small animal tissue samples and for whole carcasses.

Sample ID	date	sample type	UTM zone	latitude	longitude	animal species	tissue(s) sampled	diet	canopy position	age
		animal								
		animal								

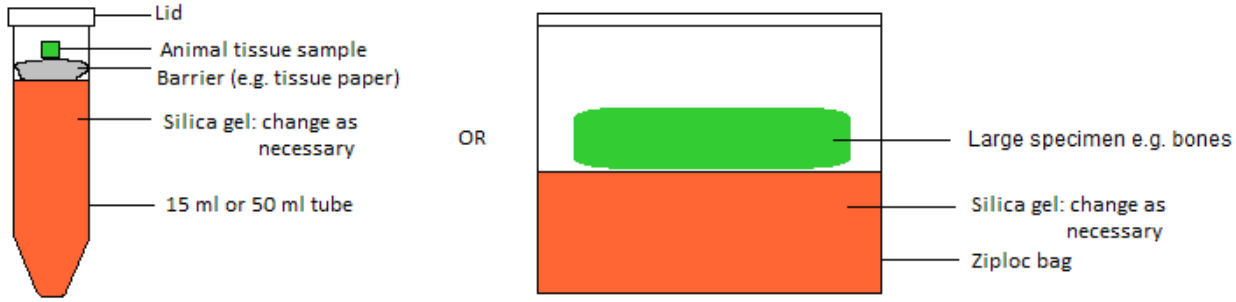

Figure 9-6 Methods for the storage of small animal tissue samples and for whole carcasses.

(i) Army Ants

Army ants (also called driver ants, in French "magnans") form huge temporary underground colonies with millions of individuals. Surface opening of the nest with loose soil are quite typical. They are nomads and raid large termite mounds regularly. Chimpanzees are known to eat the larvae by extracting them directly with the hand or by dipping for the soldiers with the help of sticks. The soldiers should therefore, they should be collected for stable isotope analyses and identification.

Nests are frequently situated at the base of trees (Figure 9-7 a). Army ant nests can be recognized by the sand pushed up onto the surface from underneath the ground (Figure 9-7 b-d). You may also be able to observe the ants near their nest – they are social insects and thus always occur in groups. The nests of some species are almost impossible to detect because they are covered by leaves (Figure 9-7e). You may also encounter foraging trails or migration trails (Figure 9-7f). When army ants move to another location, they carry the eggs, larvae and queen to the new nest. When they hunt, they form swarm raids, sweeping across the ground and carrying pieces of insects and small mammals to their nest along foraging trails.

[20] Added to this version of the protocol for clarification, but also see below on sampling of bees, ants and termites

Figure 9-7 a) Army ant nest at base of tree – here the army ants are visible at the nest entrance; b-d) Army ant nests noticeable by the sand pushed up from underneath the ground; e) Army ant foraging trail where sometimes parts of the trail are concealed with sand and leaves; f) Army ant foraging trail. Large soldier ants are often found on the edge of the trail to protect the smaller worker ants and the goods they carry (Photos a-e: Yasmin Moebius; Photo f: Casper Schöning)

For isotope analyses, collect them with a pair of tweezers, decapitate them, and store them in an empty 15ml tube (Figure 9-8a). Collect 2-3 ants per tube for stable isotope analyses. Collect another 2-3 ants in another tube for duplication in case of loss during transit to Leipzig. This duplicated tube will be given the exact same ID code and information as the other tube.

For later identification, collect the army ants with a pair of tweezers and transfer them into a labelled 5ml glass tube filled with 70% alcohol for species identification in Germany (Figure 9-8b). Only

collect the **largest soldier ants** for identification and not the smaller worker ant. Collect about 5 specimens of the largest soldiers.

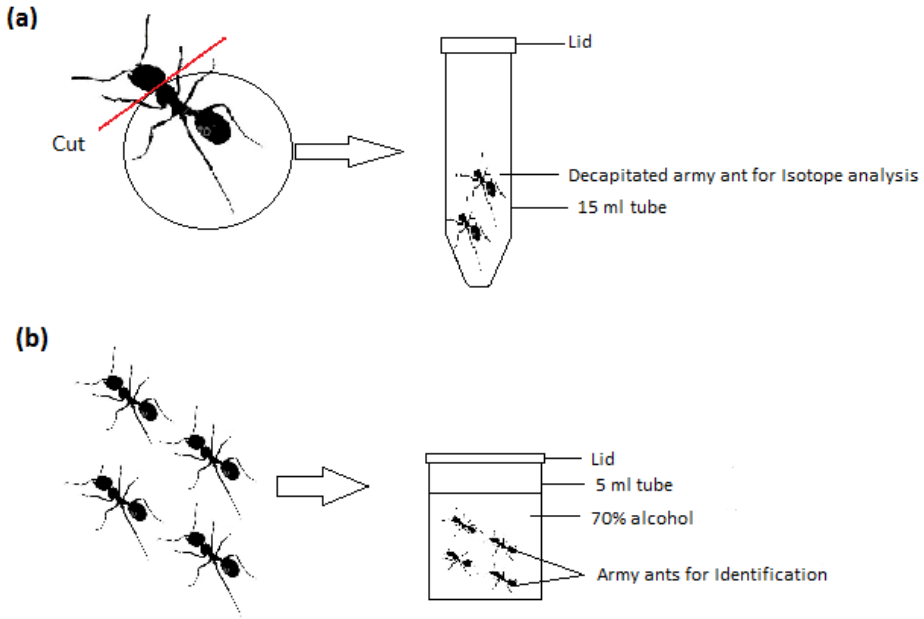

Figure 9-8 Storage methods of army ants for (a) isotope analyses and (b) identification.

(ii) Bees

Chimpanzees are known for their liking for honey and have been seen to raid beehives and extract honey with different techniques that can include up to 5 different types of tools. If honey bees are raided in all known chimpanzee populations, depending on the populations different species of stingless bees (also called "sweat bees" or in French "Mélipones"; Figure 9-9) are raided by chimpanzees for their honey. Over 30 species of stingless bees are known for Africa and 8 species are known to be eaten with tools by chimpanzees. Large stingless bees (ca. 1 cm long) and small ones (ca. 3 mm long) should be sampled. Wood-boring bees (*Xylocopa* sp.) are also regularly eaten but they build much smaller colonies and make small nest in dead above the ground hanging branches. Therefore, samples of different species of stingless bees as well as honey bees will be taken in labelled tubes with 70% alcohol for later species identification in the lab and in an empty tube for isotope analyses (Figure 9-10). Collect 2-3 bees per tube of the specimen for isotope analysis and make a duplicate copy in another tube with exact same ID code and information in case of loss during transit to Leipzig.

As for all observations record the distance from the start of the transect, time of day, observation, number of objects, and GPS coordinates.

Figure 9-9 Stingless bees (*Meliponae sp.*) in their nests. In the centre the honey can be seen (Photo: Christophe Boesch)

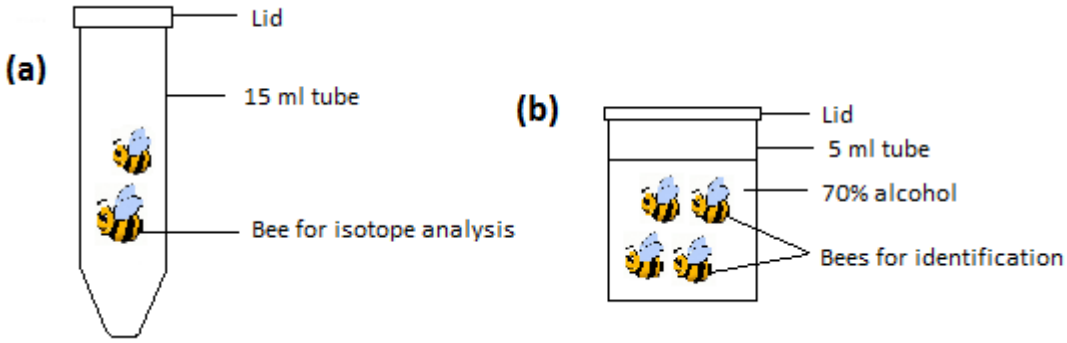

Figure 9-10 Storage methods of stingless bees for (a) isotope analyses and (b) identification.

(iii) Termites[21]

Collect termite specimens per termite mound from different types of termite mounds that are counted in the strip transects and from each habitat type. For isotope analyses collect 2-3 termites per mound with tweezers, decapitate them and store in an empty 15 ml tube (Figure 9-11a). For later species identification in the lab, 5 specimens of termites are collected per mound and stored in 5 ml glass vials with 70% alcohol (Figure 9-11b). As for army ants and bees, make duplicate copies of the isotope analyses tube, the tubes being given the exact same ID codes and information.

Make sure to collect at least the following three species that are known to be frequently eaten by chimpanzees (see section 13.2.2 for illustrations):

- *Macrotermes* species
- *Thoracotermes* species
- *Cubitermes* species

[21] Also refer to Annex IV-6 and IV-8 regarding identification vials and termite collection

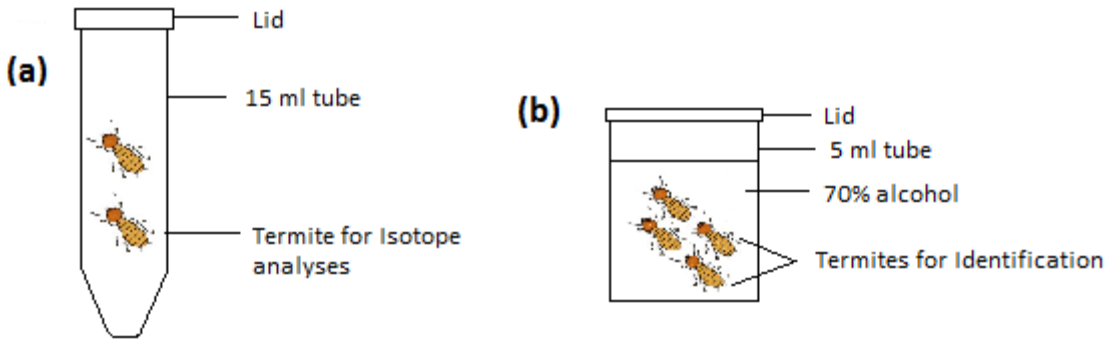

Figure 9-11 Storage methods for (a) isotope analyses and (b) identification, of termites.

9.2 Geology reference samples[22]

One open mystery in chimpanzee life is how far females disperse away from their natal group. The strontium isotope analysis has the potential to answer such a question, as the isotope properties of different areas within a region can be quite diverse allowing to track down where an individual has spent its youth. Therefore, a mapping of strontium isotope (86Sr/87Sr) within the grid and especially on different sides of larger rivers can be very informative.

a) Sampling methodology

We seek to detect locality using strontium isotopes; with the samples collected we determine the local signatures that are dependent on the underlying bedrock/geology. Collect one nut, snail shell and termite earth sample for every cell in forests, and in savannah habitats. Sample sizes can be small. Remember to record the accurate GPS location of the sample found. Collect water samples opportunistically[23].

b) Target sample number[24]

3 per sampling unit, including:

(i) Snail shell

Find an empty shell of dead snails e.g. *Achatina fulica* (Figure 9-12) and take the exact GPS coordinates at the spot where you found it. You can break the shell into small pieces to fit a 15 ml tube.

(ii) All nuts species

Take nuts that do not have any fruit flesh anymore on them, but that still are hard, dry, and not completely rotten. Take the GPS coordinates at the spot where you found them. Try to break the nut(s) to fit in to a 15 ml tube.

(iii) Soil sample

Find termite mounds that are above the ground (see some examples in Figures 9-13, 12-8, 12-9). Break off small pieces of the mound and half fill up a 15ml tube with them. Take the GPS coordinates at the spot where you found the mound and write down the coordinates. Remember when storing soil or termite mound samples, that you do NOT put the silica directly in the tube with the soil (otherwise separation for analysis is very difficult!). Store the tube of soil sample in a bag with silica.

(iv) Water samples

Collect water samples in a 15ml tube whenever you find surface water (see Annex IV-10).[23]

[22] See Annex IV-10 for water samples

[23] In the previous version of the protocol this was missing in the isotope section and only mentioned in the sample summary table.

[24] For clarity: one of each (1 snail shell, 1 nut shell, 1 termite soil) for each cell of your grid

Figure 9-12 *Achatina fulica* snail shell

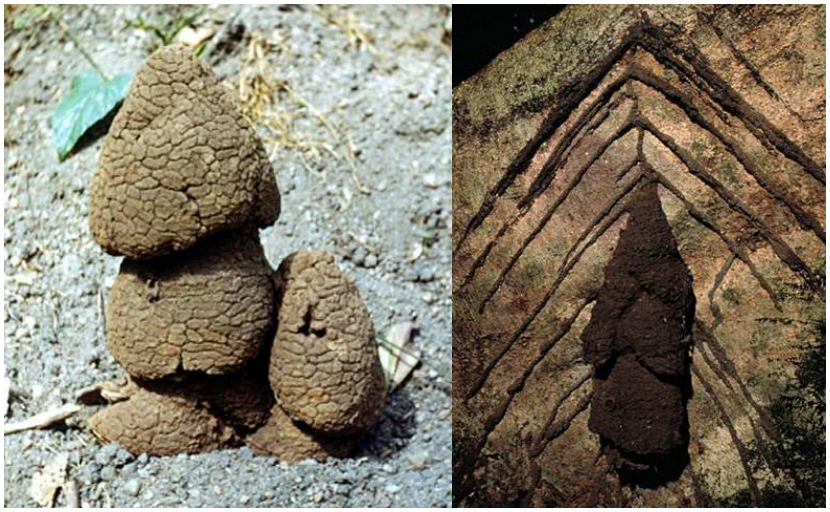

Figure 9-13 Nest of termite, *Procubitermes*

c) Sample treatment

Break the shell (both from snail and nut) in small pieces and half fill a 15 ml tube with the shell pieces; 1 g of each sample is sufficient (Figure 9-14). Cover the sample with tissue or a piece of paper, and top up the tube with silica to keep samples totally dry. Change the silica if the silica gel becomes saturated (turns from orange to colourless). Remember to include a note with all relevant information (Table 9-4) inside the tube, and also to label the tube on the outside and to cover with a piece of tape. See section 9.3 on sample ID coding system.

Table 8-4 Label for use on geology reference sample tubes

Sample ID	date	sample type	UTM zone	latitude	longitude	sample
		geology				

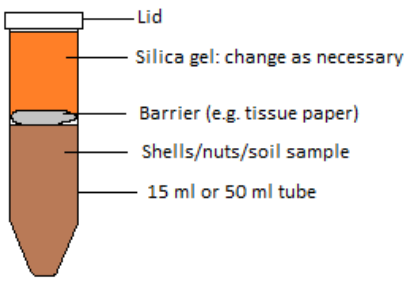

Figure 9-14 Storage method of geology reference samples.

9.3Coding system for samples

Apply the following unique coding system for all samples:

"TRS code" _ "sample ID number"

The sample ID number is unique and no sample receives the same code (exception below). So the third and fourth samples collected at Nathia would receive the code: **Nat_003** and **Nat_004**, respectively.

When more than one sample is taken from one tree/dung pile/termite mound for different analysis types, then a further extension code is given:

"TRS code" _ "sample ID number" _ "extension"

These are given the same TRS code and unique sample ID number but differ in their extensions. For example if the sixth sample collected at Nathia was a termite for stable isotope analysis and species identification, they would receive the following codes:

Stable isotope analysis: **Nat_006_a**
Species identification: **Nat_006_b**

9.4References

Oelze, V.M., Fuller, B.T., Richards, M.P., Fruth, B., Surbeck, M., Hublin, J. and Gottfried, H. 2011. Exploring the contribution and significance of animal protein in the diet of bonobos by stable isotope ration analysis of hair. *PNAS*, doi: 10.1073/pnas.1018502108.

Sanz, C., Morgan, D., Strindberg, S.Onononga, J.R. 2007. Distinguishing between the nests of sympatric chimpanzees and gorillas. *Journal of Applied Ecology* **44**, 263 – 272.

10 Genetic and Pathogen samples

Purpose: To collect samples for genetic and parasite content analyses

IMPORTANT

- Collect only fresh samples as both DNA and pathogens are very sensitive to time decay and humidity
- Store samples rapidly to prevent degradation of DNA
- Always wear gloves when collecting faecal samples
- Regularly enter information on collected samples in the prepared excel file in order to always be up to date with the state of sample collection – this is crucial for timely application of export permits.
- Use data entry sheet 'Organic samples' (see annex III)
- Refer to Annex IV-5 for an update on genetic sample collection[25]

Fresh chimpanzee faecal samples are collected whenever you encounter them. However you should in addition, conduct targeted sampling. On days that are not specifically allocated to other data collection, walk around the grid, particularly areas not covered by other data collection, in order to try and find fresh faecal samples.

10.1 Genetic samples

The collection of faecal and other organic samples such as sperm plugs and food remains of chimpanzees represents a non-invasive method for obtaining DNA for genetic analysis of paternity, relatedness, and dispersal. Additionally, information on genetic structure, sex ratio, group structure and composition, home range size, habitat use, and diet may be derived from these samples. Samples should be collected during recce surveys, line transect surveys **(i.e. whenever you find ape dung, record this observation and take a sample!)** and opportunistic or targeted sampling. Dung yields a much higher extraction success rate than food wadges (Figure 11-1) for genetic analyses. Collect food wadges only if no dung can be found.

Sampling and storing samples for genetic analysis:

Stage 1
Materials needed:

- 50 ml tubes containing silica gel beads (new i.e. not reheated silica)
- Denatured ethanol (pharmacy grade, 97% (the higher the better))
- Empty 50 ml tubes

Preparation:

- Pour approximately 40 ml of ethanol into empty tubes for sample collection.

Collection:

Collect each fresh faecal sample (approx. 5 g, size of a thumbnail) into a tube containing ~ 40 ml ethanol. Remove large particles like seeds before transferring faeces into the tube. Always wear gloves. It

[25] Addendum to original protocol: Annex IV

is important not to touch the sample directly to avoid contamination. Samples stay in alcohol over night to dehydrate the faeces before it is transferred to silica gel (see below).

Stage 2 (after 24 hours in ethanol)

The bolus of the faecal sample (or other organic material such as sperm plugs) collected in ethanol is transferred into fresh silica tubes for further drying. Again be careful not to touch the sample. This is done by carefully pouring off the ethanol with the tube loosely capped and transfer solid material to the new tube. This tube should be labelled and stored at room temperature.

It is important that all information associated with each sample (identified by a unique ID number) is recorded in a data sheet and then entered in the computer in the appropriate excel sheet. Tubes must be labelled with the below table (Table 10-1)[26]. When hairs are found in the faeces, these should be dried and stored separately in a paper envelope for isotope analysis (see section 9.1.1).

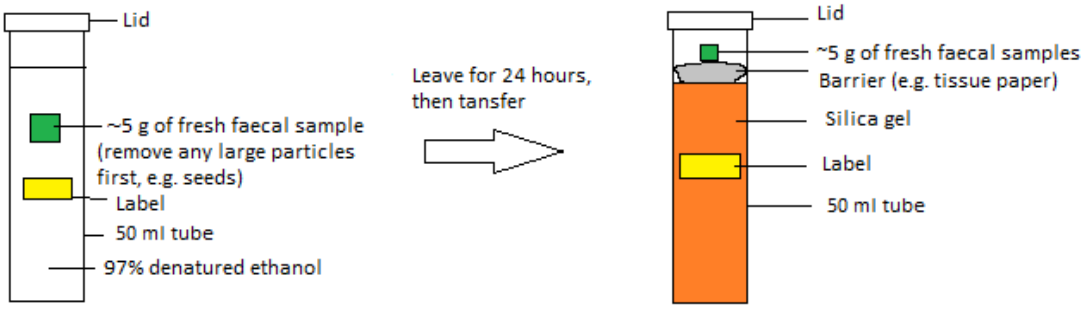

Figure 10-1 Process for the storage of genetic samples.

10.2 Pathogen samples

Fresh faecal samples are also collected for pathogen analysis. It is very important to follow these instructions carefully to ensure that the samples are collected properly so that they can be used for analysis.

From the **freshest faeces possible** put samples in the tubes.

How to fill the tubes:

Pour 10ml of RNAlater in a 15ml tube. RNAlater is **very expensive** therefore make sure not to spill anything and not to use more than indicated. Put a piece of faeces of the size of 1 small bean in the tube and shake very well.

- **Do not collect more faeces than indicated per tube as otherwise there is not sufficient RNAlater for the amount of faeces in the tube.**
- **Do not touch the inside of the tubes.**
- **Shake the tubes well, at least 20 times vigorously.**

Close the tubes well and store as cool as possible, out of the sun, ideally in a fridge or freezer. Make sure that tubes are stored upright to prevent leaking.

[26] An error in previous versions stated "and insert a piece of paper inside with all necessary information". Genetic samples are highly susceptible to contamination and all efforts should be made to assure no human contamination of the samples! LABEL THE OUTSIDE OF THE GENETIC TUBES ONLY!

10.3 Labelling samples

Table 10-1 Label for use on tubes of genetic and pathogen analysis samples

Sample ID	date	sample type	UTM zone	latitude	longitude	species	analysis
		faeces					genetic
		faeces					pathogen

Important: in the field, all samples collected have to be entered into a data sheet along with all associated information.

10.4 Coding system for samples

Apply the following unique coding system for all samples (sample IDs):

"TRS code" _ "sample ID number"

The sample ID number is unique and no sample receives the same code (exception below). So the third and fourth samples collected at Nathia would receive the code: **Nat_003** and **Nat_004**, respectively.

When more than one sample is taken from one tree/dung pile/termite mound for different analysis types, then a further extension code is given:

"TRS code" _ "sample ID number" _ "extension"

These are given the same TRS code and unique sample ID number but differ in their extensions. For example if the sixth sample collected at Nathia was a termite for stable isotope analysis and species identification, they would receive the following codes:

- Stable isotope analysis: **Nat_006_a**
- Species identification: **Nat_006_b**

10.5 References

Nsubuga A.M., Robbins M.M., Roeder A., Morin P., Boesch C. and Vigilant L. (2004) Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. *Molecular Ecology* **13**: 2089-2094.

11 Diet and Urine samples

Purpose: To identify the diet of chimpanzees through faecal sample collection and analyses, and determine their nutritional status from urine samples

IMPORTANT

- Quick identification of the diet helps also in making the phenology collection include important food trees.
- Always wear gloves when collecting urine or faecal samples to avoid contamination
- Burn any material that came into contact with chimpanzee dung
- Use data entry sheet ' Faecal samples diet' for diet, 'Organic samples' for urine (see annex III)
- Also refer to Annex IV-9 on medicinal plants and Annex IV-7 on temporal sampling[27]

Chimpanzee faeces and urine are collected whenever you encounter them throughout the study. However you should also conduct targeted sampling on days not specifically allocated to other data collection, in order to make these collections. Walk around the grid, particularly areas that are not covered by other data collection.

11.1 Chimpanzee diet[28]

When fresh or old chimpanzee faecal samples are encountered, these are washed and sieved to determine chimpanzee diet at the site. If the faecal sample is fresh, samples are also taken for genetic and pathogen analysis and labelled and stored appropriately. After these samples have been taken, the remainder of the faeces is collected in aluminium foil, carried back to camp and washed (e.g. in a small stream): the faecal material is placed in a sieve and rinsed until all soft material is gone. Large particles are removed with tweezers, identified and information recorded in the appropriate datasheet. Plant material that cannot be identified in the field is dried as much as possible and transferred into tubes or plastic bags with silica gel and labelled for later identification by an expert. All identified seeds are dried and stored in a 50 ml tube that is two-thirds filled with silica gel for archiving and future analyses[29].

Faecal samples map also contain ape hair which can also be used for isotope analyses and should be stored appropriately. Store all hair samples in a sealed envelope, which should then be kept in a Ziploc bag containing silica gel (see section 9.1.1).

Animal remains such as skin, bones or feathers that are found in the faeces will be removed, then washed, dried and stored in a 15 ml or 50 ml tube with silica gel for isotope analyses and identification if it can not be identified immediately. Note that they were found in faeces.

Feeding remains from chimpanzees can also provide precious information on food that may leave no trace in the faeces. Chimpanzees are well known for preparing extensively their food before ingestion and to do so more than any other primate species that may be sympatric to them in your grid. So that is an important source of information.

How do you recognize **chimpanzee food remains**?

- Larger fruits, like *Parinari*, *Sacoglottis* or *Klainedoxa*, are often eaten when on the ground. Chimpanzees would gather some of them and then peel the **skin of the fruits** before chewing them. So finding only fruit skin remains on the ground more or less in a pile is a good sign of chimpanzee feeding.

[27] Addendum to original protocol: Annex IV

[28] Also refer to Annex IV-7 – the importance of temporal sampling

[29] A previous version of the protocol incorrectly stated that identified seeds were used for isotope analyses. To be clear: ALL unidentified AND identified material from the dung is collected and stored in tubes containing silica.

- Second, chimpanzees make regularly "**wadges**" (Figure 11-1) from fleshy fruits, like *Parinari* and *Sacoglottis*, to extract the juice without ingesting the pulp. Wadges look like dentist imprint of your mouth and teeth. Therefore, finding wadges on the ground is a sure sign of chimpanzee remains.
- Similarly, finding piles of fruit **kernels** of which the flesh has been removed is also a sign of chimpanzee remains.
- Chimpanzees eat THV mainly by extracting the white soft pith of the plants: the only part they eat. Therefore, you will find the greener part of the THV on the ground split open in such a way as to extract the white part.
- Finally, chimpanzees when eating on small fruits that are numerous on thin branches of a tree crown, like *Dialium* and *Tricoscypha*, will tend to break off a twig bearing bundles of fruits, and sit on larger branches in the tree crown to eat them. Broken pieces of branches with most fruits eaten will therefore typically be found lying under these trees.

Figure 11-1 A food wadge made by a chimpanzee (Photo: Sonja Metzger/WCF)

11.2 Chimpanzee urine

Urine is a very informative product of the body but rather rare and difficult to find in the wild. So if you are lucky to find some try your best to collect it.

Urine samples will be collected to determine the nutritional status of apes. Fresh urine will be collected from under chimpanzee nests, on leaves or other objects, with a disposable pipette and transferred into a 1.5 ml eppendorf tube. Urine from different individuals (i.e. from different nests) is stored in different tubes. After labelling the tube, the sample will be stored in a freezer at camp. Where

freezing facilities are not available pipette as much urine as possible onto filter paper (Figure 11-2). Then dry and store these filter paper samples with a small amount of silica gel (see Marshall & Hohmann, 2005).

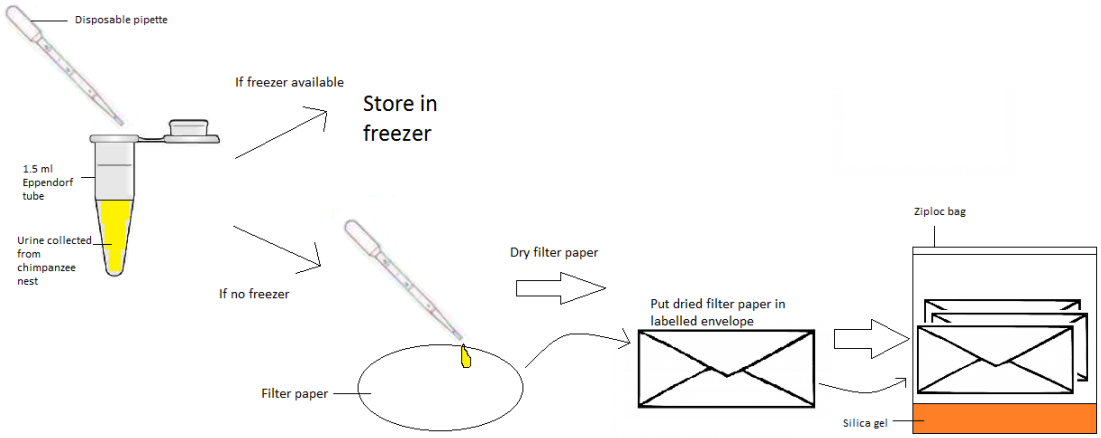

Figure 11-2 Storage process of chimpanzee urine.

11.3 Labelling samples

Table 11-1 Label for use on tubes of diet and urine samples

Sample ID	date	sample type	UTM zone	latitude	longitude	species	analysis
		faeces					Diet
		urine					

Important: in the field, all samples collected have to be entered into a data sheet along with all associated information.

11.4 Coding system for samples

Apply the following unique coding system for all samples:

"TRS code" _ "sample ID number"

The sample ID number is unique and no sample receives the same code (exception below). So the third and fourth samples collected at Nathia would receive the code: **Nat_003** and **Nat_004**, respectively.

When more than one sample is taken from one nest or faeces for different analysis types, then a further extension code is given:

"TRS code" _ "sample ID number" _ "extension"

These are given the same TRS code and unique sample ID number but differ in their extensions. For example if urine and fresh faeces were found at one nest, i.e. one individual, the samples would receive the following codes:

- Urine sample: **Nat_010_a**
- Faeces for genetic analysis: **Nat_010_b**
- Faeces for pathogen analysis: **Nat_010_c**
- Faeces for diet analysis: **Nat_010_d**

11.5 References

Marshall, A.J. & Hohmann, G. 2005. Urinary testosterone levels of wild male bonobos (*Pan paniscus*) in the Lomako Forest, Democratic Republic of Congo. *American Journal of Primatology* **65**, 87-92.

12 Traps

Purpose: To identify the abundance and diversity of bees, and use blowflies to evaluate the species diversity in the region

IMPORTANT

- Put out the traps only on sunny days
- Use the data entry sheet 'Traps'

12.1 Stingless bees

Bee nests are extremely difficult to spot, especially the underground nests as the entrance on the surface are often very small (Figure 8-4 b; Figure 12-1). Baited traps therefore allow for a more accurate way of estimating abundance and species diversity.

Figure 12-1 Surface *Meliponae* nest entrances (Photos: Christophe Boesch)

Trap preparation

1. Cut the top of a plastic 1 -2 litre bottle and fill with 200 ml of bait liquid: Bait liquid can be any of the following and different things work at different sites, so you may have to try different baits. You can test baits near your camp before setting up traps: Scented laundry soap (eg: Omo), human urine, crushed up fruits and/or flowers (with a strong scent), flowery perfume, honey, human sweat placed around the 'funnel' or ask your assistants for what they think may work.[30]
2. Replace the top of the bottle inverted (Figure 12-2) and seal the top with tape
3. Attach hooks to top

Trapping

Four traps should be set up per habitat type, ideally evenly distributed across the grid, twice before and twice during the rain seasons. Once the locations have been identified, each trap will be set 1 m above the ground on one day, and 10 m above the ground on another day. The traps will be setup for exactly 24 hours, after which the number of bees in the trap will be counted and recorded, and the bees transferred to 15 ml or 50 ml tubes. Once back at camp, clean, dry and store the bees back in the cleaned tubes with silica gel.

[30] Previous versions suggested using 50% sugar water. However this bait was unsuccessful at all sites, so the list above summarizes the solutions different people have found. Also refer to Annex IV-2

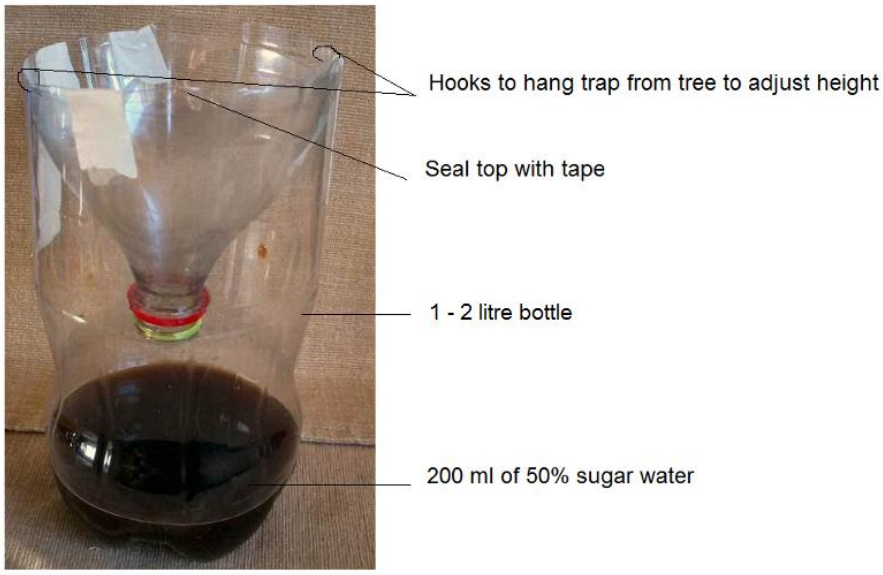

Figure 12-2 Stingless bee trap to be placed in different habitat types at 1 m above the ground, then 10 m above the ground on another day.

12.2 Blowfly

Blowflies can serve as sentinels for various pathogens but also indicate presence of carcasses in the area. Fly traps will therefore be placed at all sites within the grid, in different habitat types. Flies will be analysed for various pathogens and the origin of last meals.

Trap preparation

1. Take a medium sized plastic tub (with well closing lid for transport)
2. Empty a sachet of bait powder into the tub, add water until the powder is well soaked and close the lid
3. Store the tub for 5 days before use. Refill the tub with more water if it evaporates after a couple of days. The bait will remain effective up to 4 weeks.

Trapping

1. Put up a mosquito net, holding the edges out, and lifting one corner up (Figure 12-3)
2. Open the tub, place a small piece of net on top of the tub but not touching the bait (so that the flies do not come in contact with the bait)
3. Place the tub inside the mosquito net on the ground. If it is attracting a lot of ants, raise the tub off the ground
4. As soon as some flies have gathered in the trap, corner the flies in the net and transfer them directly into a 15 ml tube with alcohol
5. Repeat for a maximum of 20 minutes. If you capture 20 flies, stop and record the duration of capture (if there was no fly for 20 minutes note this as well on the table).
6. Within one hour, transfer the flies from the alcohol into a 50 ml tube with silica gel. Store up to 20 flies from a single trap in the same silica tube and label. **Important:** Do NOT touch the flies. The alcohol tube can be reused for all trapping locations by refilling them with alcohol.
7. Repeat the trapping procedure every 1 km along all the line transects, biasing towards different habitat types. Remember to take GPS coordinates of each location.
8. Conduct once in the dry season, and once in the wet season, at the same locations.

Sample number

Collect one tube (i.e. 20 flies) at each sampling location. At least 25 tubes will be collected per season, so at least 50 silica tubes in total.

Hygiene

Always wear gloves when sampling, handling the mosquito nets and bait tubs. Transport the net and tub in a plastic bag and wash the equipment after each sampling period with soap and detergent.

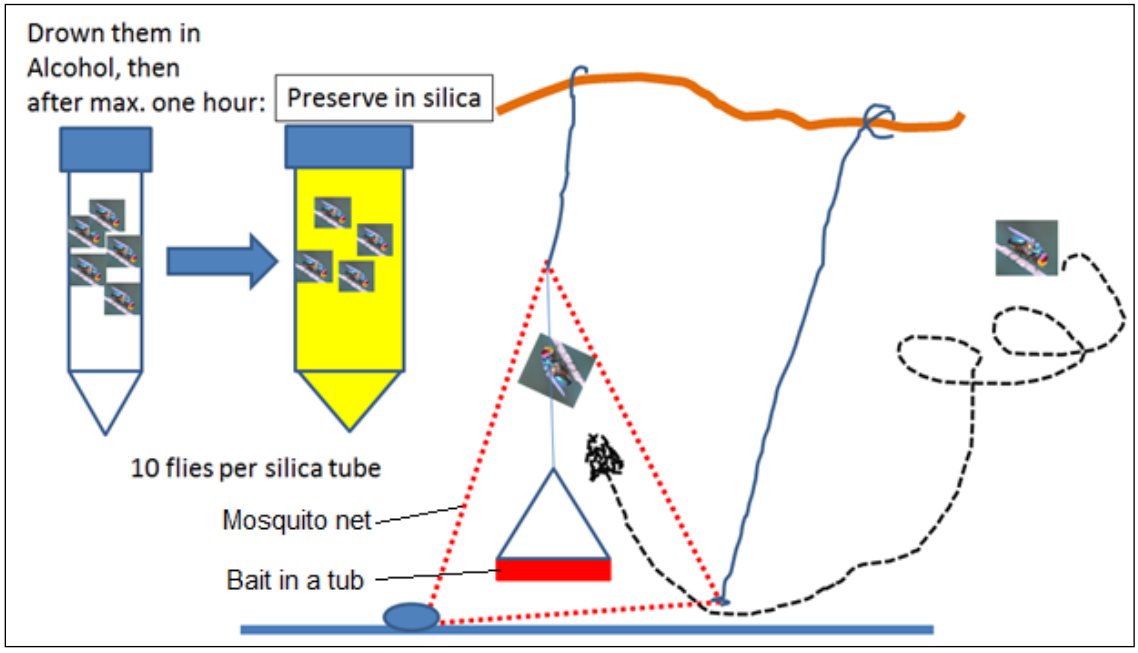

Figure 12-3 Blowfly trapping method

13 Line and strip transects

Purpose: To collect data on animal abundance on line transects, and to assess insect and tool use availability on strip transects.

IMPORTANT

- Line transects are straight lines made/cut to cross through the middle of all cells in a North/South or East/West direction across the grid.
- Strip transects are 2 m bands laid 1 m on each side of the transect
- To carefully consider the four basic assumptions of distance sampling on line transects
- Survey line transects once every 4 months during the study
- Use the data entry sheet 'Line transects' and 'Strip transects'
- Also refer to Annex IV-11 for the strip transect protocol for sites with high densities of rocks[31]

13.1 Line transects

13.1.1 Assumptions of sampling line transects

Line transect sampling relies on the skills of human observers. Humans differ a lot in their skills. To make data comparable, four basic assumptions must therefore be met:

1. 100% of the observations on the transects line and very close to it are detected
2. Animals are detected before they move
3. Perpendicular distances are measured accurately
4. Observations are independent

13.1.2 General

Line transects will be walked three times per study, once every 4 months (due to time constraints it is not always possible to walk the transects in evenly spaced increments, transects can be walked as close as 1 month apart, although a good overall temporal spacing is desirable)[32]. For reasons of visibility, it is best to collect data between 7:30 in the morning and 16:00 in the afternoon. When it is raining you cannot walk transects as rain may affect the ability to detect objects. Likewise, if it starts raining heavily while you are walking a transect you have to stop and continue the next day. When the transect could not be completed within one day, teams continue collecting indirect signs of animals the next day at the spot where they stopped the previous day. However, direct observations of animals have to start again at the very beginning of the transect to avoid double counting. When a member of the survey team detects something the team stops and everybody comes together to record the necessary data. After data collection, everybody returns to their original positions on the line.

At the start of the transect, attach the topofil, take a GPS position from the point of departure and record the position on the data sheet. Remember to activate the **tracklog** on the GPS. Once the survey team has been positioned correctly begin your observations at point 0 m. Walk the transect line at an average speed of **500 m/hour** and record data until the topofil indicates the distance that equals the length of the transect line (e.g. 4000 m). Only stop surveying when the last observer has reached the end of the line. Take the GPS position at the end of the transect line, record it on the data sheet under "end transect" and cut the topofil.

[31] Addendum to original protocol: Annex IV

[32] Addendum 2014, few people managed to reach the 4 month target and so transects are not evenly spaced across time at most sites. Transect walks can be done as close as 1 month apart.

If your TRS is located in forest with dense understorey do not cut transect lines when also collecting observations. The transects are the same as those used for the habitat structure surveys so you should not need to make further cuttings. Cutting transect lines is very noisy and animals will flee before you even see them.

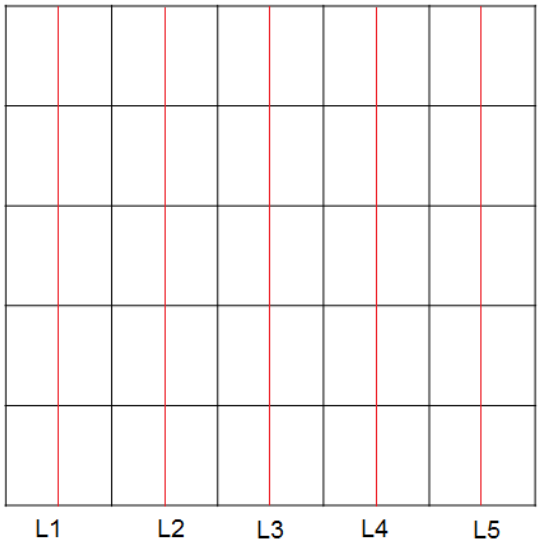

Figure 12-1 Grid with line transects (red lines) that run through the centre of cells (L1 to L5).

13.1.3 Team formation and role

Line transects will be conducted with **three members** in the following formation (Table 12-1; Figure 12-1):

Table 12-1 Positioning, role and equipment carried by each member

	Positioning and Role
Person 1	Uses a compass and GPS to guide the team in a straight line
Person 2	Max. 10 m behind person(1), carries a pair of binoculars and records all observation in a data sheet
Person 3	Max. 5 m behind person (2), carries a topofil
All	Look out for all signs and observations

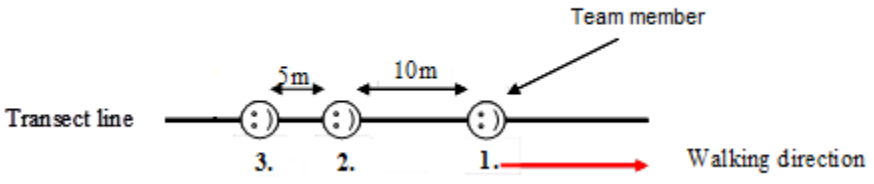

Figure 12-1 Positioning of members of the survey team along the line while walking the line transect with 3 people.

13.1.4 Using the topofil

The topofil (also known as hipchain) is an important tool with which one can determine the distance travelled even without a GPS. In addition, the GPS is not totally accurate and always has some

error (about 15 m on average) associated with every reading. However, care needs to be taken when using the topofil as wrong installation of the topofil may yield incorrect information about the distance travelled along the line transect.

Use the topofil as follows:

Make sure that the topofil displays correct distance values by measuring a length (e.g. 10 m) with the tape measure and walking the same distance with the topofil to check that the value displayed by the topofil is correct.

When you are at the beginning of your line transect, tie the thread to a tree and make sure the counter is on zero. Always have the thread wound 3 three times around the small spool. Be careful with the thread, it tears easily. If you cut the threat by accident, walk back to the point where the thread broke and start with a new thread by tying it to a tree at this point (Figure 12-2). If you have been walking for a while without the topofil without noticing that it broke, go back to the last waypoint and measure the distance to this point, then reset the counter and walk the remaining distance to get to the end of the transect. Note this on your data sheet, i.e. that the topofil broke, the distance at which it broke and the distance to the end of the transect line.

For instance, when walking a 500 m transect (Figure 12-2), your topofil breaks and the last GPS waypoint you had taken, was at 200 m. You walk back to the waypoint and measure that you walked 150 m, which means that your topofil broke at 350 m. You tie the thread to a tree at 200 m, reset the counter and walk back to 350 m (which will show on the counter as 150 m). You then start surveying again and walk the remaining 150 m to the end of the transect line at which point your counter will show 300 m (instead of 500 m). To avoid such delays, always walk slowly with the topofil in order to not break the thread before reaching the end of the transect line.

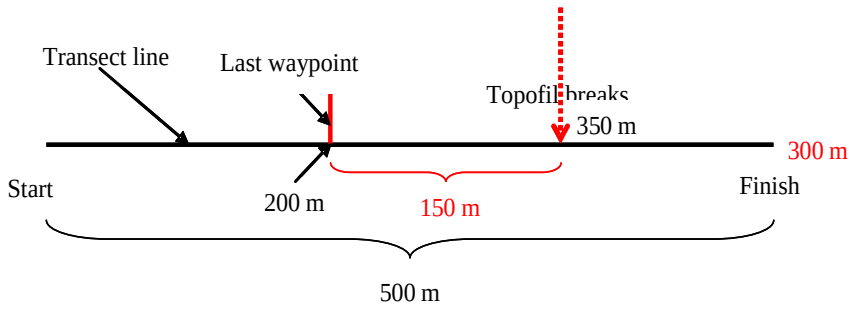

Figure 12-2 Protocol to follow when you accidentally cut the topofil.

13.1.5 Measuring perpendicular distances

Perpendicular distances are not measured for all observations. Measure perpendicular distances for the following observations from the transect line to the middle of the observation (Figure 12-3):

- Chimpanzee tools and tool use sites
- Direct observations of monkeys

All other observations are only recorded without perpendicular distances, e.g. human signs, indirect observation of monkeys, direct/indirect observation of other mammals, chimpanzee faeces, habitat type.

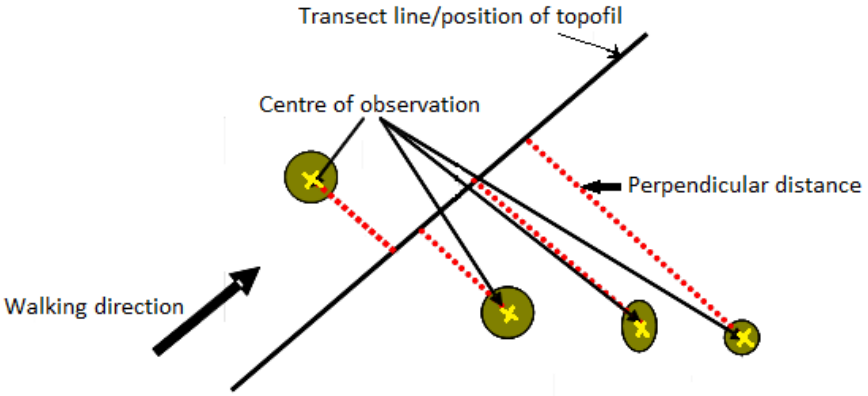

Figure 12-3 Measuring perpendicular distances (red dashed lines) to the centre (indicated by yellow crosses) of individual observations (indicated by green circle).

Perpendicular distances should be measured accurately and precisely with the tape measure. It is important to always measure at a 90 degree angle to the transect line, i.e. the topofil. Use the tape measure to measure the distance from the transect line to the detected object. Make sure that you lay the tape measure on the ground if possible and that you accurately read the distance. One team member should stay on the line and hold the tape measure on the ground. Use the compass to determine a 90 degree angle to the transect line. If you are walking North, then either East or West would be perpendicular to the line. If you are walking at 71° (North-East), then the detected object should be measured at 161° (71° + 90° = 161°), if the object is East of the direction of the line and at 341° (71° - 90° = - 19 and 360° - 19° = 341°) if the object is West of the direction of the line (Figure 12-4).

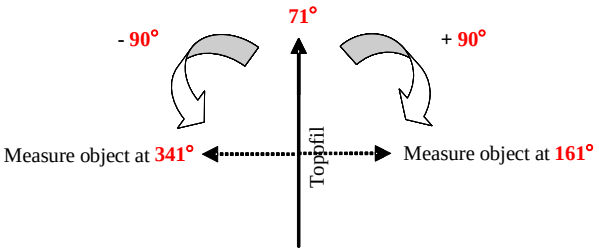

Figure 12-4 Using the compass and the topofil to measure perpendicular distance to a detected object.

13.1.6 Obstacles on the line

When you encounter an obstacle on the transect line that cannot be traversed (stream, big tree, etc.), you have to navigate around it (Figure 12-5). When you get to the obstacle, cut the topofil and walk around the obstacle until you get back to the transect line. To do this, walk exactly 90° or 270° from the direction you were travelling. When you have gone beyond the obstacle, measure the distance that you have deviated from the transect. Then turn again and proceed along your original compass bearing. When it is possible, turn 90° or 270° again and walk the same distance that you deviated back to the original line transect. Then turn one last time back to your original compass bearing to follow your original course. At

this point you tie the topofil to a tree and start surveying again. Do not collect data while moving around the obstacle.

The distance that you did not survey due to the obstacle will be subtracted from the transect length. Take a waypoint at the location when you cut the topofil, and when you start surveying again. Calculate the distance between the waypoint when you cut the topofil and when you started surveying again with your GPS. Proceed in the same manner when encountering further obstacles until you reach the end of the transect line i.e. when your topofil/GPS indicates that you have reached the end of the line transect. In order to determine the end point subtract the distance that you did not survey due to the obstacle from the original length of the line transect. Thus, in the end, a 500 m long transect may be only e.g. 464 m in length, because you excluded 36m that you did not survey due to an obstacle on the line. When you encounter obstacles, such as bushes or small trees, you can look into the bush/ tree etc. to make sure that you did not miss any nests, walk around it and back onto the line. This kind of obstacle is not subtracted from the transect length as you were able to determine whether there were nests along the line transect.

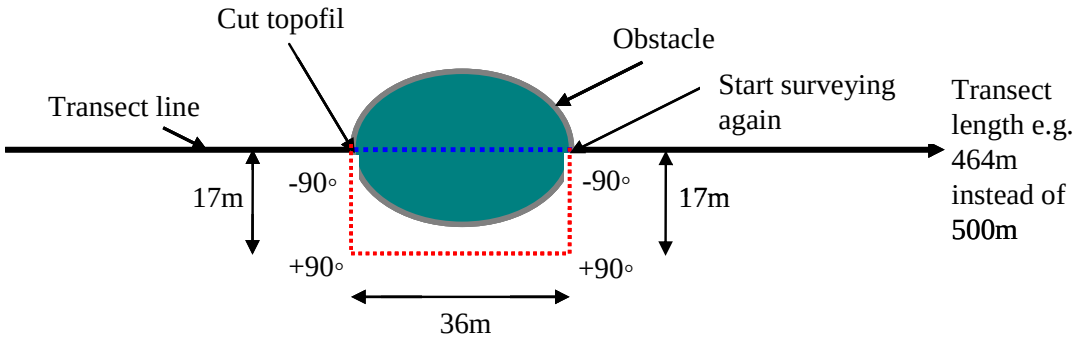

Figure 12-5 Protocol to navigate around obstacles when walking line transects.

13.1.7 Recording information on transects

On all line transects the following information must be recorded:

- Date: date when data are collected
- Transect line ID
- Start time: time at beginning of data collection on transect on a given day
- End time: time at end of data collection on transect on a given day
- Sheet number: data sheet number of data, given continuously
- Weather conditions:
  - Sunny
  - Light cloud: cloudy in patches (some blue sky visible as well as cloud)
  - Cloudy; complete cloud cover
  - Rain: rain
- Data recorder: name of person recording the data on data sheet
- Team members: team members that collected data on a given day
- GPS position and waypoint number from start to end of transect
- Start and end times, and GPS position of any break
- Any change in habitat type
- Information associated with observations:
  - distance from start of transect as indicated by topofil
  - time of day
  - number of objects
  - perpendicular distance
  - GPS coordinates

- Comments

13.1.8 Observations

a) Great apes

Collect information on ALL ape signs[33] (nests, feces, tools and tool use sites). For tools and tool use sites (see below), record the distance from the start of the transect, time of day, observation, number of objects, and GPS coordinates.

b) Human signs

Record all direct and indirect signs of human activity along the entire transect. As for all observations record the distance from the start of the transect, time of day, observation, number of objects, and GPS coordinates). Perpendicular distance is NOT required.

c) Monkeys

(i) Direct observation

Record any observations of all monkey species and record perpendicular distances to the individuals or groups. Measure perpendicular distance to the location where you **first** saw the individual. If it was a group of monkeys, estimate the centre point of the group. Count the number of individuals that you saw in the group. If you cannot count the animals note the minimum number of individuals that you saw and note in "data entry excel file" in column "no_obj_detect_explanation" that there were more individuals than detected. As for all observations record the distance from the start of the transect, time of day, observation, number of objects, and GPS coordinates.

(ii) Indirect observation

Record any first indirect observations of monkeys along the line transect in order to confirm the presence of a species. Perpendicular distance is NOT required. As for all observations record the distance from the start of the transect, time of day, observation, number of objects, and GPS coordinates.

d) Non-primate mammals

Record direct and indirect observation of non-primate mammal species once along the transect to identify biodiversity, distance from the start of the transect, time of day, observation, number of objects, and GPS coordinates. Perpendicular distance is NOT required.

e) Chimpanzee tools

Interesting or unusual tools may be collected, stored and shipped to Leipzig (see section 7.6 on coding of samples).

f) Habitat

Record all changes in habitat type along the transect, and mark a GPS coordinate at the start of each (see chapter 14 on different habitat types).

[33] Error in previous version read: "As information from camera traps will be used to identify individuals and estimate community, demographic structure etc., additional data on great apes along transects are not required EXCEPT for"

13.1.9 Army ants on return from transects

Evaluating the density of army ants along the transect whilst looking out for other observations is extremely difficult as the army ant nests and trails are below the surface of the soil. To allow an accurate evaluation, focus needs to be made only on the ground to find these trails and nests, and hence should be done when returning from the transect. Walk back along the transects whilst scratching the ground surface with a stick and record, together with their GPS coordinates, all nests and trails.

13.1.10Permanent water source

If permanent water sources are NOT encountered along the line transect, waterhole mapping needs to be conducted separate to the transect, as this indicates low density of water source in the grid. To do this, walk along valleys and any other likely areas within the grid to identify waterhole locations and take GPS coordinates, as with any other observation.

13.2 Strip transects

The strip transect, that are 2 m wide with 1 m on each side to the line transect, is applied to determine the density of objects that occur at high frequencies. Once per study the density of termite mounds and availability of tool material is determined along strip transects.

13.2.1 Tool material availability

All material such as stones, branches and roots that could POTENTIALLY be used as hammers or anvils for nut-cracking will be recorded. Potential tool material has to meet the same criteria as described for tools in the tool section.

Record the number of the following categories of potential tool material:

- Rocks: weight 100g to 8.9kg
- Rocks: weight of 9kg to 20kg
- Rocks: more than 20kg
- Emergent rocks: stuck in ground for anvils that cannot be lifted
- Roots (defined as emergent roots, ie: the tree it belongs to is unclear)[34]
- Bases of trees trunks (includes the buttress)[35]
- Branches (see section 7.2.1 on hardness test of wooden hammers)

13.2.2 Termites

All termite mounds within a width of one meter on each side of the transect line (i.e. total width of 2m) will be recorded along the strip transect. Sample termites are stored in labelled tubes with alcohol for later species identification or for stable isotope analyses (see section 9.1.3 for collection and storage methods). The following termite genera/species are frequently foraged upon by chimpanzees and important to be collected:

- *Macrotermes* sp.
- *Thoracotermes* sp.

[34] Clarifications added in 2014 - roots belonging to a known tree are therefore consider bases of tree trunks
[35] Clarification added in 2014

- *Cubitermes* sp.

Figure 12-6 a) Piece of a *Thoracotermes nest*, broken by gorillas, with many larvae (Photo: Isra Deblauwe), b) Workers and soldiers of the soil-feeding *Cubitermes severus*, from Nigeria. In the worker caste, the thin-walled abdomen is swollen to accommodate a long, convoluted intestine in which soil organic matter is digested by a complex microbial community. Soldiers in this species also digest soil, although it is fed to them by the workers. Soil-feeding termites are cryptic and soldiers are relatively rare within the colony (text by Professor David E. Bignell, www.biology.qmul.ac.uk/research/staff/bignell/) (Photo: anonymous, www.biology.qmul.ac.uk/research/staff/bignell/).

Figure 12-7 a) A major termite soldier of *Macrotermes malaccensis* (Photo: anonymous www.termiteweb.com) and b) *Macrotermes subhyalinus* (Photo R. Leuthold).

Figure 12-8 a-d) Examples of *Thoracotermes* termite mounds found in Tai National Park and from which termites are frequently extracted by chimpanzees (Photos: Lydia Luncz).

Figure 12-9 a) Characteristic mushroom-capped mounds of the soil-feeding species *Cubitermes severus*, in burned moist savannah in Cote d'Ivoire. The termites work through the surrounding soil, improving its drainage and fertility, and will survive the fire if the burning temperature is not too nigh (Photo: R. Leuthold. Copyright, Kluwer Academic Publishers); b) Workers and soldiers of the soil-feeding *Cubitermes severus* from Nigeria. In the worker caste, the thin-walled abdomen is swollen to accommodate a long, convoluted intestine in which soil organic matter is digested by a complex microbial community. Soldiers in this species also digest soil, although it is fed to them by the workers. Soil-feeding termites are cryptic and soldiers are relatively rare within the colony (text by Professor David E. Bignell, www.biology.qmul.ac.uk/research/ staff/bignell/; Photo: anonymous, www.biology.qmul.ac.uk/research/staff/bignell/).

13.3 References

Burnham, K.P., Anderson, D.R. and Laake, J.L. (1980) Estimation of Density from Line Transect Sampling of Biological Populations. *Wildlife Monographs*, 72, 3-202.

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Plumptre, A.J. (2000) Monitoring mammal populations with line transect techniques in African forests. *Journal of Applied Ecology*, 37, 356-368.

14 Habitat types

IMPORTANT

- Record with every observation during line and strip transects, camera traps and organic sample collection

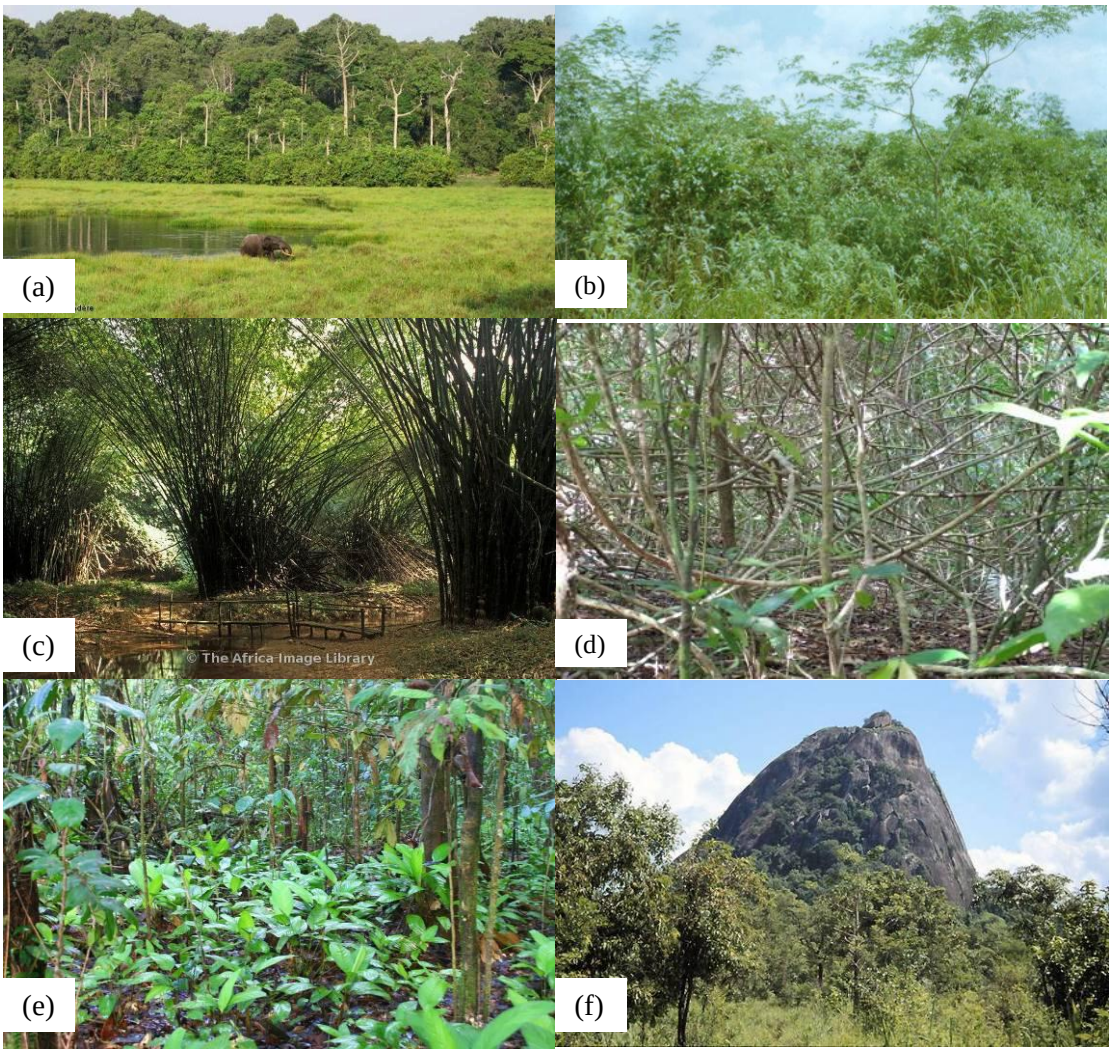
Not all habitats occur everywhere, some are typical for West Africa others for Central Africa. For more information on habitat types or classification refer to White and Edwards (2000). Habitat types are not necessarily exclusive. When in doubt, use the most specific one.

14.1 Definitions

Definitions of habitat types:

Habitat type	Definition
Bai	Marshy clearing in forest dominated by sedges (Figure 14-1a)
Fallow	Abandoned plantation (Figure 14-1b)
Forest - bamboo	Forest with exclusively bamboo growing (Figure 14-1c)
Forest - colonising	Edge of mature forest expanding into natural non-forest habitat e.g. savannah; not the same as secondary forest
Forest - lianas	Forest dominated by liana (Figure 14-1d)
Forest - *Marantaceae*	Forest dominated by *Marantaceae* (Figure 14-1e)
Forest - mixed, closed understorey	Primary forest with many large trees but dense vegetation cover on the ground
Forest - mixed, open understorey	Primary forest, with many large trees, a high, unbroken canopy and sparse vegetation cover on the ground, consisting mostly of shrubs
Forest - monodominant	Forest with the structure of mixed forest but where one species of tree is noticeably dominant
Forest - old secondary	Areas with large trees but showing evidence of past disturbance by humans, without food crops but sometimes with oil palm or mango trees still present.
Forest - seasonally inundated	Forest which is flooded during the wet season, but dries completely at other times of the year
Forest - young secondary	Areas recently or currently cultivated by humans, where some food crops persist
Forest fragment	Isolated natural woodland patch in a savannah
Forest on rock	Forest on rocky ground
Gallery forest	Running along a river or stream, found both within larger blocks of forest, or isolated in savannah vegetation
Human	Camp, market, house etc.
Inselberg	Large granite outcrops surrounded by forest (Figure 14-1f)
Liana marshes	Swamps dominated by lianas
Marshes	Swamps that are flooded all year round
Plantation	Active plantation
Raphia marshes	Swamp dominated by raphia (Figure 14-1g)
River	River

Road abandoned	Abandoned road
Road active	Active road
Rocks	Just rocks, no savannah, no inselberg
Salt lick	Saline, areas where elephants and other animals come to eat mineral-rich soil, creating opening in the forest
Savannah - bushy	Savannah (areas dominated by grasses or sometimes by ferns) - bushy
Savannah - herbs	Savannah (areas dominated by grasses or sometimes by ferns) - herbs, prairie-like
Savannah - wooded	Savannah (areas dominated by grasses or sometimes by ferns) - wooded
Savannah on rock	Savannah on rocky ground
Village	Village
Village - abandoned	Abandoned village



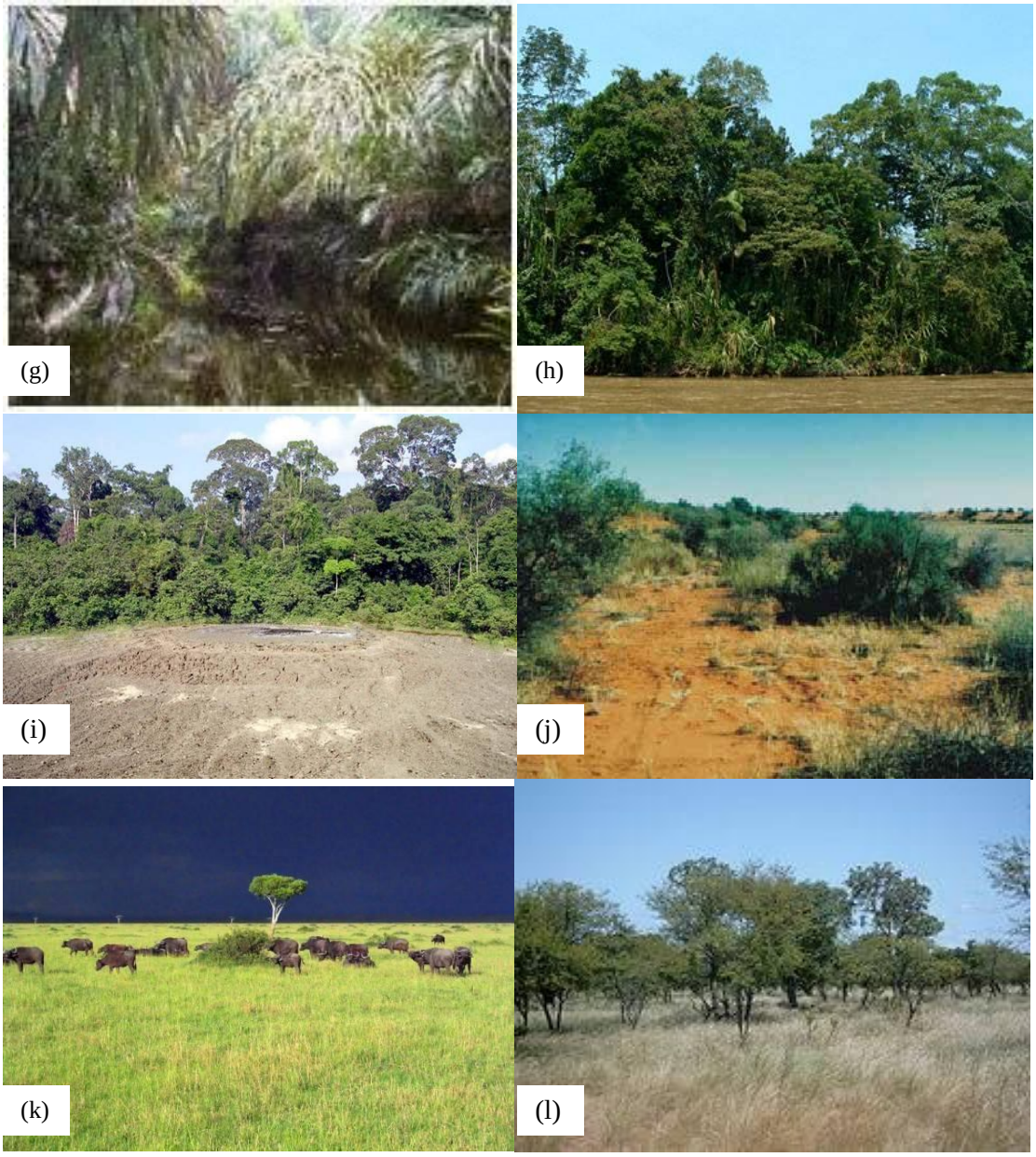


Figure 14-1 Vegetation examples for a) bai; b) fallow (Urhobo Historical Society); c) bamboo forest; d) liana forest (S. Schnitzer); e) marantaceae forest; f) inselberg; g) raphia marsh; h) gallery forest (fssbirding.org.uk); i) salt lick (www.etawu.com); j) savannah – bushy; k) savannah – herbs; l) savannah – wooded.

14.2 References

White, L. and Edwards, A. (2000) Conservation research in the African rain forests – a technical handbook. Wildlife Conservation Society.

15 Opportunistic sampling

IMPORTANT

- Record all new observations, e.g. mammal species, tools and tool use sites
- Use data entry sheet 'Recces' (see annex III)
- Also refer to Annex IV-4 – Opportunistic Sampling[36]

Whenever you find a new observation such as carcasses, tools and tool use sites when walking in the forest for whatever purpose, record these data and collect samples where appropriate. Use the data sheet 'Recces' and select 'ad libitum' from the drop-down list in 'datasheet_type'. Record the corresponding data as always, such as habitat type, GPS coordinate, time and date.

Direct or indirect observation of new mammal species which had not yet been identified through line and strip transects should also be noted with the GPS coordinates, time, date, and habitat type.

Remember that you should also conduct targeted sampling for all organic samples and tools as described in each of the sections.

[36] Addendum to original protocol: Annex IV

16 Monthly Reporting

IMPORTANT

- Send a short standardized monthly report via email
- Do not wait until submitting the report to inform of problems and/or issues in the field

A short table containing the number of data and sample collected as well as expenditures must be sent via email to the coordinator of the programme as informed, on a monthly basis (Table 16-1).

Table 16-1 Monthly report table to be completed and sent via email every month.[37]

Monthly TRS update	
Site name	
Month	

Data	Data category	Item	Quantity this month	Total quantity since beginning of data collection
Cameras		No. cameras deployed		
		Total number of videos*		
		No. chimp videos*		
		No. chimp videos using tools*		
Samples	chimpanzees	No. chimp genetic samples		
		No. chimp hair samples		
		No. chimp pathogen samples		
	gorillas	No. gorilla genetic samples		
		No. gorilla hair samples		
		No. gorilla pathogen samples		
	general	No. isotope samples		
	traps	Number of bee traps		
		Number of bees collected		
		Number of fly traps		
		Number of flies collected		
Transects		Distance of line transects surveyed (km)		
		Distance of strip transects surveyed (km)		
Plots		No. habitat plots surveyed		
Phenology		No. trees identified		
		month ALL phenology trees were identified (monitoring start month):		

* if you have not counted yet - make a rough guess

if no gorillas at site NA

[37] This monthly report table has been modified from the one that appeared in the original protocol

17 Quality Control

The phrase 'Garbage in garbage out' refers to the fact that **results can only be as good as the data are**. For this project it is absolutely essential that collected data have the highest quality possible. Make sure that data are recorded as accurately as possible and check the quality of data collection and entry regularly.

Once a TRS study is completed and the collected data are at MPI, the datasheets will be used directly for data upload to the Pan African database. Therefore you have to make every effort that entered data reflect precisely your observations in the field.

17.1 During data collection

1. Make sure that all your team members have read and understood the field protocol document.
2. Be silent when collecting data and avoid cutting the vegetation with the machete unless the vegetation is extremely dense.
3. Check that for each observation, a GPS point is recorded. When you record a position with the GPS, you have to stand still a while to record the location accurately.
4. For line and strip transects, take a new sheet for each new transect walked.
5. For line transects, check that perpendicular distances are accurately measured and that they are taken at exactly perpendicular angles. Observations close to the transect have to be measured and must not be considered as being on the transect ($\neq$ 0m).
6. Stay aware of potential novel chimpanzee tools and keep an open mind about what you might find.

17.2 Datasheets

1. Check five lines of data entry randomly on a datasheet. Check the waypoint noted (coordinate system UTM), the time and the observation with the recorded waypoints in QuantumGIS.
2. Check data using "pivot tables" and "filter" function. Check if there is no incorrect data, typos or missing data, i.e. blank cells and check that all names etc. are spelt exactly the same for each entry. If the same object is spelt differently or given a different name they are treated as different observations, e.g. if the species is entered once as "*Pan troglodytes verus*" and for another time as "chimpanzee", these two entries will be regarded as two different species. **Consistency is crucial.**

17.3 Re-training

Team members should constantly be made aware of problems and be re-trained to remind them of proper data collection, sample storage and data entry,

Re-train sessions should include the following points:

- General review of data collection methodology
- Discussion about issues encountered
- Retrain for GPS and compass if needed
- Retrain for sample collections (tools, isotope, genetic, pathogen and diet)
- Retrain on species identification
- Reviewing of the material used during surveys
- How to handle equipment and how to fix/repair used equipment

18 Shipment and Permits

IMPORTANT

- Start organising permits at least one month in advance as these procedures take time
- Have all of your sample data entered correctly to facilitate the process
- Also refer to Annex IV-14 – When you Travel in Country and to Leipzig [38]

In some countries you will need to get permits to collect data and samples prior to beginning of the study. Permits are most commonly obtained from the ministry of environment or an authority within the ministry. However, the different ministries, authorities, types of required documents and permits as well as procedures will vary among countries.[38]

Required permits and documents for sample shipment:

(i) CITES

One permit each in the country of origin for export and in Germany for the import of hair, tissue and bone samples of animal species listed (information on all species and their status can be found here: http://www.iucnredlist.org/)

(ii) Vet certificate

A document has to be obtained in the country of origin from an authorised vet who certifies that:

- the shipment consist of sample material (type and amount and period of samples) from a specific area (exact specification of area or reserve), from a certain species (exact specification of all species), which is duly packed and shipped at the MPI for scientific purposes;
- there has been no officially reported onset of an animal epidemic in the animals in the area (exact specification) in the period of sampling.

(iii) Plant import

Claudia Nebel (nebel@eva.mpg.de) needs a list with the type (fruit, lead, seed), amount, and type of storage medium (alcohol, dry, silica gel) of each plant species, 2 weeks prior to shipment in order to obtain permits in Germany for the import of these samples.

(iv) Animal import

Likewise, Claudia Nebel (nebel@eva.mpg.de) needs a list with the type (hair, faeces, urine, bone, skin, etc.), amount, and type of storage medium (alcohol, RNAlater, dry, silica gel etc.) of each animal species 2 weeks prior to shipment in order to obtain permits in Germany for the import of these samples.

(v) Isotope samples

A list with any non-plant/animal samples such as water and soil also has to be sent to Claudia Nebel 2 weeks prior to shipment.

[38] Addendum to original protocol: Annex IV

19 Rules

IMPORTANT

- Please keep in mind during the entire study that we have responsibility for what we do and that we are impacting the place where we set up TRSs and its wildlife
- Try to reduce any negative impact as much as possible
- If the TRS has to close early, focus the capture of chimpanzee tool use behaviour on the video cameras, and most importantly on organic sample collection – resume normal full protocol as soon as possible

19.1 General

- No loud talking while in the forest
- No hunting or killing of wildlife
- No bushmeat consumption
- No pets at camp
- No taking of illegal drugs
- No littering
- No leaving behind any food remains or packaging

19.2 Additional specific rules

Do not confiscate live animals from humans. If you see captive individuals of endangered animal species contact the authority responsible for this case. Ideally they are the ones taking care of animals. You can advise if you see an opportunity to bring it to a sanctuary or some other place where they will be professionally looked after.

At the village:

- It is forbidden to consume alcohol during working hours or to be under the influence of alcohol during working hours, i.e. the next day
- Always use local latrine facilities
- Never participate in any illegal activities.
- Be very sensitive about cultural differences and perceptions and do your best to respect them.

At the forest camp:

- No drinking of alcohol
- No spitting neither in rivers nor on the ground to prevent transmission of human diseases to animals
- Never walk alone in the forest and **always** carry a **compass** and map and a **GPS** when leaving camp
- Try to set up camp near a river
- Check for trees or branches of trees that could potentially fall down before you set up camp
- Hygiene rules: Also in a forest camp certain hygienic standards need to be met.
    - Latrine
    - Wash hands regularly
    - Allocate cutlery per person

- Clean dishes
- Filter drinking water or boil it
- Store rubbish together and keep the campsite clean

During the survey:

- Never walk alone and one person must always have a **compass** and a **GPS**
- No smoking
- No spitting neither in rivers nor on the ground
- No fires
- Dig a hole (minimum of about 30 cm deep) and cover your excrements during surveys
- Do not litter

19.3 Planning

Step	Topic	Options	Action	Additional actions
1	Known chimp territory	Yes	Go to step 4	
		No	Go to step 2	
2	Start organic sample collection	-	Go to step 3	
3	Do recce surveys	-	Go to step 4	
4	Chimpanzee activity hotspots identified	Yes	Go to step 5	
		No	Contact MPI	
5	Place grid and check with MPI	-	Go to step 6	
6	Place lines for habitat plots, strip and line transects	-	Go to step 7	
7	Do habitat plots	-	Go to step 8	Place cameras
8	Select trees for phenology	Sufficient trees	Go to step 10	Place cameras
		Insufficient trees	Go to step 9	
9	Do recces and search for trees	Sufficient trees	Go to step 10	Place cameras
		Insufficient trees	Contact MPI	
10	Start phenology	-	Go to step 11	Monthly phenology thereafter
11	Deploy remaining cameras	-	Go to step 12	Camera maintenance thereafter
12	Do strip transects	-	Go to step 13	
13	Do line transects	Permanent water source recorded	Repeat every 4 months	
		No permanent water source along transect	Go to step 14; repeat transect every 4 months	
14	Search for waterholes in grid	-	Map locations	

Important:

At any stage if there are insecurities in staying for a long duration at the site due to e.g. political instability, focus on organic sample collection. With the video camera traps, focus on the capture of tool use behaviour rather than the systematic methodology. Once the situation returns to normal, resume applying the full protocol as soon as feasible. Contact MPI if unsure.

20 Annexes

Annex I: Tree species for phenology

ID	Location	Species name	Family	Part consumed
1	All	Celtis mildbraedii	Ulmaceae	fruit
2	All	Elaeis guineensis	Arecaceae	nut, pith, flower, fruit
3	All	Ficus mucuso	Moraceae	ripe fruit
4	All	Klainedoxa gabonensis	Irvingiaceae	fruit
5	All	Parinari excelsa	Chrysobalanaceae	fruit, nut
6	All	Treculia africana	Moraceae	fruit
7	West	Adansonia digitata	Bombacoideae	fruit, seed
8	West	Albizia zygia	Mimosaceae	gum
9	West	Allophylus africanus	Sapindaceae	fruit
10	West	Beilschmiedia manii	Lauraceae	fruit
11	West	Calpocalyx aubrevillei	Mimosaceae	pod
12	West	Ceiba pentandra	Bombacaceae	flower, seed
13	West	Cola cordifolia	Sterculiaceae	fruit, seed
14	West	Detarium senegalense	Caesalpiniaceae	fruit
15	West	Dialium aubrevillei	Caesalpiniaceae	fruit
	West	Dialium guineensis	Caesalpiniaceae	fruit
16	West	Diospyros sanza-minika	Ebenaceae	fruit
17	West	Erythrina mildbraedi	Fabaceae	flower, leaf, fruit
18	West	Gilbertiodendron splendidum	Caesalpiniaceae	fruit
19	West	Musanga cercopoides	Moraceae	fruit, flower
20	West	Nauclea latifolia	Rubiaceae	fruit
21	West	Pachystela pobeguiniana	Sapotaceae	fruit
22	West	Pterocarpus erinaceus	Papilionceae	flower, leaf, bark
23	West	Saba senegalensis	Apocynaceae	fruit, pith
24	West	Spondias mombin	Anacardiaceae	fruit
25	West	Uapaca esculenta	Euphorbiaceae	fruit
26	West	Vitex madiensis	Verbenaceae	fruit
27	West & Central	Afzelia africana	Caesalpiniaceae	fruit, seed
28	West & Central	Coula edulis	Olacaceae	seed, leaf
29	West & Central	Diospyros mannii	Ebenaceae	fruit, flower
30	West & Central	Ficus ingens	Moraceae	fruit
31	West & Central	Irvingia gabonensis	Irvingiaceae	fruit
32	West & Central	Irvingia grandifolia	Irvingiaceae	fruit, flower, seed
33	West & Central	Mammea africana	Guttiferae	fruit
34	West & Central	Myrianthus arboreus	Moraceae	fruit

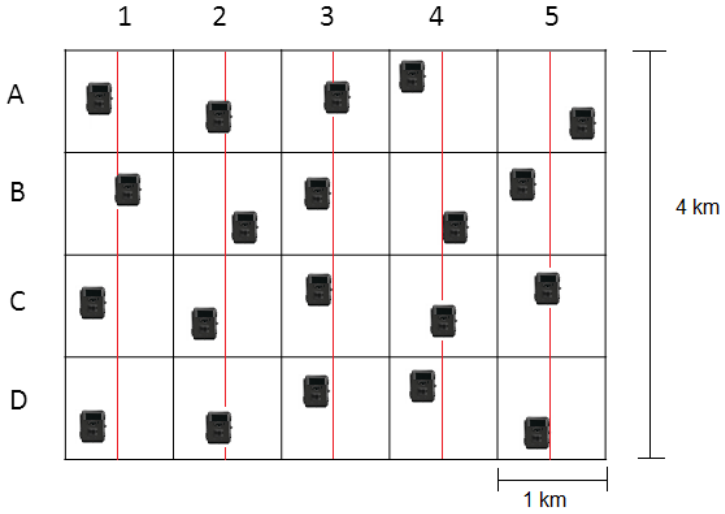
35	West & Central	Nauclea didderichi	Rubiaceae	fruit
36	West & Central	Panda oleosa	Pandaceae	seed
37	West & Central	Parkia bicolor	Mimosaceae	fruit
38	West & Central	Pentaclethra macrophylla	Mimosaceae	pod
39	West & Central	Sacoglottis gabonensis	Humiriaceae	fruit
40	West & Central	Santiria trimera	Burseraceae	fruit
41	West & Central	Sterculia tragacantha	Sterculiaceae	leaf, bark
42	West & Central	Strombosia glaucesens	Olacaceae	fruit
43	West & Central	Tetrapleura tetraptera	Mimosaceae	leaf
44	West & Central	Triplochiton scleroxylon	Sterculiaceae	flower
45	West & Central	Uapaca guineensis	Euphorbiaceae	fruit
46	West & Central	Xylopia quintasii	Annonaceae	fruit
47	West & East	Aningeria altissima	Sapotaceae	fruit
48	West & East	Entandrophragma angolense	Meliaceae	seed
49	West & East	Ficus capensis	Moraceae	fruit
	West & East	Ficus exasperata	Moraceae	fruit, leaf
	West & East	Ficus variifolia	Moraceae	fruit, leaf
50	West & East	Lannea welwitschii	Anacardiaceae	ripe fruit, wood
51	West & East	Morus mesozygia	Moraceae	fruit
52	West & East	Pseudospondias microcarpa	Anacardiaceae	fruit
53	West & East	Strychnos aculeata	Loganiaceae	fruit
54	West & East	Syzygium guineense	Myrtaceae	seed
55	Central	Staudtia gabonensis	Myristicaceae	fruit
56	Central & East	Monodora angolensis	Annonaceae	pulp
57	East	Beilschmiedia ugandensis	Lauraceae	fruit
58	East	Mimusops bagshawei	Sapotaceae	fruit
59	East	Uvariopsis congensis	Annonaceae	fruit

Annex II: Glossary

Camera coverage: The zone in front of the camera where animals will be detected

Camera settings: The options found in the camera menu which control specific recording elements, e.g. recording duration and sensitivity level

Data collection zone: The area defined by a site-specific grid with 1x1km cell size within which data collection takes place (figure below, illustrating cameras and line transects within each 1 x 1 km cells).



Diameter at Breast Height (DBH): Measurement of a tree trunk at 1.3 m above ground on the up-hill side of the slope calculated using the equation, DBH = circumference / π

Event: The capture of an animal by the camera device (false triggers are also registered as events on each device)

False triggers: Triggers that are not caused by wildlife heat or motion and instead are triggered by sun and/or wind

Grid: divided up into 1x1 km cells that is placed over a cluster of chimpanzee signs recorded during recces

Grid cell: 1x1 km cell within the grid

Habitat plot: 20x20 m quadrate that is placed centrally on the transect every 100 m, where all tree species with a DBH ≥ 10cm are measured and identified

Line transect: Straight lines made/cut to cross through the middle of all cells in a North-South or East-West direction across the grid

Opportunistic sampling: Method of identifying new observations such as new mammal species, and locating tools and tool use sites that had not been found during line or strip transects

Perpendicular distance: Distance from the line transect to an observation measured exactly 90^{o} from the line using a compass and a tape measure

Phenology: Study of the annual cycle and productivity of chimpanzee feeding tree species

Recce: A path of least resistance through an area following a compass bearing. In rainforest about 1-2km can be walked/hour, in woodland savannah up to 4km/hour.

Sampling grid: A grid with 1x1 km cell size covering the entire data collection zone. Grid rows are labelled with letters starting with 'A' in the top row, and numbers for columns running left to right starting with '1'.

SD card: Memory card used to store video clips (in .avi format) at the camera location

Strip transect: 10 m band laid centrally on the transect, 5 m on each side, along which insect and tool use availabilities are recorded

Test mode: Camera setting that allows for testing where the animal will be detected, indicated by the blinking red LED

THV: Terrestrial herbaceous vegetation that includes monocotyledons and woody sapling < 2 m in height

THV plot: 1x2 m plots placed consistently in one of the corners of the habitat plot, in which the number of herbs (monocotyledons) and the number of woody saplings are separately counted

Tool: Any natural object that presents signs of intentional modification(s) in the raw material to change its shape, and length (e.g. cut to correct length, side branches removes, bark pealed, extremities narrowed or sharpened with teeth). Principal exception to this definition is a hammer that can be unmodified stones and wooden branches, but the second criteria for **used tool** need to be satisfied.

Topofil: Device containing a topofil thread used to measure accurately, the distance travelled

Transect: Linear line that extends across the grid through all of the cells in the data collection zone, where habitat structure, THV, line and strip transects are conducted

Used tool: Tool that shows clear signs of use such as traces of hitting, wear from use, and remains of sand, honey, or termites, etc.

Visitation rate: The number of visits per week of a species of a particular camera location

Annex III: Field data entry sheets

The following data sheets are to be used in the field to record all data. Remember to type up the data into the Excel file as soon as possible after data collection.

Datasheet: RECCES

Date: Start time: End time: Weather: Sheet number:

Data recorder: Team members:

Time of observation (hh:mm)	Observation / Habitat	Genus	Species	No. of objects detected	Nest ID	Nest Decay Stage	Slope	Wpt N°	Longitude (UTM)	Latitude (UTM)	Unique ID code of photo or organic sample	Comments; measurement of tool (diameter, length, weight); Genus/Species of food; Genus/Species of root anvil; DBH

Datasheet: HABITAT STRUCTURE

Date: Start time: End time: Weather: Transect ID: Sheet number:

Data recorder: Team members:

Transect ID	Qua-drate No	Time (hh:mm)	THV plot: monocots/ woody saplings	Genus	Species	DBH (cm)	Unique ID code of sample	Habitat type	Wpt No	Longitude (UTM)	Latitude (UTM)	Comment

Datasheet: PHENOLOGY – Initial observations

Date: Start time: End time: Weather: Sheet number:

Data recorder: Team members:

Scoring: 0 = 0% 1 = 1 to 25% 2 = 25 to 50% 3 = 51 to 75% 4 = 76 to 100% / Fruits on Ground: 0 = none; 1 = little; 2 = some; 3 = many

Qua-drate No.	Tree information								Habitat	Flowers		Fruits			Leaves		Comment
	Tree ID	Time (hh: mm)	Genus	Species	DBH (cm)	Wpt No.	Longitude (UTM)	Latitude (UTM)		% Total	% Buds	% Total	% Ripe	On ground	% Total	% Young	

Datasheet: PHENOLOGY – Monthly observations

Date: Start time: End time: Weather: Sheet number:

Data recorder: **Fruits on Ground: 0 = none; 1 = little; 2 = some; 3 = many**

Scoring: 0 = 0% 1 = 1 to 25% 2 = 25 to 50% 3 = 51 to 75% 4 = 76 to 100%

Qua-drate No.	Tree ID	Flowers		Fruits			Leaves		Comment
		% Total	% Buds	% Total	% Ripe	On ground	% Total	% Young	

Datasheet: CLIMATE DATA

Location: Sheet number:

Data recorder	Date			Time		Rainfall (mm)	Temp. max. (°C)	Temp. min. (°C)	Humidity max. (%)	Humidity min. (%)	Comment
	year	month	day	hour	min						

Datasheet: VIDEO CAMERAS

Data recorder: Sheet number:

Start date	Start time	End date	End time	Distance of width (y)	Perp. distance to mid-y (x)	Blind-spot distance (m)	Cam bearing (°)	Cam height (m)	Sensor level	Location type	Cell ID	Habitat type	Wpt No	Longitude (UTM)	Latitude (UTM)	Cam no	SD card no	Comment

Datasheet: ORGANIC SAMPLES (faeces, plant samples, insects, tools, etc.)

Data recorder: Team members: Sheet number:

Date	Weather	Time (hh: mm)	Sample information						Geographical position			Comments; type of analysis; Nest: height & dist. to stem for ape hair
			Type of sample	Genus	Species	Habitat type	Unique ID code of sample	Storage medium	Wpt N°	Longitude (UTM)	Latitude (UTM)	

Datasheet: FAECAL SAMPLES FOR DIET STUDY

Data recorder: Team members: Sheet number:

Date	Weather	Time (hh: mm)	Sample information				Geographical position			Feeding remains			Comments
			Genus	Species	Habitat type	Unique ID code of photo OR sample	Wpt N°	Longitude (UTM)	Latitude (UTM)	Particles found in faeces	Genus	Species	

Datasheet: TRAPS

Date recorder: Trap type (Bees/Blowfly): Sheet number:

Date	Time set up (hh:mm)	Trap ID	Cell ID	Trap height (m)	Bait	No. target species trapped	Duration of trapping	Habitat type	Wpt	Longitude (UTM)	Latitude (UTM)	Comment/ Sample ID

Datasheet: STRIP TRANSECTS

Date: Start time: End time: Weather: Transect ID: Sheet number:

Data recorder: Team members:

Time (hh:mm)	Observation	Genus	Species	No. of objects detected	Unique ID code of photo or organic sample	Wpt N°	Longitude (UTM)	Latitude (UTM)	Comments

Datasheet: LINE TRANSECTS

Date: Start time: End time: Weather: Transect ID: Sheet number:

Data recorder: Team members:

Distance from start of transect (m)	Time of observation (hh:mm)	Observation / Habitat	Genus	Species	No. of objects detected	Perpendicular distance (m)	Wpt N°	Longitude (UTM)	Latitude (UTM)	Unique ID code of photo or organic sample	Comments; measurement of tool (diameter, length, weight); Genus/Species of food; Genus/Species of root anvil; DBH

Annex IV: Addendums to the Protocol[39]

Jan 2013-July 2014

IV-1. BONE COLLECTION

We would like everyone to make an effort to get at least 1 body bone (leg is ideal) and 1 skull from a chimp. If for some reason a whole skull is not possible, then try pulling a tooth to get a molar out with an intact root (with enough wiggling any tooth will come out). But try your best to get as much of the skeleton as possible. Also, please record the sex of the individual and the approximate age (infant, juvenile, adult) at death if it is known. A full skull or even a complete skeleton of a chimpanzee (or gorilla) is of enormous value to our institute. Please make effort to get as many bones as possible and to get as much information on their origin too (where the animal was killed, in which year).

Please remember to **never pay** for any bushmeat and keep a low profile when requesting to sample or have any bushmeat product, you don't want to drive any demand for poaching! If you need any support from our site to convince local authorities to be allowed to export the bones, we will try our best to explain why we need them and what research we plan to do.

NB: If there are already rules at your site for collecting bones, those should supercede our rules, we do not want to violate any of our collaborators protocols. However, if bones are treated (as per the original protocol) we cannot use them for any genetic analyses, so if this becomes an issue, please email me and CC our collaborator so we can see if any compromise can be reached.

Bones should NEVER be touched and handled without wearing appropriate protective clothing and following the below precautions.

Protective clothing:
- Gloves up to the long sleeved shirt
- 2 surgical face masks in front of nose & mouth
- goggles to protect the eyes
- long trousers
- long sleeved shirt
- (rubber) boots

Other equipment
- GPS, notebook, pen
- Biohazard bags (or strong plastic bags)
- forceps, pliers
- RNAlater tubes
- hand- sanitizer
- paraffin + lighter (to burn contaminated gloves etc.)

How to safely collect the bones?

1. BEFORE closely approaching or touching the bones, put on your protective clothing

2. Take a GPS coordinate of the location where the bones were found and write down some notes (e.g. date, vegetation, any obvious signs re: cause of death, any other bones/carcasses found in the area?)

3. If you find any maggots on the bones, transfer a few of them into a tube with RNAlater using a pair of forceps. Close the tube and shake it vigorously. If possible freeze it or store it as cold as possible. Disinfect the forceps overnight in 2-5% formalin (or bleach).

4. Use forceps to transfer all bones you can find including the skull into a Biohazard bag, add a piece of the absorbant tissue and close it properly. If a Biohazard bag is not available take strong plastic bags. For very small bones you can also use the 50ml tubes.

[39] This entire section added in July 2014

5. Take off your gloves by turning inside out, so that the contaminated side is on the inside. Dispose of gloves and masks by burning them on site or put them in a plastic bag, transport them back to camp and burn them there.

6. Before taking off the mask, place the bag with the bones into a second plastic bag. Do not touch the bag containing the bones while doing so as its outside might be contaminated. Simply pull the second bag inside out over the first bag and close it. Now the bones can be safely transported.

7. Take off your mask taking care to only touch their straps, then dispose of the mask (burn it).

8. Wash your hands and use hand-sanitizer.

9. Disinfect the outside of your boots overnight in 2-5% formalin (or bleach) and wash your clothes in bleach.

What if the carcass is not yet decomposed?

1. - 3. see above

4. If the body is not too decomposed yet, take a nose swab: Insert a swab into one of the nostrils and turn it a few times to swab the inside of the nose. Then place the swab in RNAlater and store the tube as described above.

5. Afterwards cover the carcass with some soil, but in such a way that there are some passages for flies or ants to consume the meat. Maybe additionally mark the place with e.g. flagging tape.

6. Burn or disinfect your protective clothes as before.

7. After a few weeks or months, depending on the stage of decomposition, come back to the carcass to retrieve some of the bones following the protocol described above.

IV-2. BEE TRAPS

Do not use the sugar water in the protocol instead try any of the following until you find something that works:

a) Washing powder (like Omu). Try making up a soapy solution that smells enough and seeing if that attracts the bees
b) Human urine
c) Bait the traps with fragrance, some sort of fruity or floral scent should work. An alternative is to try honey if nothing else is available (you can also mix honey into the sugar water if you have it available to you). A rather strong smell from vanilla extract, or peppermint, or banana flavoring all work very well- I have used them extensively in Gabon, Borneo and the Amazon. Salt solution, that's right, table salt and water,
d) Salt solution (to mimic sweat) of 1/2 tsp of salt:200ml of water (feel free to try some other concentration if you want) with a little human touching to spread some skin fatty acids on the vial or bottle, also attracts bees very well. You can also try a salt and sugar solution.

The bees should then be washed in a mild detergent and then stored in 70% ethanol or dried on silica

IV-3. TOOL COLLECTION

Please bring back a few examples of each tool type from every TRS (this was mentioned in the workshops). However, it would also be great if you could collect tools you find at your camp and before you leave take a picture of the collection (something like the right photo) to show the chimp tool kit at your TRS. This would include, stones (don't take more than 1 or 2 since chimps reuse these and you don't want to steal their tools from the forest) and hammers (which are probably way to big and heavy for you to bring back to Leipzig anyway). Something in the picture should be a reference to size (a person, or ruler or get creative).

Cleve Hicks with his Bili-Uele, DRC chimpanzee tool collection

IV-4. OPPORTUNISTIC SAMPLING

Please make sure you are recording the data for the opportunistic sampling (p73 of protocol) - this includes all novel mammal species (including primates of course) so that even if you do not observe them on your line transects, we still know they are in the forest.

IV-5. GENETIC SAMPLING

As we are further developing the ideas for genetic analyses of the samples it has become apparent that sampling also from outside the TRS would be beneficial to our collection. This is because for population history analysis it would be good to have non-relatives in the samples and this is easier achieved for chimpanzees from different communities. If you are able to do some chimpanzee genetic fecal sampling outside your TRS you may count these samples towards your 200 minimum total and it would be very much appreciated.

IV-6. IDENTIFICATION VIALS:

For the glass identification vials: please write the sample ID (not all the other infos, just the sample number) with a pencil on a small piece of paper and put it in the tube/vial with the insect and alcohol for identification. The glass tubes seem to leak and it wipes away the label, this way there is a back up. Additionally you should seal the lid with parafilm, and/or put each vial in a small plastic bag.

IV-7. THE IMPORTANCE OF TEMPORAL SAMPLING

We want to emphasize the importance of temporal sampling at all of your sites and that the targets in the protocol are all MINIMUMS.

So even if you, for example, collect 50 dungs in the first month for diet/genetics/pathogen analyses - you should not then stop collecting because we need to see what the chimps are eating/where they are ranging/with what they are being infected with across the entire timespan of the TRS. I think in most of your cases it is hard enough to find dung so you have temporal sampling by default, but for those of you reaching your targets early, please remember to keep sampling every month if possible: about 10 samples/month would be great.

This is also important for the **chimp hair** and **plant food** samples (especially as fruit and nuts can ripen only seasonally). At least 10 chimp hair samples should be collected at the very end of your field work. So if you collect a lot of chimp hair, wait another 2-3 months till you go on, try to get samples from wet and dry seasons, **target for fresh nests** and always make notes when you find a fresh chimp nest (nest age 1) and got hair from it.

IV-8. TERMITES

Every time you inspect a termite mound, please make sure to record it as opportunistic data - even/especially if you do not find any evidence for tool use! This way we know that there is no evidence for termite fishing and not simply that it was overlooked.

IV-9. MEDICINAL PLANTS

Keep an eye out for medicinal plants in feces (eg: Manniophyton fulvum leaves from a washed dung below). This also ties in nicely with the importance of having a good temporal sampling of fecal/diet samples.

In general the evidence for medicinal plant use - and in particular leaf swallowing - can only be seen once you wash the dung so keep an eye out for it and sift often please. Normally, you can see that the leaves are undigested and folded in large chunks and very often bright green (sorry, did not have a picture of this, but you can see how the brown leaves in the picture are also undigested). This is what you should keep an eye out for. If you suspect leaf swallowing then please take a picture and send it to us plus dry the sample and have the leaves identified by a botanist (and keep the leaves on silica to bring back to Leipzig).

As a reminder, please also look out for termites and ants in the feces as well.

IV-10. ISOTOPE UPDATE

Some additional remarks for clarification how to collect organic samples for stable isotopes:

Great ape hair[40]

a) Sampling methodology (-timing is everything-)

Hair samples should be collected over the entire course of the field stay to get representative samples across the 12-month period at the TRS. Sampling should occur at least every 3 months to allow for a good temporal representation of hair samples. Collect hair from nests whenever you find afresh chimpanzee (or gorilla) nest, especially when you find a group of nests. Hairs collected from fresh nest groups are very useful as they represent several individuals at the same point in time. Hair samples from older decayed nest are really difficult to date, so nests older than stage 2 should only be sampled if no fresh nests are encountered at all. If you can make more precise estimates on the age of a nest in days and weeks please do so and use the comment column to enter this information. Sample ALL nests from a nest group. Climb up to the nest and put the nest in a rice bag so that it can be taken to the ground for all team members to search for as many hairs with intact hair roots as possible. Put all hair samples found in a single nest together in a pergamin envelope and note an estimated age or the nest (stages 1-4; Figures 9-1 to 9-4), and the nest ID (call the nest from a nest group nest#1, nest#2, nest#3 etc.) on both the envelope AND in the organic data sheet (in the "comment" column) . When climbing the tree is impossible, try to shake down parts of the nest and find hairs that may have fallen to the ground.
Where sympatric gorillas exist in the study site, remember to also collect gorilla hair samples.

b) Target sample number

Collect hair samples from 50 nests (minimum is 20 fresh nests with at least 10-15 hairs with a good temporal spread across your 12-month stay at the TRS. Try to obtain hair from at least one nest group during the field stay. Try to obtain at least 5 good hair samples in the very last few weeks that you are in the field (unless this falls into the rainy season, when climbing trees is difficult). These last hair samples will resemble the last 6 months of the chimpanzees' diet and will match most of your other recorded data.

Plants

c) Conduct plant sampling mainly during phenology and habitat plots

Collect samples of plants that are common foods for chimpanzees (see Annex I for list of tree species) based on what you know about chimpanzee diet at your site (publications, local knowledge) or also from the chimp dung washes you conduct. Always sample from the part of the plant which is eaten, not from the discarded part, and enter information on what plant part this is in the 'sample type' column of the datasheet (e.g. fruit, fruit pulp, herbs, leaves, nut, seed, mushroom, flower, pith or bark) as this will not be easily recognizable once the sample has completely dried. Also use the comment column to add any specifics. When collecting hard shelled fruits/nuts, break or cut open the fruit and collect the pulp i.e. the part that is actually eaten by chimpanzees. We only need about 4 mg per sample. In the case of big fruits there is no need to collect the entire fruit. If you find leftovers of chimpanzee plant foods, identify them, note these down and make sure to sample this plant food item either on this occasion or later. In the comment column also note its position in the canopy (ground, mid-height or high in canopy). To allow for a good temporal spread and to sample plants with different fruiting phases collect most plant food items repetitively during monthly phenology (particularly fruits, leaves, nuts) or during habitat plots (herbs, other plant items) and make clear in the 'sample' column what you sampled (fruit, seed, leave, herb, pith, nut). In addition, and to evaluate potential temporal variation across seasons in plants, systematically sample edible tree leaves from 5 tree species during monthly phenology (see below).

[40] Also refer to Annex IV-7 – the importance of temporal sampling

d) Target sample number

- Collect 5 terrestrial herbs (ground vegetation, lower than 2m) species collected in 3 different habitat types and/or during different seasons → 15 herbs
- Collect 15 key fruit species: try to collect them systematically in 3 different habitat types and/or during different seasons so you would end up with at least 45 fruit samples (if you have little fruit species diversity you can also collect a fourth and fifth sample of a fruit you already have) → absolute *minimum* of 30 fruits, but aim to collect 50 or more.
- other plants: any interesting and edible mushrooms, flowers, piths, bark, liana, bamboo, savanna grass, water/swamp plants → collect 5 more of these 'unusual' plant food items if you have evidence that they are chimpanzee foods
- for nut cracking populations sample nuts the chimpanzees are known to crack and eat (e.g. Parinari excelsa, Coula edulis, Panda oleosa, Detarium senegalense, Sacoglottis gabonensis and Elaeis guineensis
- Collect tree leaves opportunistically: collect at least 10 edible leaves of trees and shrubs that grow in different habitat types → You should reach at least 20 leaf samples

e) NEW: Monthly leaf sampling of 5 individual trees during phenology

Collect a selection of tree leave species monthly: during phenology select 5 individual trees of different species with edible leaves you already marked for your phenology data collection. On these 5 trees, visibly mark a branch you can reach/climb so you can resample the leaves from the same branch in the subsequent 12 months. During monthly phenology, re-visit the tree and sample a leaf every month. Give the resulting 12 samples per tree a unique sample ID consisting of a new organic sample number (e.g. Sapo_001) and the tree ID you have given the tree for phenology (e.g. A1) and the additions "a, b, c, …" etc. for the resulting 12 samples (= Sapo_001_A1_a, Sapo_001_A1_b…etc). Add these sample IDs in the organic data sheet as with all the other plant samples. Store these leaves together in envelopes in a ziplock bag with some silica to keep them dry.

f) Sample treatment

Only a small parts of the plant item is needed as a sample: this can be as small as 10 g. Fresh fruit commonly consist of 80-90% water so a 10 g sample of fruit will be equal to 1 g of dry weight. Whenever possible, firstly dry the samples in the sun before transferring to a 50 ml tube filled 2/3 with silica gel to dry the plant part and prevent it from rotting or moulding. The sample should not come into direct contact with the silica gel so make sure that some barrier such a piece of toilet tissue or paper is put on the silica before placing the plant sample (Figure 9-5). Fruit and other moist samples are best stored in tubes with silica. Leaves have much less moisture and can be stored in paper envelopes when they are kept dry in a ziplog bag with silica which is regularly checked. Change the silica if it loses colour and is saturated with humidity (particularly in the tubes!). Reuse the saturated silica gel by reactivating (heating) for other isotope samples only. Samples can then be stored at room temperature Add a note inside the tube/envelope with the sample, with the information included in the table below (Table 9-2). Then label the tube/envelope again on the outside. For sample ID coding system see section 9.3.

Animals

g) Sampling methodology

Collect tissue samples of animals whenever you find animals and identify the species. Never touch a fresh decomposing carcass (bio hazard!) - only sample dry skeletons and attached tissues (see below). Collect insect samples that are relevant to chimpanzee diet (i.e. bees, ants, termites, see below). Hair from prey mammal species is generally desirable, but may be difficult to obtain, get creative. Try to sample hair from resting spots of animals (e.g. duikers, leopard). Focus also on sample tissues of other primates (arboreal monkeys, baboons and gorillas) including skeletal remains. Tissues can include hair, feathers, scales, insect chitin and many other body tissues. Try to estimate if the animal is a sub-adult or an adult

(this is highly relevant for isotopes). Indicate if the animal is either a herbivore, omnivore, carnivore or an insectivore, and if the animal feeds on the forest floor or high in the canopy. Indicate if the animal tissue was found in the forest or it was sampled from a poached individual. Please use the comment column in the organic data sheet for this. The more notes you make here, the more reliable the background of the sample is for our analyses.

Water samples
Collect 5 water samples: when you encounter water sources on transects or recces. Fill up a 15ml tube with water and keep the sample dark (e.g. in a zarges box). Make detailed comments on what kind of water you sampled. Was it a pond, a lake, a river, a large stream, lagoon, swamp, a puddle or a spring? Can you estimate how deep the water was and is it there all year round or does this water source dry out?

IV-11. STRIP TRANSECT PROTOCOL FOR SITES WITH HIGH DENSITIES OF ROCKS

If you are at a site with a high density of rocks (hundreds every meter) you can undertake a modified strip transect protocol. In this modification, you only count rocks (potential hammers and anvils) and possible wooden hammers and anvils the first 50m of every kilometer of strip transect. However the termite data should be collected along the whole length of the strip transect. ie:

0m-50m - record stones and potential tree anvils and termite mounds
51m-999m -record termite mounds only
1000m-1050m - record stones and potential tree anvils and termite mounds
1051-1999m - record termite mounds only
2000m-2050m - record stones and potential tree anvils and termite mounds
2051-2999m - record termite mounds only …and so forth for the full length of each transect.

IV-12. TREE DRUMMING WITH STONES: Stone throwing and accumulation

Stone throwing has first been seen in Sangaredi chimpanzees, Guinea, a dry savanna-woodland area. The behaviour has been confirmed with stone accumulation in tree trunks in Boe, Guinea-Bissau.

Why are stones accumulated in the tree trunk? Is this due to active accumulation (i.e. intended behavior)? or rather due to passive accumulation (i.e. stones thrown against trees just remain inside the tree). Do they reuse the stones within the trunk? What determines this behavior (abundance of stones, distance of stones, hollowness of trees)?

Picture on the left from Boe shows traces of impact of stones (white arrow), when thrown against the tree trunk, with stone accumulation inside the hollow trunk. Some more stones laying on the ground is seen on the ground nearby.

To answer these questions, we would like those of you who are in areas with dry habitats and/or savannah-woodland areas to collect the following data (if you are not sure if this applies to you, just ask). This behavior may not be present in all sites, but that is also very important to determine if there are some ecological conditions that trigger the presence of this behavior.

Recce survey for the following signs (record all data in the recce worksheet)

1- Presence of traces of stone impacts on the bark of hollow trees.
   a. **If present:**
      i. Make some drawings and take multiple photos from reproducible positions and heights so that you can take photos again from exactly the same positions when visiting the location next time. At your second visit you can then determine if new marks have been made. In Sangaredi, we did not see accumulation of stones in trees, but we observed the stone throwing behavior and therefore it is important to control for stone impact traces on tree trunks. I am not sure, but I am inclined to think that chimpanzees select tree trunk which are either hollow or open along their length for the stone throwing behaviour. It might have something to do with sound propagation that is better the more hollow the tree trunk is.

      ii. Take and send as many photos of the trees with stone accumulation as possible, from many angles and also many context shots (the tree with all the surrounding rocks).
      iii. Record the tree species
      iv. Send a map of the location of all the stone accumulation trees.
      v. Place a camera at any sites where you think the behaviour may occur.

   b. **If absent:** Record that tree was inspected with way point and any/all relevant comments.

2- Presence of hollow trees on stone rich grounds.
   a. Take some good photos, so that we can also try to understand why accumulation of stones is not done at all hollow trees.
   b. Take some pictures of the area around the tree (10 x 10 m), in such a way that you can determine the number of stones available and if they have been moved at your next visit.
   c. For every stone around the tree in the 10 X 10 m area, measure their distance and direction (in degrees) to the tree.
   d. All stones should be marked and weighed and put back in their original locations

3- If stones are present inside the trunk, count the number of stones and mark them in such a way that you can determine if they have been reused or moved when you return for your second recce. Take good photos of each stone and their position within the tree. Place a camera at the site.

4- If your camera shows chimps are stone throwing or you already know that they are stone throwing, then place 2-3 camera traps at some of the sites so that we can obtain sequences to describe this surprising behavior with some detail. Cameras should be placed such that they cover the potential area of stone collection, but also show in detail the potential re-use in the hollow trees

5- Hollow trunk trees should be revisited at regular time intervals to understand how stone accumulates and if the stones within the trunk are reused. If the stones are reused, it raises intriguing questions as to why some stones are preferred over others!

IV-13. ALGAE FISHING: Niche construction in drier habitats?

Algae fishing with tools has only been described so far in Bossou Chimpanzees (Guinea Forestiere) (e.g. Matsuzawa et al. 1999, Humle et al. 2008). There are only two reports of algae eating without tools in Mahale in Tanzania (one individual) and in Odzala NP in Congo (3 individuals). In Bossou, chimpanzees scoop the surface of the water for Spirogyras sp. algae in non-running water. Thus, the new Pan Af observation from the Bakoun chimpanzees in Guinea are very interesting, as they confirm algae consumption is much more general in chimpanzees, extends it to underwater fishing of algae, and includes new aspects of tool uses in that species. Therefore, at all sites but especially in DRY SITES (Ugalla, GEPRENAF, Bakoun, Bafing, Boe, Foutah Jalon, Mt Sangbe, Bateke, Marungu) it would be important to look for:

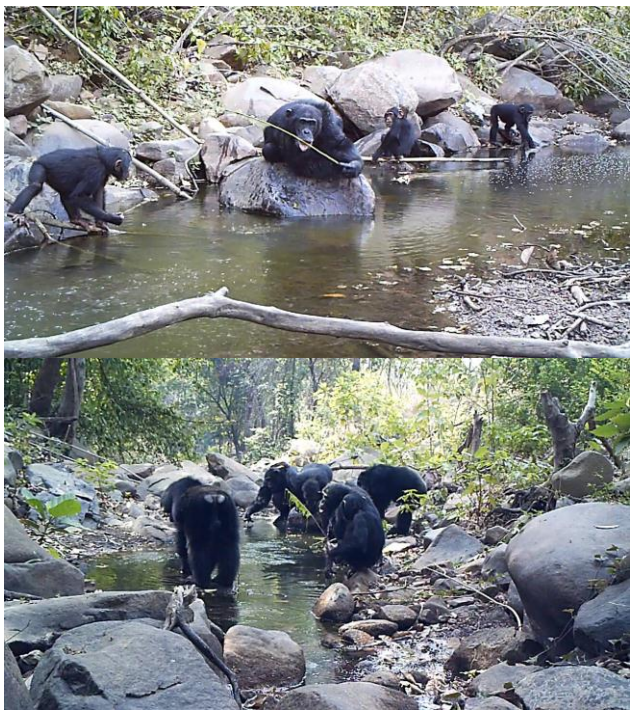
1- How widely distributed this behavior is?
2- If there are variations in the behavior we see?

We suggest to look for the presence of this behavior in your site as follows, FOR ALL SITES please record all inspected pools of water on your recce data sheet (no matter if you find something or not):

1. Look for the presence of surface or underwater algae in streams and pools. If you do not see algae at first scoop, out a large volume of water in a transparent container to look for algae in the water.
   -Record every body of water inspected and whether algae was present or not

2. Look for long twigs that have had their leaves removed and could have been used to fish algae, in the water or at the water's edge.
   -Place a camera at the site if you also find evidence of tools.

Place the cameras so that we can have a view of the section of the water where the remaining tools were found. See below two examples of placement allowing us to see both a section of the water's shore as well as enough of the environment so that we can see some of the tool making that could happen (type of sapling/branches selected and from how far away they are made).



3. Map the different sites inspected - differentiate between: no algae present, algae present but no tools, and algae and possible tools present.

4. In a new excel file record the following (Make sure to also enter this information into your recce data sheet):

Tool/ Sample ID	Date	GPS location & waypoint	Plant species from which tool is made	Length	Diameter	Shape	Modification
						Smooth	stripped of "bark"
						Barbed	one frayed end
						Other*	both ends frayed
							one cut end
							both ends cut
							sub-branches removed
							Other*

* if other please contact Mimi to discuss

5. Take pictures of the different tools so that we can confirm potential shape differences. In Bossou, smooth (top, picture below) and hooked (bottom, picture below) fishing tools have been described and researchers have suggested that there seasonal differences in the frequency of use of the two types ("the less algae are present, the more hooked tools are used").

6. Collect several tools to bring back to Leipzig, and make sure to collect tools that represent the diversity you observe in the field.

7. Collect algae specimens in three ways: **ethanol (15ml or 50ml tubes), dried on silica** (50ml tubes), **and dried and pressed** with other botanicals (to preserve structure). Describe if the algae filaments are branched, if they are slimy filaments and if you can observe (or not) other organisms within the filaments (eg: *Spirogyras* algae filaments do not branch and are slimy, while *Cladophora* algae filaments are branched and are not slimy and sometimes contain other organisms (eg: snails, bugs, crabs))

8. Collect water samples from every pool of water where you collect algae.

9. As a reminder in general: Regularly check for the presence of new tools in sites even when you cannot place cameras so that we can get an idea of the frequency and the seasonality of the behavior.

IV-14. WHEN YOU TRAVEL IN COUNTRY AND TO LEIPZIG

PLEASE carry your netbook, harddrives, receipts and data sheets in your carry-on when you travel. The samples take quite some time to get out of customs and if you pack your netbook/drives with them, you will be sitting in Leipzig with not a whole lot to do. Furthermore, it is much safer for you to have all that irreplaceable material on you JUST in case your luggage gets lost, stolen or confiscated!

When storing and packing:
Sort samples by sample type (all plants together, all pathogens together, all hair samples together, all snail and soil samples together, etc) and put them together in plastic bags, label all bags clearly! This will make it easy for you to find a sample and to keep track of your samples, and will keep the labels on tubes in good condition.

All tubes containing liquid should be wrapped with parafilm and then placed in self-sticking security pags (clear bags with blue writing on them)

To date, we have had a very successful collaboration with German custom and veterinary authorities here in Leipzig regarding clearing our PanAf samples at the airport.
We want to continue these good relations and have been asked to improve our sample processing/data transparency in the following ways. Please obey the following instructions *EXACTLY* while preparing your samples for export (either with DHL or on passenger plane):

- Sample transport should ONLY be done in metal (ZARGES) boxes - no cardboard cartons, no bags.
- We now need to provide the data from the organic and bee trap worksheets from the master datasheet prior to your departure and upon your arrival (in addition to all the other documents). So please make sure to make copy all the data from these sheets into 1 worksheet and sort the list by sample type (the same way you have packed the samples). Send this list along with your other documents before applying for export permits.
- labeling of each sample needs to be done properly and match 100 % with this sample list .
- packing of samples NEED to be done as properly as possible: NO leaking (sealing of fluid sample units), multiple packings, all bags labeled with its contents, etc.