5 Lifetime Commitment between Social Insect Families and Their Fungal Cultivars Complicates Comparisons with Human Farming

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Farming is always a form of mutualistic symbiosis in which farmers' efforts are rewarded by edible produce, and crops benefit because farmers propagate cultivar genes to future generations. As Darwin (1859) realized, human farmers practice artificial selection, both directly by manipulating differential reproduction of cultivars and indirectly by propagating close kin of preferred individual crop plants or livestock. Over time, human farmers adapted to the nutritional landscapes created by their farming practices, but only as a secondary population-level response. Rapid cultural evolution implied active nutritional niche construction (Scott-Phillips et al. 2014) and the risk of subsequent mismatch when the rate of cultural innovation exceeded the pace by which natural selection could respond with novel genetic adaptations (Tishkoff et al. 2006; Lucock et al. 2014). This human scenario differs fundamentally from the social insect fungus-farming symbioses that are often highlighted for their analogies with human farming. In contrast to humans, the fungus-growing ants and termites co-adapted with their crops in a direct pairwise manner. This is because each social insect farming family commits to a single cultivar for life (Poulsen and Boomsma 2005: Aanen et al. 2009) so that natural selection directly targets the joint reproductive success of each symbiotic colony as a whole (figure 5.1).

This chapter explores the implications of always living in lifetime matrimony with a cultivar and develops a set of analogies with mating system evolution in the social insects. These explorations show that social insect farms are bilaterally symmetrical forms of exploitation for mutual benefit, while human farming symbioses are unilateral forms of exploitation once crop-domestication has been achieved. The niche construction metaphor is useless in the fungus-farming social insects because there is no constructing agent that asymmetrically creates conditions for a partner species. Instead, each matrimonial partnership exploits, and copes with, a joint environmental niche that is fixed for the lifetime of a colony. That difference does not necessarily make farming-practice analogies between social insects and humans invalid, but it changes the causal perspective of such comparisons.

A Hamiltonian Gene's Eye Perspective on Cooperation and Conflict in Mating and Farming

I began studying Panamanian fungus-growing ants in the early 1990s, driven by a natural history interest in the manifestations of social cooperation and conflict. My initial questions
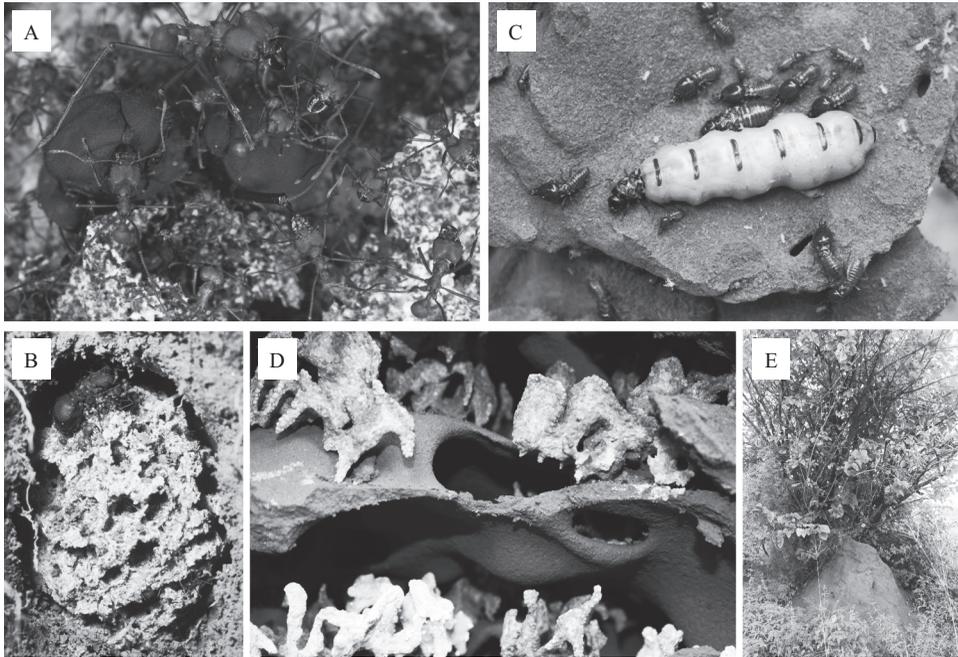
Figure 5.1
Lifetime commitment in sex and symbiosis. (A) A queen of *Atta colombica* from Panama sitting on one of the fungus gardens in a laboratory colony while being guarded by a retinue of minor workers, medium workers, and a large soldier. The queen is > 20 mm long, and her abdominal gaster contains a sperm storage organ of ca. 5 mm diameter with several hundred million stored spermatozoa that will allow her to fertilize up to a hundred million eggs during her maximally achievable life span of 20–30 years. (B) A one-year-old field colony of *A. colombica* exposed from the side with the queen on top, showing the tight fit of the excavated cavity around the colony's fungus garden, on the surface of which small newly added leaf fragments are visible. A fully mature *Atta* colony may contain hundreds of similarly sized fungus gardens, all belonging to the same cultivar clone. (C) A freshly excavated and opened royal chamber of South African *Macrotermes natalensis* showing the queen with her hugely swollen abdomen, her monogamous mate (the colony's king) just above her, and a retinue of guarding adult soldiers and immature (white) nymphs. The queen and king that founded the colony years earlier are recognizable by their blackish thoraces on which the remnants of their shed wings—used during the dispersal flight that brought them together—are still visible. At that point in time they had the same body size so the dark segments on the queen's abdomen illustrate how much her ovaries, needed to lay thousands of eggs each day, have grown. In the image she is ca. 10 cm long. These *Macrotermes* colonies are believed to have a maximal life span of several decades, similar to *Atta* colonies. (D) Fungus-garden cavities of *M. natalensis* showing the older (brown) fungus comb, recently added (grey) fungus comb, and the small white fungal nodules growing from the oldest comb (bottom), consisting mainly of asexual spores that the termites eat so that they are deposited with their primary feces to form the new inoculated (grey) comb material. (E) A medium-sized colony of *M. natalensis* near Pretoria, South Africa. Photos: David Nash (A), Michael Poulsen (D), and the author (B, C, E).

did not relate to farming but they shaped many of the hypotheses that my colleagues and I came to test in later years, because sexual conflicts between females and males and reproductive conflicts between hosts and symbionts turned out to have intriguing parallels (Frank 1996; Poulsen and Boomsma 2005; Boomsma 2007). Given our pioneering studies of the use of genetic markers for mapping pedigrees and mating systems (Van der Have, Boomsma, and Menken 1988), I found the leaf-cutting ants to be intriguing because queens of *Atta* fungus-farming ants had been observed to always mate with multiple males (Hamilton 1964). In the 1980s, many considered multiple insemination of queens to be an explanatory challenge to sterile-worker altruism, but not Hamilton, who wrote that "if the

trend to multiple insemination occurs after the firm establishment of the worker caste, its threat to colonial discipline is a rather remote one" (Hamilton 1964, 33–34).

By the end of the 1990s, we had shown that exclusive single-queen mating is ancestral across the fungus-farming ant genera of Panama and that only the crown-group of *Acromyrmex* and *Atta* leaf-cutting ants always has multiply-inseminated queens (Villesen et al. 2002). It later appeared that Hamilton's prediction implied the deeper principle that strict lifetime monogamy could be conjectured to have been a necessary condition for the evolution of permanently unmated nursing castes in general (Boomsma 2007). This idea was confirmed by a large-scale comparative analysis across the ants, bees, and wasps (Hughes et al. 2008), and it also appeared to be valid in the termites (Boomsma 2009; Hartke and Baer 2011; Boomsma 2013). A total lack of ancestral promiscuity thus appeared to explain the evolutionary origins of colonial superorganismality as defined by Wheeler (1911), with pre-imaginal caste differentiation as the sole criterion for having reached that irreversibly enhanced state of organizational complexity. Because strict monogamy maximizes relatedness between siblings, this was a gratifying confirmation of inclusive fitness theory, but it also raised irresistible new questions about the expression and regulation of sperm competition conflicts when obligate multiple queen-mating evolved as a secondary superorganismal elaboration, as had happened multiple times in the ants, corbiculate bees, and vespine wasps (Boomsma, Kronauer, and Pedersen 2009). In following Hamilton's lead in never believing one had understood cooperation without having stared the possible corrupting forces in the face, it seemed obvious to ask why sperm competition had not rendered multiply-inseminated queens infertile.

The queens of ants, social bees, and social wasps are known to never re-mate later in life (Boomsma, Baer, and Heinze 2005), so it seemed reasonable to imagine that multiple ejaculates entering a queen's sperm storage organ on the same afternoon would compete for later paternity. Strife for a higher share of sperm storage space could thus become a tragedy of the commons when ejaculates would continue to kill each other's sperm, leaving a queen without options to fertilize eggs well before her colony would be large enough to reproduce. We therefore hypothesized that leaf-cutting ant queens must have evolved molecular mechanisms to regulate what seemed to be a serious sexual conflict, and we realized that this conflict was mostly a female challenge to manage "endosymbiotic" sperm for maximal overall quality and diversity (Boomsma and Ratnieks 1996; Boomsma, Baer, and Heinze 2005). Over the years we could show that these expectations were correct. Unrelated ejaculates of phylogenetically basal attine ants with obligate single queen-mating peacefully coexisted on microscope slides, consistent with there never having been selection for aggressive sperm competition traits. However, the same *in vitro* confrontations between ejaculates of polyandrous leaf-cutting ants (and honeybees) gave rapid sperm mortality. And, as predicted, adding secretion from queen sperm storage organs terminated mutual sperm killing, at least in *Atta* leaf-cutting ants (Den Boer et al. 2010) (figure 5.1A, B).

We recently characterized the (rather few) proteins involved in this conflict and in its ultimate resolution and showed that they likely evolve rapidly as expected in antagonistic arms races (Dosselli et al. 2019). The bottom line of this study was that, in spite of damaging potential conflict, multiple insemination appears to be evolutionarily stable because queens have the agency to remain in control over a genetically variable set of unruly sperm

"symbionts." Ultimately, both males and females came to benefit from the advantages of more genetically diverse colonies (Hughes and Boomsma 2004, 2006) after the potentially devastating tragedy of the stored-sperm commons had been tamed. We thus inferred that for millions of years any new male mutant producing more competitive ejaculates had likely been countered by an effective female response before it could drive populations extinct. It then seemed logical as well that a crucial condition for queens having gained the upper hand must have been that the stored-sperm commons remained closed for newly arriving ejaculates: ancestral lineages with monogamous, full-sibling colonies could thus give rise only to derived lineages with chimeric (multiple patriline) colonies when queens retained their ancestral habit of never re-mating later in life (Boomsma, Baer, and Heinze; Boomsma 2007). What had seemed to be an idiosyncratic peculiarity of social insect mating systems now appeared to make beautiful sense. Lifetime commitment between the sexes—ancestrally between a single queen and a single male and later by a single queen and a specific set of males—had to be a closed game to keep the reproductive interests of the sexes aligned, albeit with the stored ejaculate symbionts in a powerless role devoid of any independent agency.

Very similar Hamiltonian predictions of cooperation and conflict turned out to apply to interspecific host-symbiont interactions, such as those between farming ant families and their fungal cultivars, as was consistent with theory by Frank (1996). Such interactions should be stable if they were as exclusive as the lifetime association between a mitochondrial clone and two sexual gametes in a zygote. However, if symbionts were acquired promiscuously, conflict between genetically different lineages would be expected to threaten symbiotic stability. This is because all garden cultivars need to grow, both in their own interest and to be worth their keep as mutualists, so that multiple cultivars coexisting in the same host-provided niche would inevitably compete for farmer attention. Strife between symbiont lineages would then reduce their overall crop service to the farming colony, in a way that is analogous to sperm competition compromising male fertilization services to queens and their colonies unless inter-ejaculate conflicts are suppressed. Frank's logic thus implied the expectation that farming ants had evolved mechanisms to avoid mixing symbionts in chimeric fungus gardens and that no worker should thus be allowed to introduce a novel unrelated cultivar—for example a fragment from a vacated garden of a newly founded colony nearby.

In this case the conceptual logic again delivered. We could show that *Acromyrmex* colonies always have a single clonal fungus garden, that the fungal cultivars express incompatibility reactions when confronted with mycelium from other fungus gardens, that worker fecal fluid contains nourishing proteins from the resident cultivar that cannot be smoothly absorbed by unrelated cultivars, and that foraging workers are reluctant to adopt alien cultivar fragments (Bot, Rehner, and Boomsma 2001; Poulsen and Boomsma 2005; Ivens et al. 2009; Kooij, Poulsen, et al. 2015). As it turned out, there is lifetime commitment throughout, not only between a queen and the single or multiple male(s) that inseminate her, but also between every ant family and its clonal fungal cultivar (figure 5.1A, B). It appeared that once colonies have workers, a cultivar can neither be replaced nor be secondarily mixed with other cultivars under natural conditions (Poulsen and Boomsma 2005; Howe et al. 2019). Thus, potential conflicts between cultivar symbionts and farming hosts were either avoided or regulated, but, as in sperm storage after queen-mating, at the

expense of parties losing all options for later promiscuous exchange. It seemed likely, in fact, that the very origin of ant fungus farming had been conditional on the enforcement of lifetime commitment between a core family of ant farmers and a single cultivar in each colony.

Comparing Exclusively Committed Insect Farming with Promiscuous Human Farming

Conceptually, the findings summarized above cannot be reconciled with the practices of human farming. We would not dream of being dependent on a single clonal cultivar for life, and human farmers are not lifetime unmated altruists comparable to ant workers. There are surely many analogies between farming practices across the human and social insect domains (chapter 14, this volume), but it makes a difference whether we evaluate the deeper roots of unilateral human management to maximize yield or the sustainability of a co-adapted mutualism over evolutionary time. Because the farming ants have practiced the mutual co-adaptation model during millions of years of relentless natural selection on joint performance, they often surpass us in specific efficiency targets. Not only did ants in general evolve sperm banks at ambient temperature that last a queen's potential life span of two to three decades (Den Boer et al. 2009), but they also somehow prevented the evolution of resistance by specialized *Escovopsis* garden pathogens against biocontrol compounds obtained from Actinobacteria that they rear on their cuticles (De Man et al. 2016; Holmes et al. 2016; Heine et al. 2018) (chapter 11, this volume). Recent work has further indicated that the fungus-growing termites are equally efficient in keeping their colonies as free from pathogens as the leaf-cutting ants appear to be (Otani et al. 2019; see also figure 5.1C, D, E). Relative to the extreme specialization of social insect farmers, human farmers are jacks of all trades in their interactions with domesticated crops, and we remain extremely vulnerable to endemic and epidemic diseases of our cultivars.

Given at least a partial understanding of the power of superorganismal farming symbioses, it seems also important to note that the impressive achievements of fungus-farming ants and termites came with an important tradeoff that should make us very reluctant to adopt them as role models. To phrase it provocatively, admiring any superorganismal colonies for their productivity benchmarks is analogous to singing the praise of Nazi Germany because the Third Reich was superbly efficient in building motorways. Defined as they are by stringent and unconditional commitments for life, superorganismal colonies practice what we would conceive in the human domain as *Blut und Boden* principles from the cradle to the grave. As already noted by Wheeler (1911), all individual agency in the sense of independent decision-making has disappeared out of their family lives no matter if one is a queen, a worker, or a soldier (Boomsma 2013; Boomsma and Gawne 2018). Lifetime unmated workers are as committed to their family as somatic cells are to a metazoan body. The main difference is that every somatic cell analog has a brain and six legs (figure 5.1).

It is a truism that the two sexes need each other to reproduce and that the same applies to the farming families of ants and their cultivar clones. However, the notion that none of them can pass on any genes to future generations without a thriving mutualistic partner undermines anthropomorphic extrapolations of human farmer agency to farming insects. A narrative based on fungal cultivars having enslaved an addicted ant family is equally

credible. The same is true for the fungus-growing termites. Here, the founding pair establishes a colony and produces its first cohort of workers, who begin to forage, digest, and defecate while creating a fungus comb where sexual *Termitomyces* spores germinate because they were mixed with the termite excrements (Aanen et al. 2009; Nobre et al. 2011). Different *Termitomyces* haplotypes then merge into dikaryons and compete for being propagated asexually so that invariably only a single clone remains. This requires only that the farming termites continue to harvest and eat garden nodules with asexual spores and to produce new inoculated comb material (figure 5.1D). That process ultimately produces an optimal match between a single cultivar clone and a termite family, but it is then equally reasonable to maintain that the garden symbionts domesticated the farming termites as it is to emphasize that the termites domesticated *Termitomyces* (Aanen et al. 2009; chapter 4, this volume). Symbiotic farming agency is evenly split between the insect partner and the cultivar, and none of them can opt out to join a neighboring colony once their host-symbiont marriage has been consummated (see below for this not yet being the case during colony foundation). Strict lifetime commitments thus unambiguously ensure cooperative harmony via monogamous sex and exclusive symbiotic partnership, and they secure a joint interest in suppressing any secondary events of sexual or symbiotic promiscuity.

It seems obvious to consider the fungus-growing termite symbiosis as symmetrically mutualistic (Aanen et al. 2002; Korb and Aanen 2003; Nobre et al. 2011), but fungus-growing ants have always been considered to have unilateral farmer agency because dispersing queens vertically transmit a cultivar clone to the next generation of colonies. However, we actually do not know how vertical the colony-founding matchmaking between a farmer ant queen and a cultivar actually is. Our recent work has challenged the general vertical transmission belief by showing that founding queens of two Panamanian *Acromyrmex* species often acquire their cultivar from nests of other founding queens (Howe, Schiøtt, and Boomsma 2019). This is possible because these queens need to forage from open burrows so they frequently succumb to predation, while other queens lose their incipient garden because of mold or fungus-feeding predators and start looking for a replacement garden. Molecular marker data from natural colony-founding aggregations of *Acromyrmex* made us conjecture that horizontal cultivar acquisition may well be common throughout all attine ant lineages except for *Atta*, which is the only genus that evolved claustral colony founding and sufficient queen fat reserves to rear a first garden and worker cohort in complete seclusion and without foraging.

The study by Howe, Schiøtt, and Boomsma (2019) revealed an unexpected parallel between the fungus-growing ants and the fungus-growing termites where horizontal (*h*) cultivar acquisition is phylogenetically basal and where two species/genus-level lineages secondarily evolved vertical (*v*) cultivar acquisition (Aanen et al. 2002). The difference in cultivar transmission between the independently evolved farming systems of ants and termites now appears to be a matter of degree rather than of principle. A colony-founding *Acromyrmex* queen can principally acquire a single cultivar at the time in some *v, h, h, . . .* sequence, and she would then commit for life to the last of these before her first workers hatch. Only when no accidents happen will she remain with her original (*v*) cultivar (Poulsen et al. 2009). The fungus-growing termites acquire multiple haplospores from the leaf litter that the first workers bring in, then passively stage competition between the emerging dikaryons in their first garden substrate, and finally let positive frequency-

dependent selection sort out which single diploid clone will obtain the monopoly of termite care for the lifetime of the colony (Aanen et al. 2009). Foraging for cultivars—creating h, h, . . . sequences—thus occurs in both symbioses. The same strategy must also have applied to early human farmers when they tested plants for their suitability as crops (Smith 1998). The difference is that our own culturally evolving ancestors could easily retain their unilateral exploitative agency as farmers—mixing and replacing cultivars as they saw fit—while the insect farming symbioses could remain stable over evolutionary time only by exclusive mutual commitment and symmetrically shared agency in every pair of farming family and cultivar crop.

Rethinking the Natural History of Insect Fungus Farming

The logic outlined above implies that there is host-cultivar recombination across the generations in both ant and termite fungus-farming colonies. Recombination between the fungus-growing termites and their *Termitomyces* cultivars is unconstrained, but two limitations apply in the fungus-growing ants. First, vertical cultivar transmission will often result in vertical cultivar acquisition—all Howe, Schiøtt, and Boomsma (2019) showed is that we cannot take this for granted even in ecological time. There remains, therefore, a significant difference of degree between conditional swapping of cultivars in the fungus-growing ants and the rampant and essentially random host-symbiont recombination across the generations in most fungus-growing termites. This is related to almost all fungus-farming termite genera having retained sexual reproduction of cultivars via spore-producing mushrooms (chapter 6, this volume), while the evolutionarily derived attine cultivars may well be completely asexual and thus unable to recombine genotypes within the fungal cultivar populations (Nobre et al. 2011; Shik et al. 2020). Interestingly, the cultivars of *Acromyrmex* and *Atta* became polynucleate chimeras (Kooij, Aanen, et al. 2015) around the same time that the farming ants evolved chimeric families through multiple queen-insemination (Nygaard et al. 2016). Both parties in the leaf-cutting ant symbioses can therefore effectively express genes from a polyploid set of chromosomes. It is important to stress that this secondarily evolved bilateral form of partner chimerism between the ants and their crops does not challenge the lifetime commitment principle—it just implies that more partner genomes became involved on both sides.

A consequence of stringent "matrimonial" commitments in sex and symbiosis is that there are—at each moment in time and in each population—an equal number of mature termite farmer families alive as there are *Termitomyces* fungal cultivar clones. This also applies throughout the attine ants and their cultivars, although only approximately so in the mutually chimeric colonies of the leaf-cutting ants (Kooij, Aanen, et al. 2015). The same is true for the number of males and females alive in all committed royal pairs of termites and in all attine genera with singly inseminated queens, as long as one acknowledges that single colony fathers remain functionally alive when they are represented by stored sperm (figure 5.1A, B). As I have argued elsewhere (Boomsma 2009, 2013), lifetime commitment is a very powerful mechanism for enforcing evolutionarily stable altruism within species, because promiscuity never intervenes and the offspring produced by both partners always have maximal possible relatedness to each other. Family life of such stringent coherence provides the highest possible incentive for older siblings to raise younger siblings

rather than their own offspring. That same logic should also tie different species together in obligate mutualisms when their partnerships remain "monogamously" exclusive (Wyatt, West, and Gardner 2013). In all these cases, partner commitment is so strong that one dies with the only sexual or mutualistic partner(s) that one will ever have (figure 5.1A–D). The contrast with human mate choice and our interactions with domesticated crops and livestock could hardly be more striking.

A corollary of my "vertical transmission unless . . ." interpretation of attine symbiotic commitment is that vertical (parent-offspring) cultivar transmission may have become obligate only in claustral colony-founding *Atta* (e.g., Baer, Armitage, and Boomsma 2006) and in *Macrotermes bellicosus* and the genus *Microtermes* as far as the fungus-growing termites go (Aanen et al. 2002). That would make strict vertical cultivar acquisition a consistently derived evolutionary trait, emerging either from ancestries whose horizontal cultivar transmission was merely rare (*Acromyrmex* and likely all other phylogenetically more basal genera of attine ants) or omnipresent (all other fungus-growing termites). Such a perspective would make fungal cultivar transmission in *Atta* comparable with the secondary loss of sex and meiosis in multicellular organisms, because host-symbiont recombination becomes impossible or significantly compromised when vertical transmission becomes obligate, just as intragenomic recombination ceases when parthenogenesis is no longer alternated with bouts of sexual reproduction. The extent to which such a transition to pure vertical transmission has increased genetic load in fungal cultivars (i.e., reduced average fitness due to accumulating deleterious mutations) is an empirical question, because rare sex and recombination can be disproportionally effective in preventing this problem (D'Souza and Michiels 2010). The degree of year-to-year horizontal cultivar transmission now documented for *Acromyrmex* (Howe, Schiøtt, and Boomsma 2019) is likely to be sufficient to avoid cultivar symbiont deterioration by mutation accumulation (also known as Muller's ratchet (Kondrashov 1994)). This is not because the cultivars fail to accumulate deleterious mutations, but because host-symbiont recombination is frequent enough and subsequent colony-level selection is strong enough to remove cultivars of compromised quality. Even populations of *Atta* cultivars may be protected against Muller's Ratchet, perhaps not so much by rare host-symbiont recombination when the nest cavities of founding colonies might sometimes meet each other underground, but because *Atta* and *Acromyrmex* have been assumed to rear the same species of *Leucoagaricus gongylophorus*. Although high resolution genetic markers showed that the symbionts of these two ant genera in Panama belong to distinct sympatric populations (Kooij, Poulsen, et al. 2015), it might be that founding *Atta* colonies occasionally acquire a fitter *Acromyrmex* cultivar.

My main argument so far has been that horizontal cultivar acquisition is possible whenever founding queens (ants) or first worker cohorts (termites) need to forage, because that will inevitably imply exposure to, respectively, unrelated incipient gardens or sexual cultivar spores. Evolved defenses against chimerism should then imply that secondary cultivar introductions will not be able to replace established gardens (Poulsen and Boomsma 2005; Aanen et al. 2009), but that new cultivars will have a chance of successful take-over in the colony founding phase (Aanen et al. 2009; Poulsen et al. 2009) before matrimonial permanency is established. In this context it is interesting to note that a third group of highly diverse small-scale fungus farmers, the ambrosia beetles, are permanently confined to their nest galleries, which are excavated almost invariably in dead or dying wood. The complete absence of foraging outside the gallery, both by helping offspring and by nest-

founding parents, might imply that ambrosia beetles may be the only clade of fungus farmers in which vertical cultivar transmission is both ancestral and has remained obligate ever since (Kirkendall, Biedermann, and Jordal 2015). This is consistent with these beetles being the only fungus-farming lineage where specialized mycangia organs for cultivar co-dispersal are always found (Mayers et al. 2015; Hulcr and Stelinski 2017; Smith et al. 2018; chapter 7, this volume). Even here, however, foundresses may sometimes tunnel next to each other in the same piece of wood so closely that gallery systems could secondarily merge and exchange cultivars.

Retrospectively, it probably makes good sense that cultivar transmission in fungus-farming insects is almost never completely set in stone. Farming mutualisms are all ecto-symbioses (Law 1985) (figure 5.1), where partners can survive without each other's company for at least short periods. This is the key factor enabling rare but statistically predictable cultivar swapping in ecological time. In contrast, microbial endosymbionts have such faithful transmission that punctuation of vertical inheritance by horizontal swaps is detectable only on an evolutionary time scale (McCutcheon, Boyd, and Dale 2019). Transfers at the latter time scale have also been documented in attine ants (Green, Adams, and Mueller 2002: Mehdiabadi et al. 2012). However, ecological and evolutionary time scales are different by orders of magnitude and should not be confused because faithful vertical transmission over evolutionary time implies that we do not expect defensive traits against chimerism to have evolved. That reasoning is again analogous to mixed ejaculates from species with exclusively singly inseminated queens not expressing hostile sperm competition, whereas ejaculates of males from species with multiply inseminated queens do (Den Boer et al. 2010; Dosselli et al. 2019). Only chimeric encounters on an ecological time scale will establish natural selection for defense, enabled by random mutations that can be coopted for expressing mutually hostile phenotypes, be it among ejaculates or fungal cultivars. Vertically transmitted ectosymbionts are thus expected to have defensive traits against competing strains that offer similar services to hosts, while vertically trans-mitted endosymbionts are likely to be devoid of independent competitive agency. However, the evolution of defensive traits against chimerism will proceed only when there are no other a-priori mechanisms that remove selection for such hostile traits. It took us a while to understand this logic. We initially looked for incompatibility reactions between *Termi-tomyces* clones similar to those expressed between *Acromyrmex* cultivars, until we realized that positive frequency-dependent propagation of asexual spores within the farming termite colonies selects for rapid cultivar growth and maximal production of edible nodules with asexual spores, rather than for active direct defenses against competing strains (Aanen and Boomsma 2006; Aanen et al. 2009). For these aspects of farming the termites are just passive spectators and defecators of inoculated comb (figure 5.1D).

Finally, it is worth noting that insect farmers differ fundamentally from human farmers because they cultivate heterotrophic crops that need organic substrate rather than autotro-phic cultivars that need light and inorganic nutrients. It is interesting that farming of photosynthesizing crops is rare outside humans (Aanen 2010; Wcislo 2020). This is true even among the ants, where neither devil's gardens in the Amazon rain forest (Frederick-son and Gordon 2007) nor *Philidris* ants farming *Squamellaria* epiphytes on some of the Fijian islands (Chomicki, Kadereit et al. 2020; chapter 9, this volume) have realized adap-tive radiations remotely comparable in diversity to the fungus-farming ants, termites, and ambrosia beetles. A difference of this kind is perhaps not surprising, because farming

mutualisms with autotrophic crop symbionts depend on the plant partners providing both above-ground housing (domatia) and nourishment (floral nectaries) to ant colonies. These are then traded against anti-herbivory services or delivery of manure in the form of inorganic waste. If there is competition in these systems, it is for domatia space between alternative ant colonies, which implies that the farming analogy becomes rather indirect and that mutualistic agency rests primarily with the plants, not with the ants (Ward and Branstetter 2017; Marting et al. 2018). In contrast, fungal agriculture is almost invariably based on the farming insects excavating nest cavity "domatia" in abiotic (i.e., not co-adapting) space (figure 5.1B), in which fungal cultivars either have an unchallenged clonal monopoly of farmer care (ambrosia beetles) or gain such monopoly after outcompeting other cultivar strains that are either primarily acquired (in termites) or secondarily invading (in ants).

Conclusions

We are intuitively inclined to think of farmers as subjects and crops as objects because "agency" in the sense of who or what domesticated and maintains what or whom appears to be fundamentally asymmetric. However, it is not without conceptual problems to extrapolate this anthropomorphic narrative to insect farmers. Human farms promiscuously combine and exchange crops; they hedge their bets to secure profit even when some crops fail. In contrast, fungus-farming symbioses of ants and termites lack promiscuity: their families consist of lifetime-committed sets of parents and lifetime unmated altruistic sibling workers who are jointly committed for life to rear a single cultivar clone and make the best of that assignment in terms of reproductive success for both parties. Farmer agency seems indisputable when cultivars are exclusively vertically propagated, but strict mutual dependence and horizontal cultivar acquisition makes that notion ambiguous—with equal right one could then say that cultivars acquire a lifetime devoted nursing insect family to pass on fungal genes to future generations. Recent work shows that it has not been sufficiently appreciated that vertical transmission does not preclude horizontal cultivar acquisition as long as founding queens or first worker cohorts actively or passively forage for alternative cultivars before resident crops acquire enough agency to fight for their monopoly of farmer attention (Nobre et al. 2011; Howe, Schiøtt, and Boomsma 2019). Only the fungus-growing ambrosia beetles never forage outside their burrows, so these beetles may be the most convincing example of vertical cultivar acquisition being both ancestral and universally extant.

   Using a conceptual framework of mutualism theory that acknowledges potential conflicts between farming hosts and cultivar ectosymbionts, I have argued that the dynamics of social insect fungus farming are fundamentally different from human farming practices, in spite of interesting analogies in the optimal management of human and insect farms. With both sexual- and host-symbiont partners being committed for life, there are uncanny parallels between the ways in which sexual conflict and host-symbiont conflict were resolved in order to align reproductive interests—perspectives that are irrelevant in human farming. Rather than comparing insect fungus farming with endosymbiotic domestication processes reminiscent of how mitochondria became enslaved by eukaryote cells, it seems more appropriate to consider nest cavities with fungus gardens as artifactual farmer-built

"domatia," in which closely related competing cultivar strains always exclude each other, driven by the necessity to obtain a monopoly of care by a farmer family whose fitness is also maximized by rearing only a single clonal cultivar. No such considerations ever apply to culturally evolved human farming practices, which has left our cultivars powerless because they cannot enforce exclusive farmer commitments.

Throughout this edited volume, *domestication* is defined as the genetic modification of one species by another in ways that benefit the modifying species (the farmer) but that would have reduced the fitness of the modified species (the domesticate) in its original niche. This definition, in spite of its apparent generality, implicitly assumes that agency is fundamentally asymmetric, and I have argued that it therefore only partly applies to fungus-farming insects. In these insect farming symbioses it is equally legitimate to consider the insects as domesticates of the crop fungi as the other way around because neither of the parties can still survive in their ancestral nonfarming niche. In my elaboration of the tensions emanating from this mismatch, I have aimed to achieve three objectives: (1) to establish the notion that we should be careful when using analogies between human and insect farming—however fascinating they are—in such broad-brush narrative strokes that the fundamental difference between promiscuous culturally driven artificial selection and naturally selected one-to-one co-adaptation becomes opaque; (2) to evaluate the striking analogies between lifetime commitments in sex and symbiosis that characterize insect fungus farmers; and (3) to illustrate the usefulness of evolutionary theory for a parallel understanding of conflict regulation in mating and farming—an approach that generates both testable predictions and the perspective of a more encompassing understanding of cooperation and conflict.

Acknowledgments

References

Aanen, D. K. 2010. "As You Weed, So Shall You Reap: On the Origin of Algaculture in Damselfish." *BMC Biology* 8:81.

Aanen, D. K., and J. J. Boomsma. 2006. "The Evolutionary Origin and Maintenance of the Mutualistic Symbiosis between Termites and Fungi." In *Insect Symbiosis*, edited by K. Bourtzis and T. Miller, 2:79–95. Boca Raton, FL: CRC Press.

Aanen, D. K., P. Eggleton, C. Rouland-Lefevre, T. Guldberg-Froslev, S. Rosendahl, et al. 2002. "The Evolution of Fungus-Growing Termites and Their Mutualistic Fungal Symbionts." *Proceedings of the National Academy of Sciences* 99(23): 14887–14892.

Aanen, D. K., H. H. D. Licht, A. J. M. Debets, N. A. G. Kerstes, R. F. Hoekstra, et al. 2009. "High Symbiont Relatedness Stabilizes Mutualistic Cooperation in Fungus-Growing Termites." *Science* 326(5956): 1103–1106.

Baer, B., S. A. O. Armitage, and J. J. Boomsma. 2006. "Sperm Storage Induces an Immunity Cost in Ants." *Nature* 441(7095): 872–875.

Boomsma, J. J. 2007. "Kin Selection Versus Sexual Selection: Why the Ends Do Not Meet." *Current Biology* 17(16): R673–R683.

Boomsma, J. J. 2009. "Lifetime Monogamy and the Evolution of Eusociality." *Philosophical Transactions of the Royal Society B—Biological Sciences* 364(1533): 3191–3207.

Boomsma, J. J. 2013. "Beyond Promiscuity: Mate-Choice Commitments in Social Breeding." *Philosophical Transactions of the Royal Society B—Biological Sciences* 368:20120050.

Boomsma, J. J., B. Baer, and J. Heinze. 2005. "The Evolution of Male Traits in Social Insects." *Annual Review of Entomology* 50:395–420.

Boomsma, J. J., and R. Gawne. 2018. "Superorganismality and Caste Differentiation as Points of No Return: How the Major Evolutionary Transitions Were Lost in Translation." *Biological Reviews* 93:28–54.

Boomsma, J. J., D. J. C. Kronauer, and J. S. Pedersen. 2009. "The Evolution of Social Insect Mating Systems." In *Organization of Insect Societies: From Genome to Sociocomplexity*, edited by J. Gadau and J. Fewell, 3–25. Cambridge, MA: Harvard University Press.

Boomsma, J. J., and F. L. W. Ratnieks. 1996. "Paternity in Eusocial Hymenoptera." *Philosophical Transactions of the Royal Society B—Biological Sciences* 351(1342): 947–975.

Bot, A. N. M., S. A. Rehner, and J. J. Boomsma. 2001. "Partial Incompatibility between Ants and Symbiotic Fungi in Two Sympatric Species of *Acromyrmex* Leaf-Cutting Ants." *Evolution* 55(10): 1980–1991.

Chomicki, G., G. Kadereit, S. S. Renner, and E. T. Kiers. 2020. "Tradeoffs in the Evolution of Plant Farming by Ants." *Proceedings of the National Academy of Sciences* 117:2535–2543.

D'Souza, T. G., and N. K. Michiels. 2010. "The Costs and Benefits of Occasional Sex: Theoretical Predictions and a Case Study." *Journal of Heredity* 101:S34–S41.

Darwin, C. 1859. *On the Origin of Species*. London: John Murray.

De Man, T. J. B., J. E. Stajich, C. P. Kubicek, C. Teiling, K. Chenthamara, et al. 2016. "Small Genome of the Fungus *Escovopsis weberi*, a Specialized Disease Agent of Ant Agriculture." *Proceedings of the National Academy of Sciences* 113:3567–3572.

Den Boer, S. P. A., B. Baer, and J. J. Boomsma. 2010. "Seminal Fluid Mediates Ejaculate Competition in Social Insects." *Science* 327(5972): 1506–1509.

Den Boer, S. P. A., B. Baer, S. Dreier, S. Aron, D. R. Nash, et al. 2009. "Prudent Sperm Use by Leaf-Cutter Ant Queens." *Proceedings of the Royal Society B—Biological Sciences* 276(1675): 3945–3953.

Dosselli, R., J. Grassl, S. P. A. Den Boer, M. Kratz, J. M. Moran, et al.. 2019. "Protein-Level Interactions as Mediators of Sexual Conflict in Ants." *Molecular & Cellular Proteomics* 18:S34–S45.

Frank, S. A. 1996. "Host-Symbiont Conflict over the Mixing of Symbiotic Lineages." *Proceedings of the Royal Society B—Biological Sciences* 263(1368): 339–344.

Frederickson, M. E., and D. Gordon. 2007. "The Devil to Pay: The Cost of Mutualism with *Myrmelachista schumanni* Ants in 'Devil's Gardens' Is Increased Herbivory on *Duroia hirsuta* Trees." *Proceedings of the Royalty Society B—Biological Sciences* 274:1117–1123.

Green, A. M., R. M. M. Adams, and U. G. Mueller. 2002. "Extensive Exchange of Fungal Cultivars between Sympatric Species of Fungus-Growing Ants." *Molecular Ecology* 11:191–195.

Hamilton, W. D. 1964. "Genetical Evolution of Social Behaviour 2." *Journal of Theoretical Biology* 7(1): 17–52.

Hartke, T. R., and B. Baer. 2011. "The Mating Biology of Termites: A Comparative Review." *Animal Behaviour* 82:927–936.

Heine, D., N. A. Holmes, S. F. Worsley, A. C. A. Santos, T. M. Innocent, et al. 2018. "Chemical Warfare between Leafcutter Ant Symbionts and a Co-Evolved Pathogen." *Nature Communications* 9:2208.

Holmes, N. A., T. M. Innocent, D. Heine, M. Al Bassam, S. F. Worsley, et al. 2016. "Genome Analysis of Two *Pseudonocardia* Phylotypes Associated with *Acromyrmex* Leafcutter Ants Reveals Their Biosynthetic Potential." *Frontiers in Microbiology* 7:2073.

Howe, J., M. Schiøtt, and J. J. Boomsma. 2019. "Horizontal Partner Exchange Does Not Preclude Stable Mutualism in Fungus-Growing Ants." *Behavioral Ecology* 30:372–382.

Hughes, W. O. H., and J. J. Boomsma. 2004. "Genetic Diversity and Disease Resistance in Leaf-Cutting Ant Societies." *Evolution* 58(6): 1251–1260.

Hughes, W. O. H., and J. J. Boomsma. 2006. "Does Genetic Diversity Hinder Parasite Evolution in Social Insect Colonies?" *Journal of Evolutionary Biology* 19(1): 132–143.

Hughes, W. O. H., B. P. Oldroyd, M. Beekman, and F. L. W. Ratnieks. 2008. "Ancestral Monogamy Shows Kin Selection Is Key to the Evolution of Eusociality." *Science* 320(5880): 1213–1216.

Hulcr, J., and L. L. Stelinski. 2017. "The Ambrosia Symbiosis: From Evolutionary Ecology to Practical Management." *Annual Review of Entomology* 62:285–303.

Ivens, A. B. F., D. R. Nash, M. Poulsen, and J. J. Boomsma. 2009. "Caste-Specific Symbiont Policing by Workers of *Acromyrmex* Fungus-Growing Ants." *Behavioral Ecology* 20(2): 378–384.

Kirkendall, L. R., P. H. W. Biedermann, and B. H. Jordal. 2015. "Evolution and Diversity of Bark and Ambrosia Beetles." In *Bark Beetles: Biology and Ecology of Native and Invasive Species*, edited by F. E. Vega and R. W. Hofstetter, 85–156. Cambridge, MA: Academic Press.

Kondrashov, A. S. 1994. "Muller's Ratchet under Epistatic Selection." *Genetics* 136:1469–1473.

Kooij, P. W., D. K. Aanen, M. Schiøtt, and J. J. Boomsma. 2015. "Evolutionarily Advanced Ant Farmers Rear Polyploid Fungal Crops." *Journal of Evolutionary Biology* 28:1911–1924.

Kooij, P. W., M. Poulsen, M. Schiøtt, and J. J. Boomsma. 2015. "Somatic Incompatibility and Genetic Structure of Fungal Crops in Sympatric *Atta colombica* and *Acromyrmex echinatior* Leaf-Cutting Ants." *Fungal Ecology* 18:10–17.

Korb, J., and D. K. Aanen. 2003. "The Evolution of Uniparental Transmission of Fungal Symbionts in Fungus-Growing Termites (Macrotermitinae)." *Behavioral Ecology and Sociobiology* 53:65–71.

Law, R. 1985. "Evolution in a Mutualistic Environment." In *The Biology of Mutualism: Ecology and Evolution*, edited by D. H. Boucher, 145–170. London: Croom Helm.

Lucock, M. D., C. E. Martin, Z. R. Yates, and M. Veysey. 2014. "Diet and Our Genetic Legacy in the Recent Anthropocene: A Darwinian Perspective to Nutritional Health." *Journal of Evidence-Based Complementary and Alternative Medicine* 19:68–83.

Marting, P. R., N. M. Kallman, W. T. Wcislo, and S. C. Pratt. 2018. "Ant-Plant Sociometry in the *Azteca-Cecropia* Mutualism." *Scientific Reports* 8:17968.

Mayers, C. G., D. L. McNew, T. C. Harrington, R. A. Roeper, S. W. Fraedrich, et al. 2015. "Three Genera in the Ceratocystidaceae Are the Respective Symbionts of Three Independent Lineages of Ambrosia Beetles with Large, Complex Mycangia." *Fungal Biology* 119:1075–1092.

McCutcheon, J. P., B. M. Boyd, and C. Dale. 2019. "The Life of an Insect Endosymbiont from the Cradle to the Grave." *Current Biology* 29:R485–R495.

Mehdiabadi, N. J., U. G. Mueller, S. G. Brady, A. G. Himler, and T. R. Schultz. 2012. "Symbiont Fidelity and the Origin of Species in Fungus-Growing Ants." *Nature Communications* 3:840.

Nobre, T., C. Fernandes, J. J. Boomsma, J. Korb, and D. K. Aanen. 2011. "Farming Termites Determine the Genetic Population Structure of Termitomyces Fungal Symbionts." *Molecular Ecology* 20(9): 2023–2033.

Nygaard, S., H. Hu, C. Li, M. Schiøtt, Z. Chen, et al. 2016. "Reciprocal Genomic Evolution in the Ant-Fungus Agricultural Symbiosis." *Nature Communications* 7:12233.

Otani, S., V. L. Challinor, N. B. Kreuzenbeck, S. Kildgaard, S. K. Christensen, et al. . 2019. "Disease-Free Monoculture Farming by Fungus-Growing Termites." *Scientific Reports* 9:8819.

Poulsen, M., and J. J. Boomsma. 2005. "Mutualistic Fungi Control Crop Diversity in Fungus-Growing Ants." *Science* 307(5710): 741–744.

Poulsen, M., H. Fernandez-Marin, C. R. Currie, and J. J. Boomsma. 2009. "Ephemeral Windows of Opportunity for Horizontal Transmission of Fungal Symbionts in Leaf-Cutting Ants." *Evolution* 63(9): 2235–2247.

Scott-Phillips, T. C., K. N. Laland, D. M. Shuker, T. E. Dickins, and S. A. West. 2014. "The Niche Construction Perspective: A Critical Appraisal." *Evolution* 68:1231–1243.

Shik, J. Z., P. W. Kooij, D. A. Donoso, J. C. Santos, E. B. Gomez, et al. 2020. "Nutritional Niches Reveal Fundamental Domestication Tradeoffs in Fungus-Farming Ants." *Nature Ecology and Evolution* 5:122–134.

Smith, B. D. 1998. *The Emergence of Agriculture*. New York: Scientific American Library.

Smith, S. M., D. S. Kent, J. J. Boomsma, and A. J. Stow. 2018. "Monogamous Sperm Storage and Permanent Worker Sterility in a Long-Lived Ambrosia Beetle." *Nature Ecology & Evolution* 2:1009–1018.

Tishkoff, S. A., F. A. Reed, A. Ranciaro, B. F. Voight, C. C. Babbitt, et al. 2006. "Convergent Adaptation of Human Lactase Persistence in Africa and Europe." *Nature* 39:31–40.

Van der Have, T. M., J. J. Boomsma, and S. B. J. Menken. 1988. "Sex-Investment Ratios and Relatedness in the Monogynous Ant *Lasius niger* (L)." *Evolution* 42(1): 160–172.

Villesen, P., T. Murakami, T. R. Schultz, and J. J. Boomsma. 2002. "Identifying the Transition between Single and Multiple Mating of Queens in Fungus-Growing Ants." *Proceedings of the Royal Society B—Biological Sciences* 269(1500): 1541–1548.

Ward, P. S., and M. G. Branstetter. 2017. "The Acacia Ants Revisited: Convergent Evolution and Biogeographic Context in an Iconic Ant/Plant Mutualism." *Proceedings of the Royal Society B—Biological Sciences* 284:20162569.

Wcislo, W. T. 2020. "Fijian Farming Ants Resolve the Guns-or-Butter Dilemma for Their Crop Plants." *Proceedings of the National Academy of Sciences* 117:3357–3359.

Wheeler, W. M. 1911. "The Ant-Colony as an Organism." *Journal of Morphology* 22:307–325.

Wyatt, G. A. K., S. A. West, and A. Gardner. 2013. "Can Natural Selection Favour Altruism between Species?" *Journal of Evolutionary Biology* 26:1854–1865.