



The evolution of social parasitism in *Formica* ants revealed by a global phylogeny

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Studying the behavioral and life history transitions from a cooperative, eusocial life history to exploitative social parasitism allows for deciphering the conditions under which changes in behavior and social organization lead to diversification. The Holarctic ant genus *Formica* is ideally suited for studying the evolution of social parasitism because half of its 172 species are confirmed or suspected social parasites, which includes all three major classes of social parasitism known in ants. However, the life history transitions associated with the evolution of social parasitism in this genus are largely unexplored. To test competing hypotheses regarding the origins and evolution of social parasitism, we reconstructed a global phylogeny of *Formica* ants. The genus originated in the Old World ~30 Ma ago and dispersed multiple times to the New World and back. Within *Formica*, obligate dependent colony-founding behavior arose once from a facultatively polygynous common ancestor practicing independent and facultative dependent colony foundation. Temporary social parasitism likely preceded or arose concurrently with obligate dependent colony founding, and dulotic social parasitism evolved once within the obligate dependent colony-founding clade. Permanent social parasitism evolved twice from temporary social parasitic ancestors that rarely practiced colony budding, demonstrating that obligate social parasitism can originate from a facultative parasitic background in socially polymorphic organisms. In contrast to permanently socially parasitic ants in other genera, the high parasite diversity in *Formica* likely originated via allopatric speciation, highlighting the diversity of convergent evolutionary trajectories resulting in nearly identical parasitic life history syndromes.

brood parasitism | dulosis | Emery's rule | Formicidae | inquilinism

The complex societies of eusocial insects are vulnerable to exploitation by social parasites that depend on their host colonies for survival and reproduction without contributing to colony maintenance and brood care (1–4). Social parasitism is common among eusocial Hymenoptera and evolved independently in distantly related lineages, including bees, wasps, and ants (3–8). Many studies on social parasitism have focused on the evolution of cooperation and conflict in colonies of eusocial insects and on coevolutionary arms race dynamics between hosts and parasites (9–12). However, the evolutionary origins of social parasitism and the coevolutionary factors causing speciation and thereby contributing to the high diversity of social parasite species in eusocial insects are not well understood (13, 14). Comparative evolutionary studies of social parasites are promising, because they are expected to provide insights into the conditions associated with a behavioral change from cooperative eusociality to exploitative social parasitism as well as into the consequences of the life history transitions on speciation and biological diversification.

Social parasitism is a life history strategy that evolved at least 60 times in ants, and more than 400 socially parasitic species are known from six distantly related subfamilies (4). Despite the high diversity, three main life history strategies can be recognized across social parasites: 1) temporary, 2) dulotic, and 3) permanent social parasitism (1, 3, 15–21). The queens of

temporary socially parasitic ant species invade the host nest and kill the resident queen(s), and the host workers raise the parasite's offspring (16). In the absence of an egg-laying host queen, the host workforce is gradually replaced until the colony is composed solely of the temporary social parasite species. The queens of dulotic social parasites start their colony life cycle as temporary social parasites, and once sufficient parasitic workers have been reared, they conduct well-organized raids of nearby host nests to capture their brood (22). Some brood is eaten, but most workers eclose in the parasite's nest and contribute to the workforce of the colony. By contrast, most permanent social parasite (i.e., inquiline) species are tolerant of the host queen, allowing her to continuously produce host workers, whereas the inquiline queens focus their reproductive effort on sexual offspring (1, 13). Inquilines obligately depend on their hosts and most inquiline species lost their worker caste entirely (1, 18, 19, 23).

The evolutionary origins of social parasitism have been debated since Darwin's *On the Origin of Species by Means of Natural Selection* (24). Entomologists have long noticed that ant social parasites and their hosts are close relatives (15, 16, 25–29), an observation subsequently referred to as “Emery's rule” (30). Strictly interpreted, Emery's rule postulates a sister group relationship between host and parasite, whereas a less restrictive or “loose” interpretation signifies for example a congeneric, but not

Significance

Identifying the conditions associated with a life history transition from cooperative colony life to exploitative social parasitism is important for understanding how changes in behavior contribute to speciation. To explore the evolutionary origins of social parasitism, we reconstructed the evolutionary history of *Formica* ants because half of all species are social parasites and all socially parasitic life history syndromes known from eusocial insects are represented in this genus. We demonstrate that social parasites evolved from an ancestor that lost the ability to establish new colonies independently and that highly specialized parasites can evolve from less complex social parasite syndromes. Our findings emphasize that social parasite syndromes readily originate in socially polymorphic organisms and evolved convergently across the ant phylogeny.

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Table 1. Diversity of social parasites in the genus *Formica* compared to all other ants

	Temporary social parasites (%)	Dulotic social parasites (%)	Permanent social parasites (%)	Total social parasite diversity (%)
All ants ($n = 13,861$)	200 (1.4)	80 (0.6)	100 (0.7)	>400 (2.9)
<i>Formica</i> ($n = 172$)	68 (39.5)	14 (8.1)	2 (1.2)	84 (48.8)

Socially parasitic life histories are significantly overrepresented in *Formica* ants, except for inquilineism. The total social parasite diversity in ants is higher than the sum of species in individual life history categories because the biology of numerous social parasites remains unknown. The data are derived from published sources (1, 3, 4, 55).

necessarily a sister taxon relationship (13, 31–33). Consequently, two competing hypotheses were developed for explaining the speciation mechanisms of social parasites: 1) The interspecific hypothesis proposes that host and social parasite evolved reproductive isolation in allopatry, whereas 2) the intraspecific hypothesis postulates that the social parasite evolved directly from its host in sympatry (3, 13, 18, 20, 21, 31–37). Empirical studies of temporary, dulotic, and host queen-intolerant workerless ant social parasites generally provide support for the interspecific hypothesis (14, 38–48), whereas recent phylogenetic studies lend support to the intraspecific hypothesis for queen-tolerant inquilines (33, 36, 49–51). In some cases, host shifts, secondary speciation events of hosts and/or parasites, and extinctions obscure the original evolutionary conditions under which social parasitism originated (52–54).

To explore the origin and evolution of diverse socially parasitic life histories in eusocial insects, we reconstructed the evolutionary history of the Holarctic ant genus *Formica*. *Formica* ants are ideally suited for comparative studies of social parasitism because the genus has the highest number of social parasite species in any ant genus (84 of 172; Table 1), and all socially parasitic life history traits known from eusocial insects evolved in *Formica* ants (Fig. 1 and Tables 1 and 2). In addition, colonies of *Formica* species vary significantly in colony-founding behavior as well as in nest and colony structures, providing an opportunity to explore the interplay between colony organization and life history at the origin of social parasitism. Some *Formica* species use independent colony foundation (ICF), when new colonies are started by a single queen (i.e., haplometrosis) or a group of coop-

erating queens (i.e., pleometrosis). Queens of other species rely on dependent colony founding (DCF), cooperating with groups of conspecific workers to found a new colony (i.e., budding) or invading an existing heterospecific colony as a temporary social parasite (TSP) or a permanent social parasite (PSP) (Table 2) (1, 56–62). In contrast to other studies (63), we regard TSP as a form of DCF because the socially parasitic queen relies on the social environment of the host for colony founding and rearing of the first brood. Furthermore, *Formica* colonies can have a single or multiple functional queens (monogyny vs. polygyny) and comprise one (monodomous) or multiple (polydomous) to thousands of interconnected physical nests covering a large area (supercolonial) (64).

To infer the evolutionary origins of social parasitism and explore the behavioral transition from a social colony life to a socially parasitic life history, we reconstructed a global phylogeny for *Formica* ants and relevant outgroups from the formicine genera *Iberoformica*, *Polyergus*, *Proformica*, and *Rossomyrmex*, thus spanning the root node of the tribe Formicini (65). The comprehensive, time-calibrated phylogeny allows for 1) testing competing hypotheses regarding the origins and evolutionary transitions of social parasitism, 2) reconstructing the evolutionary and biogeographic history of the group, and 3) suggesting modifications to the internal classification of the genus.

Results and Discussion

***Formica* Originated in Eurasia during the Oligocene.** To infer the life history evolution of the diverse, Holarctic genus *Formica*, we inferred a comprehensive phylogeny for 101 *Formica* species



Fig. 1. Diversity of life history traits in the formicine ants. In clockwise direction: (A) members of the *F. fusca* group practicing independent colony founding; (B) *F. obscuripes*, representing the *Formica integra* group (Nearctic members of the paraphyletic “*rufa*” group), which practices dependent and temporary social parasitic colony founding; (C) *Formica gynocrates*, representing the facultatively dulotic species of the *F. sanguinea* group, with a worker of its *neogagates* group host species, *Formica vinculans*; (D) the highly modified worker of *Polyergus mexicanus*, representing the obligately dulotic formicine ants in the genera *Polyergus* and *Rossomyrmex*. All images courtesy of Alex Wild (www.alexanderwild.com).

Table 2. Diversity, taxonomy, life history, and evolutionary traits of *Formica* ants across currently recognized species groups, as well as of closely related formicine ants

<i>Formica</i> species group or genus	No. of described of species	Estimated no. of new species	Colony-founding behavior	Colony organization	Nest organization	Socially parasitic life history	Phylogenetic information	Geographic distribution
<i>F. dakotensis</i> gr.	2	Unknown	TSP; budding at low frequency	Monogynous, polygynous	Monodomous, polydomous	TSP, PSP(?)	Monophyletic	Nearctic
<i>F. difficilis</i> gr.	16	5 to 10	TSP; budding at low frequency	Monogynous, polygynous	Monodomous	TSP, PSP	Monophyletic	Nearctic
<i>F. exsecta</i> gr.	17	Unknown	TSP; budding at high frequency	Monogynous, polygynous	Monodomous, polydomous, supercolonial	TSP	Monophyletic	Nearctic and Palearctic
" <i>F. fusca</i> gr."	76	1 to 15	Haplo- and pleometrosis; budding rare, if present in a species, at low frequency	Monogynous, polygynous	Monodomous, polydomous, rarely supercolonial	Not socially parasitic	Paraphyletic	Nearctic and Palearctic
<i>F. integra</i> gr.	20	2 to 3	TSP; budding at low frequency	Monogynous, polygynous	Monodomous, polydomous, rarely supercolonial	TSP	Monophyletic	Nearctic
" <i>F. neogagates</i> gr."	8	2 to 3	Haplometrosis	Monogynous, polygynous	Monodomous	Not socially parasitic	Paraphyletic	Nearctic
<i>F. pallidefulva</i> gr.	5	Unknown	Haplo- and pleometrosis	Monogynous, polygynous	Monodomous	Not socially parasitic	Monophyletic	Nearctic
<i>F. rufa</i> gr.	13	None	TSP; budding at high frequency	Monogynous, polygynous	Monodomous, polydomous, supercolonial	TSP	Monophyletic	Paleartic
<i>F. sanguinea</i> gr.	14	3 to 5	TSP	Monogynous, polygynous	Monodomous, rarely polydomous	TSP, facultative and obligate dulosis	Monophyletic	Nearctic and Palearctic
<i>F. uralensis</i> gr.	1	None	TSP; budding at high frequency	Monogynous, polygynous	Monodomous, polydomous, supercolonial	TSP	Monotypic	Paleartic
<i>Iberoformica</i>	1	None	Haplometrosis	Monogynous	Monodomous	Not socially parasitic	Monotypic	Paleartic
<i>Polyergus</i>	14	Unknown	TSP	Monogynous	Monodomous	Obligat TSP, dulosis	Monophyletic	Nearctic and Palearctic

The former *F. rufa* group is divided into three clades, i.e., the *dakotensis*, *integra*, and *rufa* groups. The erstwhile *microgyna* group is properly referred to as the *difficilis* group based on name priority. Please refer to *SI Appendix, Table S2* for a detailed list of traits for individual species and references to original research. Total number of *Formica* species does not add to 172 because of one valid, poorly described species (*Formica graveleyi*) of uncertain group affinity.

representing all 10 currently recognized species groups (Table 2) across their wide geographic distribution in both the Old World (19 spp.) and the New World (82 spp.) and outgroups, using 2,242 ultraconserved element (UCE) loci per taxon. Our analyses recovered *Formica* as a strongly supported clade with the monotypic genus *Iberoformica* as its sister lineage (Fig. 2 and *SI Appendix, Fig. S1*). *Formica* and *Iberoformica* split from their sister genus *Polyergus* around 33 Ma ago (95% highest posterior density [HPD]: 27 to 39 Ma) and *Formica* diverged from a common ancestor with its sister lineage *Iberoformica subrufa* ~30 Ma ago (95% HPD: 24 to 35 Ma). The crown group age of extant *Formica* ants is ~26 Ma (95% HPD: 21 to 31 Ma). Therefore, modern *Formica* ants evolved recently and likely originated in Eurasia during the Oligocene after the global cooling following the Terminal Eocene Event (66). A similar evolutionary history was inferred for the species-rich Holarctic ant genus *Myrmica* (49).

Independent Colony Founding and Social Polymorphism Are Ancestral. To understand the evolution of social parasitism in *Formica* ants, it is necessary to recover the evolutionary origins of different life history strategies in the biologically diverse species groups. Character state reconstructions of life history traits

including nest structure, social organization of colonies, and colony-founding behaviors (Table 2 and *SI Appendix, Figs. S3–S5*) based on our phylogenomic tree (Fig. 2) show that facultative polygyny and polydomy originated early during *Formica* evolution. Purely monogynous species groups are found only outside of *Formica* (Table 2). Within *Formica*, the deepest node marks the divergence between the independent colony-founding species in the "*neogagates*" and *pallidefulva* groups plus *Formica gagates* on one side and the independently colony-founding species in the paraphyletic grade of the *Formica* "*fusca* group" on the other side of the bifurcation (Fig. 2). Accordingly, facultative polygyny, independent colony founding, and polydomy are ancestral traits that were likely present in the most recent common ancestor (MRCA) of all extant *Formica* species. The *fusca* group arises as a paraphyletic grade nested between these early diverging lineages and all other *Formica* species and consists of at least five monophyletic groups (Fig. 2). Facultative DCF via budding has been documented in certain species of the *fusca* group (*SI Appendix, Table S2*). The nesting behavior transitioned repeatedly between constructing monodomous, polydomous, and even supercolonial nests in the *fusca* group, which has important implications for population structure and population density of those species.

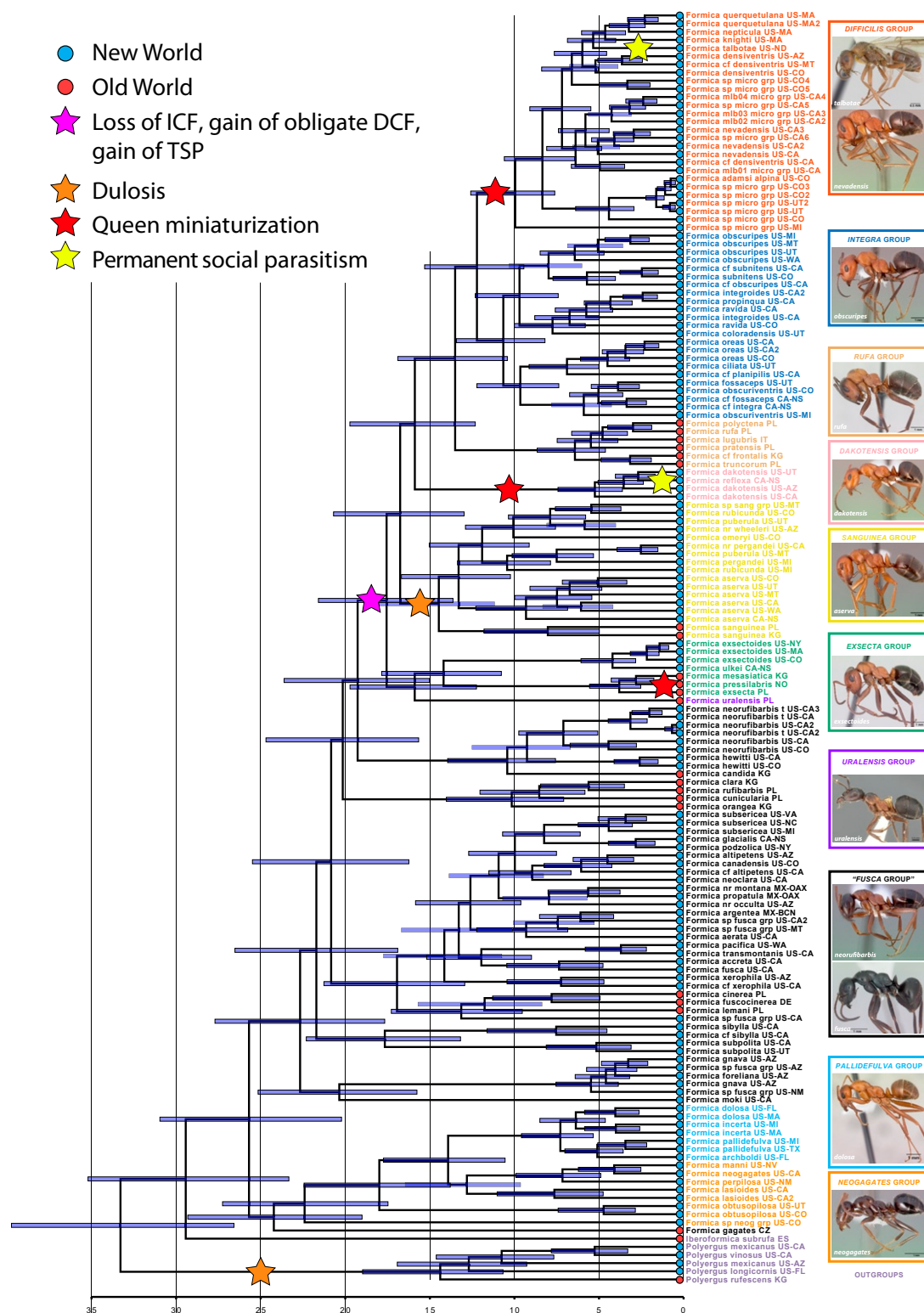


Fig. 2. A time-calibrated molecular phylogeny of *Formica*, *Iberoformica*, and *Polyergus*. Node bars indicate 95% highest posterior density. Scale is in millions of years. Abbreviations after taxa names indicate sample origin. Country codes follow the International Organization for Standardization (ISO) 3166: United States (US), Canada (CA), and Mexico (MX), in addition to noting state/province. Note single loss of independent colony foundation and gain of obligate DCF with inquilinism arising from within temporary parasites (yellow star). Taxon highlight colors signify species group membership; *neogagates* and *fusca* species groups are not monophyletic. Photographs are by April Nobile, Erin Prado, and Estella Ortega. Courtesy of <https://www.antweb.org/>.

ICF Was Lost Once, Leading to Obligate DCF. In *Formica*, obligate dependent colony founding evolved ~18 Ma ago (95% HPD: 14 to 21 Ma) (Fig. 2) when an ancestor practicing both

independent and facultative dependent colony founding via budding, as observed in some *fusca* group species, lost the capacity for independent founding. This loss was precipitated by or

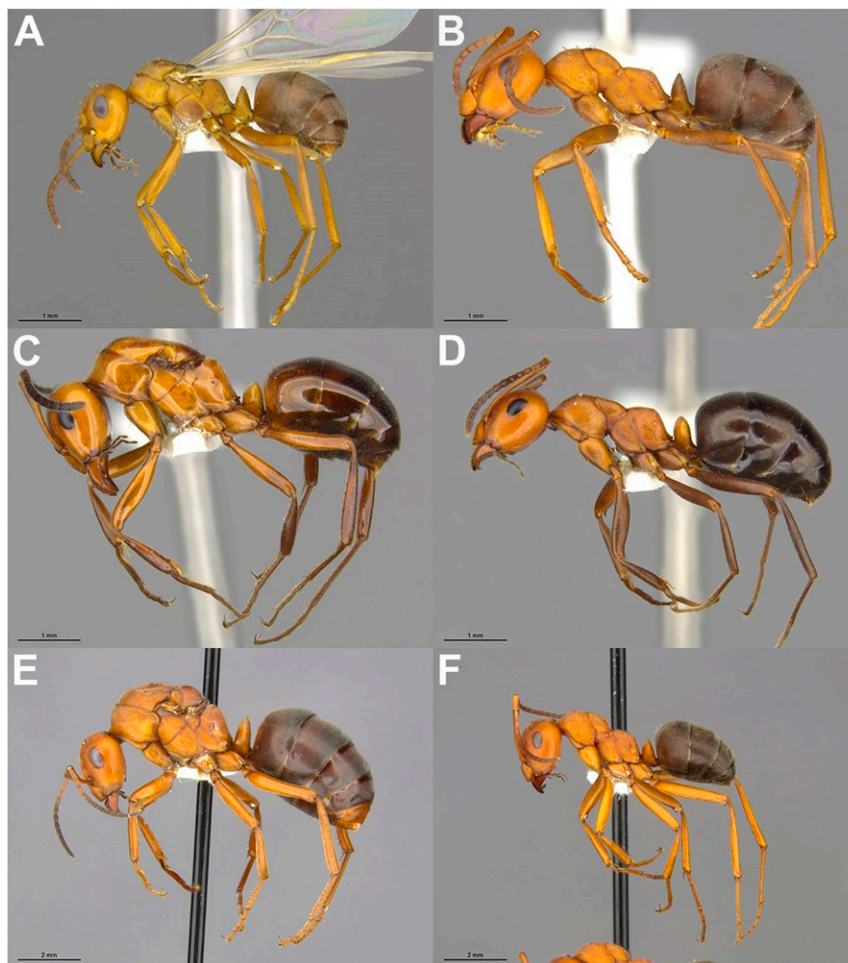


Fig. 3. Convergent evolution of queen miniaturization in temporary social parasitic *Formica* ants. Queen miniaturization evolved in the *F. difficilis* (A and B) and *dakotensis* (C and D) species groups. In comparison, queens in the *integra* group (E and F) show a pronounced queen–worker dimorphism typical for *Formica* ants. (A and B) Queen (A) and worker (B) of a hitherto undescribed *F. difficilis* group species showing one of the most extreme cases of queen size reduction known in ants. (C and D) Queen (C) and worker (D) of *F. reflexa* representing a second, independent evolutionary origin of queen miniaturization, which is less extreme than in the *difficilis* group species. (E and F) Queen (E) and worker (F) of *Formica ravidia* demonstrating a typically sized queen with morphological modifications related to wing bearing and reproduction that are absent from the worker. Note that the *F. ravidia* individuals are significantly larger than all other ants depicted here. (Scale bars, 1 mm in A–D and 2 mm in E and F.) Specimen identifiers are as follows: (A) MCZ 574034, (B) MCZ 574022, (C) MCZ 525288, (D) MCZ 525283, (E) MCZ 552096, and (F) MCZ 575163. Photographs are by Patrick McCormack. Images copyright President and Fellows of Harvard University.

coincided with using heterospecific workers for dependent colony founding (TSP), which likely evolved from budding, a strategy involving the use of conspecific workers for colony founding. This is suggested by the fact that there are no confirmed obligate dependent founding species that practice budding only, without the capacity for TSP.

The clade with obligate DCF and capacity for TSP includes species of the *exsecta*, *sanguinea*, *dakotensis*, *rufa*, *integra*, and *difficilis* groups, as well as *Formica uralensis*, a species of uncertain taxonomic affiliation that is here inferred to be the sister lineage to the *exsecta* group. Recently, Romiguier et al. (48) also recovered a single loss of ICF among Palearctic *Formica* species including representatives of 4 of the 10 species groups. Our global phylogenetic analysis confirms and significantly expands on this earlier conclusion and adds a temporal scale showing that the MRCA of this clade lived around 18 Ma ago. Furthermore, our analysis reveals that clades of socially parasitic species have secondarily transitioned to other parasitic life histories. Evolutionary reversals from social parasitism to independent colony founding were not recovered, suggesting that a transition to a socially parasitic lifestyle is irreversible. A similar pattern

was found in the ant genus *Lasius*, where temporary parasitism evolved twice but reversals to ICF are unknown (40).

Among the TSP species, two ecologically distinct life histories can be recognized. First, species in the *dakotensis*, *difficilis*, and *integra* groups are predominantly facultative temporary social parasites and practice colony budding at low frequency. Newly mated queens are unable to found new colonies independently via haplo- or pleometrosis, but instead, they seek adoption in heterospecific host colonies or readoption into conspecific colonies leading to secondary polygyny. In these species, colony budding seems to occur occasionally, which results in a characteristic population structure with smaller clusters of nests (usually less than five), whereas large, unicolonial populations are absent. In contrast, species in the *exsecta* and *rufa* groups are facultative temporary social parasites practicing colony budding at high frequency, which can result in highly polydomous and/or supercolonial populations (Fig. 2 and Table 2 and *SI Appendix, Table S2*).

The temporary social parasite species in the *Formica difficilis*, *integra*, *rufa*, and *dakotensis* groups shared a common ancestor ~16 Ma ago (95% HPD: 13 to 19 Ma), and they constitute the

sister group to the dulotic *sanguinea* group (Fig. 2). The *difficilis* group is also monophyletic and is sister to the *integra* group (Fig. 2). All species in the *difficilis* group have miniature queens not larger than their largest workers (Fig. 3 A and B), which is likely associated with the socially parasitic life history. For the *difficilis* group, we infer a single origin of queen miniaturization ~10 Ma ago (95% HPD: 8 to 12 Ma). It is important to note that most *difficilis* group species are rare and our knowledge about their biology is fragmentary at best. Therefore, the temporary social parasitic behavior remains to be observed for most species. However, the few existing direct observations on nest founding behavior, which include *F. difficilis* (16), *Formica densiventris* (67), *Formica impexa* (67), *Formica adamsi alpina* (S.P.C., personal observation), and *Formica* new species (S.P.C., personal observation), confirm temporary social parasitism.

Interestingly, queen miniaturization evolved repeatedly in *Formica*, including in the *exsecta* group, where it is present in several Palearctic species (68), and in the Nearctic *dakotensis* group. Because our sampling includes only one of the Palearctic *exsecta* group species with miniature queens (*Formica pressilabris*), we cannot ascertain whether miniaturization evolved once or multiple times in this group. In the temporary social parasite species of the Nearctic *Formica dakotensis* clade (Fig. 3), both species (*F. dakotensis* and *F. reflexa*) have small queens. *Formica dakotensis* is a facultative temporary social parasite (55) and fully independent colonies are common. In contrast, *Formica reflexa* is rare and was found only in association with *fusca* group host workers (69, 70) (S.P.C., personal observation), suggesting a unique life history including parasitic colony founding and potentially a life-long dependence on the host. Queen size reduction is frequently observed in inquiline social parasites (13, 71), but the independent origins of miniature queens in the *difficilis*, *dakotensis*, and *exsecta* groups imply that queen size reduction is adaptive for a temporary social parasitic life history syndrome in *Formica* ants.

Evolution of Dulosis. The dulotic species of the *Formica sanguinea* group are monophyletic (Fig. 2), suggesting that dulotic behavior evolved once some time prior to its inferred crown group age of ~14 Ma (95% HPD: 11 to 18 Ma). Thus, dulotic behavior and temporary social parasitism did not evolve simultaneously in *Formica*, but instead dulosis evolved secondarily from a temporary socially parasitic ancestor. The single origin of dulotic behavior in a diverse clade of temporary social parasite species supports the hypothesis that dulosis originates only under rare circumstances (3, 4, 22). In fact, the evolutionary origins of dulotic behavior in ants have been debated since Darwin's *On the Origin of Species by Means of Natural Selection* (24) and three not mutually exclusive hypotheses have been proposed to explain the origins of this highly specialized behavior: 1) predation, 2) brood transport, and 3) territorial competition (1, 3, 20, 22, 24, 35, 72–76).

Our phylogenetic results and behavioral observations indicate that the predatory behavior of temporary social parasites could lead to the evolution of facultative dulosis in *Formica*. Brood stealing would be favored by natural selection if the aid of heterospecific workers increased the parasite's fitness, although we are not aware of experimental studies demonstrating fitness benefits provided by stolen host workers to facultatively dulotic species. Additional biological factors that were associated with the evolutionary origins of dulosis, including polygyny, polydomy, brood transport, and territoriality (17, 22, 35, 77), can also be inferred for the common ancestor of the dulotic species in the *F. sanguinea* group.

It is important to note that dulosis evolved convergently and under different ecological conditions in distantly related, non-predatory ants, such as the omnivorous, scavenging species in the genera *Temnothorax* and *Tetramorium* (12, 41, 42, 44, 46, 78). This pattern suggests that alternative factors, such as territori-

ality and brood transport, likely play an important role in the origin of dulotic behavior in nonpredatory ants. Across the ant tree of life, dulosis originated at least nine times convergently in distantly related clades (22, 47, 79), including three origins in the Formicini (65) and six origins in the Crematogastrini (41, 45, 46).

Evolution of Inquiline Social Parasitism. The only confirmed workerless inquiline social parasite in the genus *Formica* is *Formica talbotae* (80, 81). *Formica talbotae* is phylogenetically nested within the *difficilis* clade (Fig. 2), suggesting that workerless permanent parasitism evolved once from a facultatively polygynous ancestor practicing temporary social parasitism. This is empirical evidence for an evolutionary transition from temporary to workerless inquiline social parasitism, a hypothesis earlier suggested by Wilson (18). *Formica dirksi* has also been repeatedly suggested to be a workerless social parasite of *Formica subaenescens* in Maine (3, 82, 83), but there are no natural history data substantiating this claim.

Formica talbotae is a distant relative of its *integra* group host, *Formica obscuripes*, with which it shared a common ancestor ~12 Ma ago (95% HPD: 10 to 15 Ma). The host–parasite relationship of *F. talbotae* and *F. obscuripes* is consistent with the “loose” interpretation of Emery's rule, where hosts and parasites can be congeners but not sister lineages, suggesting that *F. talbotae* evolved via the interspecific, allopatric route of social parasite evolution. This result contrasts with previous studies inferring workerless inquiline social parasites as directly evolving from free-living, closely related ancestors via the intraspecific, sympatric route of social parasite speciation (33, 36, 49, 50). However, and in contrast to many host queen tolerant inquiline social parasites, *F. talbotae* was found exclusively in queenless host colonies. It seems unlikely that the miniature *F. talbotae* queens assassinate the many and much larger host queens. Instead, it appears more likely that *F. talbotae* specializes on declining host colonies that lost their reproductive queen(s) (80, 81). Preferentially inhabiting queenless host colonies is a highly specialized and rare behavior that was described only for few social parasite species (84–87). It is important to note that the phylogenetic placement of *F. talbotae* was not unequivocal in our analysis, but it is important for understanding the evolution of workerless inquilinism in *Formica* ants. The museum specimens of *F. talbotae* available to us were collected in the 1950s, and from these specimens we recovered only fragments of 496 UCE loci (~8% of nucleotides in the full data matrix). Coalescent-based species tree estimation is known to suffer from missing data (88), and to test for incongruencies, we performed coalescent-based species tree estimations (89–92) using a reduced data matrix that included only the 67 loci for which at least 50% of the *F. talbotae* sequences were present. This analysis recovered *F. talbotae* as the sister lineage to the *difficilis* group (*SI Appendix*, Fig. S8), and statistical support was low across the species tree. The phylogenetic position of *F. talbotae* differs from the nested position obtained in concatenation (Fig. 2 and *SI Appendix*, Fig. S1), but the topology is more similar to the concatenated tree than the species tree analysis of the full dataset (*SI Appendix*, Fig. S6), suggesting that missing data do have a negative impact on the species tree analysis. A quartet sampling analysis (93) revealed topological conflict at the same nodes where the concatenated tree differed from the species tree, but strongly supported monophyly of the *difficilis* group including *F. talbotae* nested within (*SI Appendix*, Fig. S9).

In agreement with morphological evidence, which places *F. talbotae* within the *difficilis* group (80, 81), we also consider *F. talbotae* a member of the *difficilis* group. We interpret the placement outside of the *difficilis* group by species tree methods as an artifact caused by missing data.

Historical Biogeography. To infer the biogeographic conditions under which social parasitism evolved in the Formicini, we

conducted a historic biogeography analysis inferring repeated dispersal between the Old and the New World in the genera *Formica*, *Iberoformica*, and *Polyergus* (Fig. 4 and *SI Appendix*, Fig. S2). According to our biogeographical stochastic mapping analysis (94), at least 8 and more likely 9 or 10 such dispersal events occurred in this genus group (Fig. 4). All other genera classified in the Formicini are confined to the Old World. Therefore, early stem lineages of the genus *Formica* almost certainly lived in the Old World and subsequently started dispersing into the New World and back, likely via Beringia, which connected Eurasia and the Nearctic throughout most of the Paleogene (95). Recent trans-Beringian dispersal was also demonstrated for several Holarctic ant species, which include *Formica gagateoides* (96).

Nine fossil species of *Formica* are known from Eocene amber inclusions of Europe (47.8 to 33.9 Ma) (97–99). Several fossils have been compared to extant species in the *fusca* and *rufa* groups (100), and close examination suggests that this resemblance may be superficial (101, 102), which raises the question of whether the Eocene amber fossils indeed represent crown group *Formica*. Doubts about the correct identification of putative *Formica* fossils in Baltic amber currently limit the utility of Eocene amber fossils for calibrating divergence analyses in the genus.

Implications for *Formica* Taxonomy and Classification. The internal classification of species-rich genera, such as *Formica*, is important because the affiliation with a species group (or subgenus) can provide first clues regarding the life history and general biology of an unknown species. Of the 10 presently recognized *Formica* species groups (Table 2), the *pallidefulva* (= *Neoformica*), *exsecta*, *sanguinea* (= *Raptiformica*), and *difficilis* groups were recovered as monophyletic. In contrast, species traditionally classified in three species groups (or subgenera), the *fusca* (= *Serviformica*), *neogagates* (= *Proformica*), and *rufa* (= *Formica* s. str.) groups, form nonmonophyletic assemblages. The Palearctic species *F. gagateoides*, traditionally placed in the *fusca* species group, was inferred as the sister species to the Nearctic

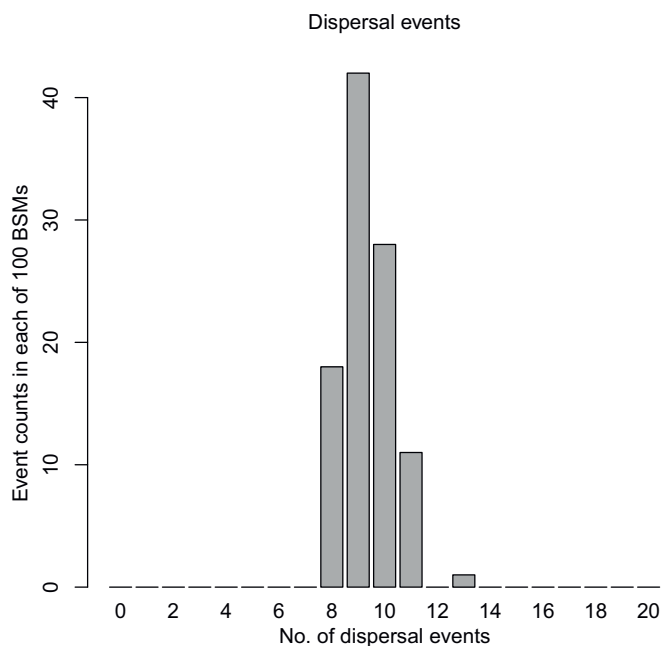


Fig. 4. Inferred dispersal events between the Old and the New World in *Formica*, *Iberoformica*, and *Polyergus* ants. Shown is a histogram of dispersal event counts from 100 biogeographic stochastic maps under the DEC+J model in BioGeoBEARS.

clade comprising the *neogagates* and *pallidefulva* groups. The *neogagates* group itself forms a paraphyletic grade outside the *pallidefulva* group. The Holarctic *exsecta* group plus the problematic *F. uralensis*, placed in a monotypic group, are sisters to all other dependent colony-founding/temporary social parasite groups, instead of *F. uralensis* being part of either the *fusca* or the *rufa* group, as previously suggested (103, 104). The rest of species traditionally classified in the *fusca* group form a grade consisting of five clades progressively more closely related to the dependent colony-founding clade. Although our sampling did not include *Formica fusca*, prior work shows that it would be placed within the Palearctic clade containing *Formica cinerea*, *Formica fuscocinerea*, and *Formica lemmani* (61). More thorough taxon sampling and a careful morphological study are necessary for a stable classification of those species. The Nearctic *Formica obtusopilosa* is closely related to *Formica neogagates* and not a member of the *sanguinea* group (105, 106), as treated by some authors (107, 108). This classification is consistent with the biology of *F. obtusopilosa*, which is not dulotic and its queens found colonies independently, like other species in the *neogagates* group (105, 107) (S.P.C., personal observation). Hence, the clypeal notch, long thought to be diagnostic of *sanguinea* group species, evolved convergently in *F. obtusopilosa*. The traditional *rufa* group is recovered as paraphyletic because the Old World *rufa* group species (now the true *rufa* species group) form a clade that is sister to the Nearctic *integra* and *difficilis* groups, and the distinctive *F. dakotensis* and *F. reflexa* form a clade (here called the *dakotensis* group) sister to *rufa*, *integra*, and *difficilis* groups. Finally, according to custom and for consistency we refer to the erstwhile *microgyna* group as the *difficilis* group, after the oldest constituent species name. The *difficilis* group species are monophyletic and sister to the *integra* group, not nested within it as previously suggested (109).

Considering that all the traditional *Formica* subgenera are nested within *Formica* and that three of the four subgenera are paraphyletic, we suggest discontinuing the use of the subgeneric names and using species group names instead. While these results clarify the internal structure within the genus, much work remains to be done on the species level. The *fusca*, *integra*, *difficilis*, Nearctic *sanguinea*, and *neogagates* groups all need taxonomic revisions.

Conclusions

Our study provides a robust phylogenetic framework for studying the evolution of the diverse and ecologically important Holarctic ant genus *Formica* and allows for testing competing hypotheses regarding the origins and evolution of social parasitism in ants. We conclude that in the formicine genera *Formica*, *Polyergus*, and *Rossomyrmex*, social parasitism originated repeatedly and convergently. In the genus *Formica*, multiple transitions to increasingly more complex socially parasitic life histories evolved. First, the capacity for occasional DCF via budding evolved in facultatively polygynous species practicing ICF. Eventually the ability for ICF was lost in the ancestor of what is now a large clade of obligate dependent colony-founding species (clade marked by a purple star in Fig. 2). Temporary social parasitism either coincided with or preceded this loss of ICF and the transition to obligate DCF. Because all species of the obligate DCF clade appear capable of TSP, it is likely that the evolution of TSP precipitated the loss of ICF. Within this obligate DCF clade dulosis evolved once in the ancestor of the *sanguinea* group. Finally, the permanent social parasites, *F. reflexa* and the workerless *F. talbotae*, evolved independently from temporary social parasitic ancestors. Across species, *Formica* social parasites likely originated via the interspecific, allopatric speciation route of social parasite evolution, emphasizing that convergent evolutionary trajectories can lead to highly similar parasitic life history syndromes across eusocial insects.

The inferred sequence for the evolution of dulosis lends empirical support to Charles Darwin's "predation hypothesis" for the origin of dulotic behavior in ants. Furthermore, our results suggest that the ancestor of the dulotic *Formica* species likely possessed all the traits associated with the evolution of dulotic behavior, namely territorial and predatory behavior, brood transport behavior among spatially distinct nests of polygynous colonies, and the capacity for parasitic and dependent colony founding. The origin of dulosis was then followed by secondary diversification into 14 species that today form the monophyletic *sanguinea* group.

Our study inferred the workerless social parasite *F. talbotae* as arising from a clade of temporary social parasites in the *difficilis* group, providing empirical evidence for a transition from temporary to workerless social parasitism. The example of *F. talbotae* underscores the importance of distinguishing between the different life history traits summarized under the umbrella term "inquiline social parasitism." Taking the evolutionary origins into account is important because the majority of queen-tolerant workerless inquiline parasites likely speciated directly from free-living ancestors, whereas most queen-intolerant workerless social parasites apparently transitioned to the workerless state from a dulotic or temporary social parasitic ancestor. *Formica talbotae* can be regarded as a queen-intolerant obligate temporary social parasite that preferentially inhabits queenless host colonies and secondarily lost its worker caste. The distant relatedness to its host, *F. obscuripes*, and its distinct life history traits suggest that *F. talbotae* also evolved via the allopatric, interspecific route of social parasite evolution, contrasting with the sympatric origins of some queen-tolerant inquiline social parasites.

We show that *Formica* evolved during the early Oligocene, representing a relatively young ant genus that diversified rapidly into a diverse, ecologically dominant group. During its evolutionary history *Formica* ants dispersed several times between the Old and the New World.

Our study outlines the life history changes associated with the transition from a cooperative eusocial to exploitative socially parasitic life history. Given the high diversity of social parasite species in the genus *Formica*, and considering the high degree of morphological and behavioral specialization, socially parasitic *Formica* species appear to be an ideally suited study organism for investigating caste determination and for exploring the genetic basis underlying behavioral and life history evolution.

Materials and Methods

Taxon Sampling. We newly sequenced 101 ingroup morphospecies from all 10 species groups of *Formica* ants that were recognized prior to our study and 8 outgroup species. Collection data associated with sequenced samples can be found in *SI Appendix, Table S1* and detailed voucher information is on Zenodo.

Molecular Data Generation. To obtain the genetic data we extracted DNA, prepared genomic libraries, and then enriched them using 9,446 custom-designed probes targeting 2,524 UCE loci in Hymenoptera (110). We submitted the enriched libraries to the University of Utah High Throughput Genomics Core Facility for sequencing on two Illumina HiSeq 125 Cycle Paired-End Sequencing v4 runs.

Data Processing. We processed the resulting reads using the Phyluce bioinformatics pipeline (111). Following alignment and trimming, we retained only individual locus alignments that had 110 or more taxa (70% of total), resulting in 2,242 loci on average 667 nt long. The resulting concatenated matrix was 1,497,044 nt long and contained 17.58% of missing data and gaps.

Phylogenetics. To infer the maximum-likelihood phylogeny, we used ModelFinder (112) as implemented in IQ-TREE (113) to select the best model for each UCE locus under the Akaike information criterion (AICc). These models were then used for by-locus partitioned analysis of the concatenated data matrix (114). To assess the robustness of this result to different analytics we performed an unpartitioned analysis and a quartet sampling analysis (93). In addition to concatenated analyses we performed coalescent-based species tree estimation using ASTRAL-III (92).

Divergence Time Analyses. For divergence time analyses we used a node dating approach, as implemented in MCMCTree, a part of the PAML package, v4.9e (115). We constrained our root node with soft bounds around a conservative maximum age estimate of 79 Ma, which corresponds to the lower bound of the 95% highest posterior density interval for that split in a previous phylogenomic study (65).

Biogeography. For biogeographic inference we used BioGeoBEARS (116). We discretized the distribution of *Formica* species into two regions, the New World and the Old World. We used 100 replicates of biogeographical stochastic maps (94) to estimate the number of times *Formica* dispersed between the Old and the New World.

Ancestral State Reconstruction. To investigate the evolution of nest structure, colony structure, and mode of colony foundation, we used stochastic character mapping (117) as implemented in the R package Phytools (118). We compared and selected best-fitting models of character evolution using GEIGER (119) with a time-calibrated tree pruned from distant outgroups and intraspecific samples as input. We based our character coding for each species (*SI Appendix, Table S2*) on literature records and 80 y of cumulative field research by ourselves and colleagues.

Data Availability. For detailed methods, see *SI Appendix*. Reads generated for this study are available at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (BioProject ID [PRJNA749764](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA749764)). Other files used in analyses are available on Zenodo (DOI: [10.5281/zenodo.4341310](https://doi.org/10.5281/zenodo.4341310)) (120).

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