Human and Insect Agriculture Codebook, Version 2 (20NOV14)

Species / Culture __Fungus-Farming Termites____________________________________
Coder __Duur Aanen____
Comments:

There is an important contrast between fungus-farming ants and termites. In the fungus-farming ant symbiosis, the ants form a monophyletic group (Brady and Schultz, 2008), but their mutualistic fungi do not (Mueller et al., 1998; Mikheyev et al). Not only have multiple fungi been domesticated independently by the ants, also some fungal domesticates have retained links with free-living relatives (Mueller et al., 1998). In contrast, in the fungus-farming termites, not only the termites form a monophyletic group, also their domesticated fungi do. So the fungus-farming termites have domesticated a single fungus, and for neither of the symbiotic partners, the termites nor their fungi, descendants have reverted to non-symbiotic lifestyles (as far as we know, at least for extant fungus-farming termites; but see below). Some innovations have occurred right at the beginning (or were present as a pre-adaptation in the last common ancestor of this fungus), for example the production of asexual spores (which is exceptional in basidiomycete fungi, but which some of the related species of *Termitomyces* do), which serve as inoculum and as food source. This early evolution implies that there is no variation within the group for some of these key innovations.

What about the sister group relationships of fungus-farming termites? The fungus-growing termites (subfamily Macrotermitinae) form a basal clade within the Termitidae (higher termites) (Bourguignon et al., 2015; Edward et al. 2007). All four subfamilies of the Termitidae have lost their flagellate protozoan gut symbionts, but have retained gut bacteria. Within the higher termites, two main specialised feeding habits have evolved upon the loss of flagellates: i) fungus farming (while retaining relatively simple gut morphology) and ii) soil feeding (with the evolution of highly complex gut morphology). According to Eggleton and co-workers (Inward et al., 2007), the first step was the acquisition of fungi, which allowed the loss of flagellate gut symbionts. Essentially, fungus-farming termites have externalised the gut so that a significant part of the degradation of organic matter occurs in the fungus-comb rather than in the gut. Wood is masticated and passes through the worker gut essentially unchanged before being defecated on to the fungus comb. Much of the digestive burden carried originally by the flagellates is therefore passed on to the fungus (Rouland-Lefe`vre, 2000) and the flagellates are no longer required. One additional consequence of the use of woody faecal material for fungus comb construction was that faecal material no longer was available as a building material (‘carton’) for nests and so fungus-growing termites build their colony structures out of soil, specifically clay-rich sub-surface soil (Noirot and Darlington, 2000). According to Inward et al. (2007) this may have favoured the transition to soil feeding. In line with this reasoning, previous research has shown that Macrotermitinae workers have substantial amounts of soil in their guts (Donovan et al., 2001a), presumably because they (intentionally or unintentionally) ingest soil particles during mound building. This might pre-adapt fungus-growing termites to soil-feeding on clay-rich soil, which has predominantly highly recalcitrant soil organic matter (SOM) that is very hard to digest.

Following this hypothesis, the proper comparison is not that between fungus-growing termites (the subfamily Macrotermitinae) and its sister group within the Termitidae (composed of the three other subfamilies), but between fungus-farming termites and the sister group of Termitidae. I have followed this logic in my scoring. Within the fungus-farming termites, I have created six columns for coding: (i) The clade composed of the genera *Acanthotermes* and *Pseudacanthotermes*. This lineage is the most basal split from all other groups in the fungus-growing termites; (ii) The clade composed of the genera *Odontotermes* and *Protermes*; (iii) The clade composed of the genus *Macrotermes*; (iiia) The clade composed of the species *Macrotermes bellicosus*; (iv) The clade composed of the genera *Synacanthotermes* and *Allodontermes*; (v) The clade composed of the genus *Ancistrotermes*; (vi) The clade composed of the genus *Microtermes*. However, as explained above, these groups do not show a very clear evolutionary pattern in the complexity of agriculture, except for some characteristics.

Like Ted, I have interpreted "community" to mean "colony" and this works out for most variables, but for some variables it may introduce problems. A termite colony is, functionally, an organism, so when it comes to, e.g., population-biological questions about birth and death rates or density dependence, it is probably better to think at the level of a colony within a population of colonies rather than an individual within a single colony. Given this problem, my scores should be considered tentative and may require correction.

I. "Agricultural" practice variables

VI.1 Selecting substrate (universal)
1 = low specificity
2 = moderate specificity
3 = high specificity
9 = missing
COMMENTS: The fungus-growing termites generally use dead plant material, ranging from herbaceous to woody, for substrate construction, and from what is known, specificity is low. However, this deserves further study.

VI.2 Internal sustainability (harvested domesticates provide source for next crop cycle)
1 = 0 to 33%
2 = 34 to 67%
3 = 68 to 100%
9 = missing
COMMENTS: Most species of fungus-growing termites acquire their symbionts via horizontal transmission. There are only two known transitions to vertical symbiont transmission, in both cases uniparental. In the species *Macrotermes bellicosus* transmission is via the male reproductives, while it is via the female reproductives in species of the genus *Microtermes* that have been studied. However, across longer time scales occasional horizontal transmission occurs even for those species.

VI.3 Planting crops
1 = low investment
2 = moderate investment
3 = high investment
9 = missing
COMMENTS: The termites are obligate agriculturalists.

VI.4 Preparing substrate
1 = low investment
2 = moderate investment
3 = high investment
9 = missing

VI.5 Dimensions of substrate
1 = 2d
2 = 3d
9 = missing

VI.6 Temporal variation in cultivation
1 = discrete (seasonal/crop rotation/fallowing)
2 = continuous
9 = missing
COMMENTS: Fungus-growing termites occur in tropical to temperate regions. Probably there is no seasonality in most species, although we have seen reduced activity in colonies of *Macrotermes natalensis* in winter time in South Africa, where temperatures can be below zero.

VI.7 Diversity of domesticates (at a single location/within a single group)
1 = single domesticate
2 = two or three domesticates
3 = four or more domesticates
9 = missing
COMMENTS: There is one domesticate strain in a given colony at a given time (and usually for the entire life of the colony; at least this is what has been found in the species studied. We are investigating this in greater detail at the moment).

VI.8 Monitoring crops for disease or thieves/predators
1 = 0 to 33% of the time
2 = 34 to 67% of the time
3 = 68 to 100% of the time
9 = missing
COMMENTS: Intensive monitoring.

VI.9 “Weeding”: Physical removal of invasive pests/predators
1 = 0 to 33% of pests removed
2 = 34 to 67% of pests removed
3 = 68 to 100% of pests removed
9 = missing
COMMENTS: This has been studied in only few species of the genera *Odontotermes* and *Macrotermes*. In those studies intensive weeding has been observed.

VI.10 Engineering for optimal growth condition (climate control, watering, etc.)
1 = low investment
2 = moderate investment
3 = high investment
9 = missing
COMMENTS: Mound-forming termites, but also some of the subterranean termites, have sophisticated climate control (e.g. Korb and Linsenmair, 2000). Most other subterranean species do not have sophisticated climate control, other than quickly responding to changing conditions by building new fungus gardens higher or lower. When we compare mound-building species from the rainforest and from the savanna, the most effective climatic buffering is found (and necessary) in savannas.

VI.11 Pests: Chemical control
1 = 0 to 33% of crops treated
2 = 34 to 67% of crops treated
3 = 68 to 100% of crops treated
9 = missing
COMMENTS: What all species of fungus-growing termites have in common, is that the substrate passes the gut before deposition on the fungus garden. This gives the opportunity for the addition of chemicals and beneficial microbes for disease control. However, it is unknown if this plays a role.

VI.12 Pests: Microbial control
1 = 0 to 33% of crops treated
2 = 34 to 67% of crops treated
3 = 68 to 100% of crops treated
9 = missing
COMMENTS: Several bacteria have been isolated that produce antibiotics (Visser et al., 2012). The question how specific these are in their activity against pathogens remains to be answered.

There is one rather specific antibiotic (Bacillaene A) produced by bacteria of the genus *Bacillus* (Um et al., 2013) that shows some specificity against antagonistic fungi.

VI.13 Fertilizing: Organic
1 = 0 to 33% of crops treated
2 = 34 to 67% of crops treated
3 = 68 to 100% of crops treated
9 = missing
COMMENTS: All substrates are organic.

VI.14 Fertilizing: Synthetic chemical
1 = 0 to 33% of crops treated
2 = 34 to 67% of crops treated
3 = 68 to 100% of crops treated
9 = missing
COMMENTS: That's a human thing.

VI.15 Reproductive isolation from free-living populations (reproductive barriers)
1 = low isolation
2 = moderate isolation
3 = high isolation
9 = missing
COMMENTS: As explained in my introduction, all extant domesticated fungi are obligate mutualists, and reproductively isolated from free-living populations.

VI.16 Controlling breeding partners (controlling recombination and sexual selection)
1 = low control
2 = moderate control
3 = extensive control
9 = missing
COMMENTS: In most species, fungal symbionts regularly form mushrooms, so sexual structures. It seems that reproduction is closely linked to alate production, usually a month later (Kone et al., 2011). The proximate mechanism may be that during the period of alate production fewer resources have been invested in workers, which are the individuals that control fungal reproduction. There are only two known transitions to clonal uniparental vertical transmission (in group IIIa via the male reproductive and in group VI via the female reproductive). In both groups mushroom formation is unknown. Nevertheless, vertical transmission is not strictly vertical over longer time scales, as we have found evidence for links with horizontally transmitted fungi in both cases (Nobre et al, 2011).

VI.17 Artificial selection for domesticate improvement
1 = no selection performed
2 = selection done, but less than annually
3 = selection common (annually or more frequent)
9 = missing
COMMENTS: By inoculating a fraction of the spores the termites harvest, they automatically will exercise selection (Aanen, 2006), so I have scored this as 3.

VI.18 Genetic engineering for domesticate improvement (e.g. GMO)
1 = absent
2 = present
9 = missing
COMMENTS: Selection as described above does not qualify as deliberate, human-style "genetic engineering." Score: 1.

II. Agriculture process variables

VII.1 Degree of dependence on domesticated resources (estimated through caloric intake or productive effort)
1 = 0 to 33% of crops
2 = 34 to 67% of crops
3 = 68 to 100% of crops
9 = missing
COMMENTS: As far as we know, the termites depend entirely on the fungus and its action on plant material.

VII.2 Sociality
1 = asocial/solitary
2 = ultrasocial/communal
3 = eusocial
9 = missing
COMMENTS: All Termitidae, including the Macrotermitinae qualify as "highly eusocial."

VII.3 Task specialization
1 = no agricultural task specialists
2 = one or two specialists
3 = three or more specialists
9 = missing
COMMENTS: This has only been studied well in the general *Odontotermes* and *Macrotermes*, where extensive specialisation exists. Among workers there is age polyethism, with older workers performing risky tasks (foraging), and young workers inside-colony tasks, and the soldiers perform defensive tasks. In the other genera less is known.

VII.4 Use of extrasomatic technology
1 = absent
2 = present
9 = missing
COMMENTS: I code this as state 2 because fungus0farming termites have specialised colonies.

VII.5 Use of somatic technology/specialization
1 = absent
2 = present
9 = missing
COMMENTS: Unknown.

VII.6 Information transmission
1 = genetic
2 = developmental
3 = traditional
9 = missing
COMMENTS: No known culture in termites, although, in addition to genetic transmission, termites can transmit information about, e.g., location of food sources, presence of danger (by ‘head banging’).

VII.7 Storage of domesticates
1 = absent
2 = seasonal but less than a year
3 = more than a year
9 = missing

COMMENTS: There is variation in the dynamics of fungus gardens among species (consumed at once after development or continuously), but all have domesticates year round.

III Uses of domesticates variables
VIII.1 Subsistence foods
1 = absent
2 = present
9 = missing
COMMENTS: The termites require their fungi for survival.

VIII.2 Secondary foods
1 = absent
2 = present
9 = missing
COMMENTS: I scored this as 1/1/1 because the termites grow only one thing and obligately depend on it.

VIII.3 “Drug” foods
1 = absent
2 = present
9 = missing
COMMENTS: The cultivar could serve to provide non-nutritive substances, but this remains unknown. I have scored this as state 1, absent, but it could alternatively be scored as state 9.

VIII.4 Raw materials
1 = absent
2 = present
9 = missing
COMMENTS: 1.

VIII.5 Utensils
1 = absent
2 = present
9 = missing
COMMENTS: 1

VIII.6 Labor
1 = absent
2 = present
9 = missing
COMMENTS: 1

VIII.7 Protection
1 = absent
2 = present
9 = missing
COMMENTS: Although the fungus garden may have a protective function for larvae, this is a consequence of the comb structure and it is unknown if the fungus itself plays a role here, so I have scored this as 9.

VIII.8 Detoxification
1 = absent
2 = present
9 = missing

COMMENTS: The fungus is mostly cultivated on dead plant material, so this is probably not very important.

IV. Biological impacts of agriculture variables

VIV.1 Population density
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: The family Termitidae is characterised by complex colony and social structures and increased size, so following the scenario outlined in the introduction I consider this a consequence of the transition to agriculture.

VIV.2 Community size
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: I am interpreting this as colony size and scoring it 3.

VIV.3 Number of communities
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Unknown.

VIV.4 Catchment area
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Moving from single-piece nesters to separate piece nesters implies foraging outside the nest, so increased foraging area.

VIV.5 Genetic changes
1 = absent
2 = present
9 = missing
COMMENTS: Relative to non-fungus-farming termites, genetic modifications are present (Poulsen et al 2-14).

VIV.6 Longevity
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: I have interpreted this to mean colony lifespan, which is equal to the reproductives lifespan. This varies across species but in general, higher termites have increased colony lifespans relative to lower termites.

VIV.7 Age distribution
1 = declines
2 = stable

3 = increases
9 = missing
COMMENTS: I don’t know.

VIV.8 Sex ratios
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Termites are diploid social insects and there is no clear pattern in the sex of the different castes.

VIV.9 Birth rate
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: This could be the rate at which individual termites are produced in a colony or it could be the rate at which new colonies (i.e., successful queens and kings) are produced. Larger colonies produce more sexual offspring and more workers per unit time than do smaller colonies, but survival rates may be different. I have scored this ?/?/?

VIV.10 Death rate
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: The "birth rate" comments apply equally here. Again, scored as ?/?/?

VIV.11 Age of reproduction
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: This varies across species, but in general higher termites have increased ages of reproduction, i.e., colonies usually reach reproductive maturity multiple years after nest-founding, so I have scored this as state 3. However, this deserves further study.

VIV.12 Density dependence
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: unknown.

VIV.13 Pathogen load
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: I am assuming this does not include pathogens of the cultivars. Stable

VIV.14 Nutrition
1 = declines

2 = stable
3 = increases
9 = missing
COMMENTS: Compared to dead plant material, the fungal cultivars have increased nutritional value.

VIV.15 Zoonotics
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: I am interpreting the termite analogue to be diseases that termites can acquire from their gardens. Unknown.

VIV.16 “Wear and tear”
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Based on input from Peter, this has to do with increased somatic stress and damage caused by an agricultural lifestyle. I think this has not changed.

VIV.17 Plastic responses
1 = absent
2 = present
9 = missing
COMMENTS: Peter indicates that this refers to changes in phenotypic traits due to agriculture. The higher termites are characterised by the most specialised caste differentiation, so I have scored this as 2.

VIV.18 Microbiota
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: The gut microbiota has declined in the higher termites, and according to the scenario outlined in the introduction, the first step was the acquisition of an ‘external rumen’, the fungus garden.

VIV.19 Ecological diversity
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: According to Peter, this refers to the effect of farming on local ecological diversity. Probably increased, especially on savannas, where termite monds can be ‘islands of fertility’.

VIV.20 Ecological assemblage
1 = declines
2 = stable
3 = increases
9 = missing

COMMENTS: Since, for the reasons mentioned in the previous comment, the number of species has increased due to termites, I think that the ecological assemblage has also increased.

V. "Sociocultural" impacts of agriculture variables
VV.1 Sedentarism
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: All termites are central-place foragers, bound in space by the locations of their nests. Certainly fungus farming termites differ little from their non-fungus-farming close relatives in this regard. Scored 2

VV.2 Intra-community communication/ coordination
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Unknown

VV.3 Inter-community communication/ coordination
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Whether fungus-farming or not, colonies of the same termite species are essentially competitors. The only need for communication may be to set territorial boundaries. Scored 2.

VV.4 Intra-community territoriality/ ownership
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Although there are inherent genetic conflicts of interest between workers in social insect colonies, this is limited due to almost strict monogamy in higher termite species due to irreversible caste differentiation. Scored 2.

VV.5 Inter-community territoriality/ ownership
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Termite colonies must control foraging territories. Scored 2.

VV.6 Intra-community violence
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Scored 2.

VV.7 Inter-community violence
1 = declines

2 = stable
3 = increases
9 = missing
COMMENTS: Scored 2.

VV.8 Intra-community exchange/ transmission/ diffusion
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: UNCLEAR HOW THIS DIFFERS FROM VV.2, AT LEAST FOR Termites. Scored 9.

VV.9 Inter-community exchange/ transmission/ diffusion
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: It's doubtful that any information is exchanged between colonies except perhaps information about territory. Scored 2/2/2.

VV.10 Kinship structure
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: The entire colony ("community") is a closely related family with, usually a single mother and father/ Scored 2.

VV.11 Size of kin group
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: The size of the kin group is identical to the colony ("community") size, so the answer to this question is the same as for VIV.2 and may be misleading in the intended context of this variable. Scored 3.

VV.12 Access to and control of resources
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Since fungus farming allowed nesting outside the food source and collecting food outside the colony, colony growth and longevity was no longer connected to the food source itself.

VV.13 Access to and control of reproduction (social and physical)
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: The Termitidae have much less flexible caste regulation, and in the vast majority of species irreversible differentiation occurs, in contrast to the Rnitermitidae.

VV.14 Access to leadership
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Irrelevant in termites, which are leaderless.

VV.15 Differential survivorship
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Some tasks in termite colonies such as foraging and colony defence are higher-risk than others such as fungus-comb tending. Among the workers, there is age polyethism, with lower-risk, within-nest tasks carried out earlier in life, and higher-risk outside-nest tasks (e.g., foraging) carried out later in life (Leuthold et al., 1989, Li et al., 2015). This has been studied in detail only in the genera *Macrotermes* and *Odontotermes*. In all groups, with the origin of a true worker and soldier class, there is an extreme difference in longevity between reproductives and helpers, so I have scored all groups 3.

VV.16 Cultural evolutionary mechanisms for selection of behaviors and their transmission
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: No culture in termites, so all 9.

VV.17 Genetic mechanisms for selection of behaviors and their transmission
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: It's all about genetics in termites, so 3.

VV.18 Diversity of tasks
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: The diversity of tasks has increased in all groups.

VV.19 Specialization of tasks
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Scored 2/3/3.

VV.20 Informal social control mechanisms (religion/ tradition)
1 = declines
2 = stable
3 = increases
9 = missing

COMMENTS: 9.

VV.21 Genetic social control mechanisms
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: I followed Ted who interpreted the spirit of this question as addressing whether genetically based mechanisms for coordinating social systems are stable, increase, or decrease, and score this as 3 since social complexity has increased in the higher termites.

VV.22 Communal social control mechanisms
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: According to Peter, this addresses traditions that control behavior and is likely irrelevant to termites, so 9.

VV.23 Authoritarian social control mechanisms
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Absent in termites.

VV.24 Traditional coordination of labor and tasks (ritual/ religion/ mythology)
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: 9.

VV.25 Genetic coordination of labor and tasks
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Since all behaviors in termites are under genetic control, this variable may be redundant with a number of others. Scored 3.

VV.26 Authoritarian coordination of labor and tasks
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: 9

VV.27 Communal decision making
1 = declines
2 = stable
3 = increases
9 = missing

COMMENTS: 9, although all "decisions" in a termite colony are in a sense "communal," certainly in contrast to authoritarian.

VV.28 Pheromonal communication
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: 3

VV.29 Tactile communication
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: 2

VV.30 Acoustic communication
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: 2/2/2

VV.31 Visual communication
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: In termites, only the reproductives have eyes, while workers and soldiers do not have eyes although they can sense light. This has not changed after adoption of agriculture, so 2.

VV.32 Linguistic communication
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: 9

VV.33 Written communication
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: 9

VV.34 Environmental information extraction--observation / interaction
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: According to Peter, this is about contrasting the ability to monitor and process information about the environment in non-farming and farming termites. Since the transition to higher termites is accompanied with increases in foraging area I scored this as 3 for all groups.

VV.35 Environmental information extraction--dedicated organic sensors
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: Unknown.

VV.36 Environmental information extraction--technological sensors
1 = declines
2 = stable
3 = increases
9 = missing
COMMENTS: 9