Review Article

Title

Speciation in ants: Unlocking ant diversity to study speciation (Hymenoptera: Formicidae)

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Abstract

Ants are among the most abundant terrestrial animals and play key roles in ecosystems across the globe. Their taxonomic and ecological diversity makes them a prime taxon for contributing to our understanding of the patterns and processes of speciation. However, studies on ants, such as those on ant diversification or taxonomy, often do not explicitly consider how their results inform or update the broader framework of speciation and reproductive isolation. Yet the wealth of taxonomic, biogeographical, behavioral, and genomic studies on ants could significantly contribute to our understanding of both micro and macroevolutionary patterns of speciation. Here, we combine microevolutionary studies on reproductive isolation and hybridization with macroevolutionary work on ant diversification to review what is known about ant speciation. We also discuss how two key features, sociality and haplodiploidy, could impact speciation and hybridization in ants. We find that, although key innovations as drivers of ant diversification have been studied, gaps exist in our understanding of reproductive isolating mechanisms in ants. However, general population features or traits, such as co-evolution within mutualistic or parasitic relationships, suggest that reproductive isolating mechanisms studied in ants may be generalizable across the tree of life. We suggest that ants could serve as valuable systems to study open questions in speciation, especially hybridization and divergence with gene flow, the genomic basis of intrinsic postzygotic isolation, and species interactions as drivers of reproductive isolation between species.

Introduction

Ants and speciation

With a conservative estimate of 20 quadrillion individuals and a total biomass of 12 megatons of dry carbon, exceeding that of wild birds and mammals, ants (Hymenoptera: Formicidae) are among the most abundant terrestrial animal families on Earth (SCHULTHEISS & al. 2022). Ants started diversifying from their wasp-like ancestors in the late Cretaceous, roughly 100 Mya, coupled with the proliferation of angiosperm forests and accompanying herbivores (MOREAU & al. 2006). Today, they play a central role in land ecosystems (HÖLLDOBLER & WILSON 1990, PARKER & KRONAUER 2021). Some ant lineages, such as wood ants, army ants, and harvester ants, are particularly important ecosystem service providers, conducting tasks such as bioturbation (i.e., reworking of soils and sediments), nutrient cycling (e.g., decomposition of organic matter), and seed dispersal (HANDEL & BEATTIE 1990, PARR & al. 2016). Moreover, some ant species shape the surrounding invertebrate and plant communities through predation and symbiotic relationships (PARKER & KRONAUER 2021). In addition to being an ecologically important group, ants are a relatively diverse group, with over 14,000 known species worldwide (BOLTON 2025), with many more still undetected or undescribed. In addition to this taxonomic diversity, ants have acquired an essentially global distribution, inhabiting nearly every biome on earth (Hölldobler & Wilson 1990). This species and geographic diversity establish ants as a prime target for taxonomic studies, naturally tying into questions of speciation. Interestingly, although ants are the focus of numerous taxonomic studies, there has been less, yet growing, emphasis on using ants as a system to understand questions about speciation (Fig. 1). Here we review what is known about the processes of speciation in ants and some of their unique features, and how this group can provide answers to open questions in the field of speciation.

Speciation as a field studies the processes that generate biological discontinuity, that is, what leads to the emergence and persistence of new species. There are several current open questions in speciation research, including 1) How do the different components of reproductive isolation (RI) evolve, 2) What is the identity and genomic distribution of barrier loci (i.e., loci that restrict gene flow between divergent lineages), 3) What are the mechanisms and rates of species persistence after divergence, and 4) How do speciation processes at the microevolutionary level connect to patterns observed at a macroevolutionary scale. Here we present these ideas from a speciation perspective as a brief introduction, and reserve further discussion of how they apply to ants for later sections.

Ever since Dobzhansky (1937), RI has played a central role in speciation research. In order to fully understand speciation, one needs to understand how RI evolves (Stankowski & Ravinet 2021, Westram & al. 2022). RI can be defined qualitatively or quantitatively. Qualitatively, mechanisms of RI are characteristics of species that reduce or prevent successful reproduction with members of other species (Kulmuni & al. 2020a); while quantitatively, RI is the reduction in gene flow between populations due to genetic differences (Westram & al. 2022). To understand how a process such as local adaptation contributes to RI and species divergence, one can test species fitness on new hosts in a transplant experiment (see Via & al. 2000) or measure the reduction in gene flow between differently adapted populations either experimentally or through simulations (see Lackey & Boughman 2017, Anderson & Weir 2022). Similarly, if strong RI between species or populations is observed, we can ask what mechanisms are reducing RI, and if they are sufficient to explain the observed strength of RI. Westram & al. (2022) argue that knowledge of both genetic and ecological processes is needed to understand speciation and the evolution of RI. Furthermore, multiple taxon pairs are needed to account for differing evolutionary histories. This is an admittedly difficult task, but quantifying the contribution of different RI components across taxa provides a way to compare and relate common isolating mechanisms.

The buildup of RI between taxon pairs or populations is opposed by gene flow. The flow of alleles between populations equalizes allele frequencies and homogenizes genomes, preventing divergence and thus slowing or reducing the buildup of RI between populations. Much research has been dedicated to understanding how speciation can happen with gene flow (Bolnick & Fitzpatrick 2007, Papadopulos & al. 2011, Martin & al. 2013). The important result of that research is an understanding that the effects of migration, an organismal level phenomenon, and the resulting gene flow, occur not only at the whole genome level, but also vary locally across the genome. That is, gene flow varies across the genome with some regions being permeable and exchanging alleles between populations, while other regions do not exchange alleles and remain distinct (Turner & al. 2005, Nosil & al. 2009, Nosil & Feder 2012). These regions that resist gene flow are known as barrier loci. Identifying such barrier loci can help to understand the genetic basis of reproductive barriers found at the organismal level, and is one of the next major steps in speciation research (Ravinet & al. 2017). The ultimate goal of this line of speciation research is to understand how a barrier locus impacts a phenotype to result in complete RI (Seehausen & al. 2014).

Speciation research has often focused on understanding the processes that lead to the initial divergence of populations. There has been less focus, however, on understanding late stages of speciation and the contexts that allow for the persistence of lineages once they have diverged (Kulmuni & al. 2020a, Stankowski & al. 2024). Identifying the conditions that promote or prevent the persistence of lineages and species is thus an important step in answering this question.

Finally, an important open question in speciation research is ‘Are macroevolutionary patterns simply generated by the accumulation of microevolutionary processes?’ (Stankowski & al. 2024). This is exemplified by Robosky and Matute (2013), who found that the experimental estimates of RI between *Drosophila* species did not correlate with the speciation rates from phylogenetic estimates. Therefore, additional data across taxa are needed to help understand and reconcile patterns of speciation across timescales.

In reviewing the literature on ant speciation research, we find that there has been a wealth of research on the taxonomic distinctions of ants (i.e., species revisions, evolutionary histories, and the classification of new species; Fig. 1). However, the results of these studies are often not put back into the broader context of speciation. Specifically, ant research makes use of the latest methods (e.g., genomic, morphological, paleontological, and behavioral approaches), and the terminology of speciation (e.g., geographic modes of speciation, especially sympatry and allopatry). However, they are used to define the histories and differences of particular species, rather than to contribute to the broad understanding of the patterns and processes of speciation and the buildup of RI (but see e.g., Savolainen & Vepsäläinen 2003, Schwander & al. 2008, Blacher & al. 2022). We see this as both a gap and an opportunity. The extensive and diverse research on ants can provide a general understanding of how speciation patterns apply to ants and beyond, and in turn, identify areas in which ants may be particularly well suited to furthering our understanding of the speciation process.

In this review, we focus on five main areas of ant speciation, bridging from microevolutionary processes to macroevolutionary patterns. We cover 1) key factors affecting speciation in ants, 2) reproductive isolating mechanisms, 3) hybridization, 4) interactions with other organisms as drivers of speciation in ants, and 5) patterns of macroevolutionary diversification (Fig. 2). We conclude with open questions and avenues to explore in future research. We hope that this review will be useful to researchers interested in answering questions of speciation in ants. To this end, we attempt to provide instances of similar analyses from other systems which may be useful. Furthermore, we hope that speciation researchers see value in using ants to address open questions in the field of speciation.

Key factors affecting speciation in ants

Ants have two key features that may impact the process of speciation and outcomes of hybridization. These are sociality and haplodiploidy, both of which could speed up the evolution of RI and lead to faster speciation under some conditions (SOCIAS-MARTÍNEZ & PECKRE 2023). The complete and lifetime morphological assignment to distinct castes from early development–one of which is a permanently non-reproductive worker caste–that occurs in most ant species, is the hallmark of obligatory eusociality (or superorganismality), one of the major evolutionary transitions (WHEELER 1986, HELANTERÄ 2016, BOOMSMA & GAWNE 2018, BOOMSMA 2022). Specifically, colony size and queen-worker dimorphism represent key evolutionary transitions promoting ant evolution (BELL-ROBERTS & al. 2024, Matte & LeBoeuf, 2025, VIZUETA & al. 2025). In ants with a distinct queen caste, queen number can vary from a single queen per nest (monogyny), to up to hundreds or thousands of queens per nest (polygyny). Queen number alters aspects of social organization and reproduction at the individual and colony level, as well as colony growth and organization; that is whether species build single nests (monodomy) or multiple interconnected nests (polydomy) (HÖLLDOBLER & WILSON 1990). An important impact of social organization is that it is often associated with dispersal traits. For example, polygyny and polydomy are frequently associated with reduced dispersal and mating within or near the natal colony in the *Formica rufa* group, leading to differentiation between populations and low effective population size within populations (CHAPUISAT & al. 1997, SUNDSTRÖM & al. 2005). A similar pattern of dispersal propensity and differentiation is found in *Mystrium* species: Genetic distance increases more rapidly with geographic distance in species that practice dependent colony formation with limited dispersal than those that practice independent colony formation (GRAHAM & al. 2016). Over longer evolutionary time scales and large geographic distances, reduced dispersal can eventually lead to the build-up of genetic differentiation and RI (SEIFERT 2010). Interestingly, supercoloniality (an extreme form of polygyny and polydomy, see

Helanterä 2022) is associated with a rapid speciation rate in the *Formica rufa* group wood ants (Seifert 2010). However, supercolonial species may not persist over macroevolutionary time scales as relatedness approaches zero, the process of kin selection and maintenance of altruistic worker phenotypes is impeded, leading to nepotism, mutation accumulation, and ultimately to the extinction of supercolonies (Helanterä 2022). In this case, there would be a mismatch between microevolutionary processes and macroevolutionary patterns, where rapid speciation does not correspond to the persistence of species rich clades over long time scales.

In at least five ant lineages, including *Formica* and *Solenopsis*, social organization is underpinned at the genomic level by supergenes (Wang & al. 2013, Purcell & al. 2014, reviewed in Chapuisat 2023b). These supergenes take the form of large inversions on a “social” chromosome. In speciation, inversions are both theoretically and empirically shown to play a role in divergence by locking together beneficial alleles responsible for local adaptation (Koch & al. 2021, Gompert & al. 2025), and delaying fusions between diverging lineages, especially with gene flow (Rieseberg 2001). Social supergenes with clear phenotypic effects in ants offer an excellent opportunity to study the importance of inversions and supergenes in speciation and how they may relate to RI barriers such as reduced dispersal. However, we still lack sufficient data on measures of RI between species with supergenes and the prevalence of social supergenes across ant taxa. This offers an important and interesting area to understand how social organization and supergenes could interact to lead to the build-up of genetic differentiation and the evolution of RI.

There are additional implications of sociality for speciation which we briefly outline here and expand upon in later sections. The division of labor between queens and workers is associated with multiple potential conflicts caused by asymmetries in relatedness (Queller & Strassmann 2002, Meunier & al. 2008). Such kin conflicts have been suggested to result in sympatric speciation via social parasitism in *Myrmica* (Savolainen & Vepsäläinen 2003) and in fungus growing ants (Rabeling & al. 2014) (see section “Interactions with plants, fungi, and other organisms as drivers of speciation”). More generally, workerless social parasite species raise interesting questions on how and when they diverged from their hosts (Wilson & Taylor 1967, Buschinger 2009, Rabeling 2021, Trible & al. 2023). Additionally, sociality changes the selective pressures operating on hybrids. Sterility of hybrids is no issue if hybrids develop into workers, as in *Pogonomyrmex* harvester ants (see section “Hybridization and its implications in ants”) (Cahan & al. 2002, Helms Cahan & Keller 2003). Cooperative breeding and polygyny also allow hybrid queens with high egg mortality to persist and reproduce (Beresford & al. 2017). This interaction between sociality and hybridization may shape ant evolution differently from other classic hybridizing taxa such as cichlids (Meier & al. 2017, Meier & al. 2023) or

Heliconius butterflies (Martin & al. 2013), and may play a role in the high rates of hybridization observed in ants.

Finally, ants as Hymenopterans belong to one of eight lineages in which haplodiploidy has evolved (Hartl 1971). Haplodiploidy in ants is a result of arrhenotokous reproduction, where unfertilized eggs from queens develop into haploid males and fertilized eggs into diploid females (de la Filia & al. 2015). The existence of haploid males allows for increased selection on recessive variants (Fig. 2b), leading to more efficient purging of recessive deleterious variants (Avery 1984, Werren 1993, Miller & Sheehan 2023) and faster fixation of recessive beneficial mutations (Nouhaud & al. 2020). The result is analogous to the faster X effect, where sex chromosomes evolve faster than autosomes (Charlesworth & al. 1987, Koevoets & Beukeboom 2009, Meisel & Connallon 2013), with haploid organisms experiencing faster substitution rates and therefore potentially faster evolution than diploid organisms (Hartl 1971, Koevoets & Beukeboom 2009). This may promote faster genetic divergence between populations, and thus a more rapid evolution of RI in ants and other haplodiploid species. However, to our knowledge, no comparative studies explicitly examine the rate of RI accumulation in haplodiploid versus diploid species. While haplodiploidy exposes generally deleterious alleles to selection in haploid males, it is also expected to influence epistatic incompatibilities such as Bateson-Dobzhansky-Muller incompatibilities (BDMIs) (see section “Mechanisms of postzygotic isolation in ants”). As BDMIs are hypothesized to be predominantly recessive (Presgraves 2003, Ayala-Lopez & Bank 2024), we expect them to be purged in haplodiploids in a similarly efficient manner as deleterious recessive alleles. This may therefore facilitate hybridization by allowing hybrid populations to be maintained by removing incompatibilities quicker than their diploid counterparts. Finally, two aspects of haplodiploidy may be leveraged to help identify the barrier loci that contain incompatibilities. These barrier loci have interested speciation research for decades, but experimental research on mapping their prevalence and distribution throughout the genome have been hampered by limited data and computational tools. Ants and other haplodiploid species can help in this. First, in haploid males, recessive and dominant deleterious alleles causing reduced hybrid fitness are selected against and can be revealed by sequencing the surviving males and determining which alleles are missing when comparing them to diploid females (Kulmuni & Pamilo 2014). Second, using haploid males avoids phasing and allows direct sequencing of haplotypes and utilization of methods relying on accurate haplotype information to map incompatibilities (Li & al. 2022, Heidbreder & al. 2025).

Reproductive isolating mechanisms in ants

Reproductive isolating mechanisms as a common framework to understand speciation across taxa

As highlighted in the introduction, speciation research investigating RI mechanisms and quantifying RI between pairs of ant species is rare. Most research on ant speciation focuses on hybridizing species (Fig. 1) (SCHWANDER & al. 2008, NOUHAUD & al. 2020, BLACHER & al. 2022, PORTINHA & al. 2022), potential sympatric speciation between socially parasitic species and their non-parasitic host species (SAVOLAINEN & VEPSÄLÄINEN 2003, RABELING & al. 2014), and the divergence of entire genus and species groups using a macroevolutionary perspective (MOREAU & al. 2006, GOROPASHNAYA & al. 2012). In this section, we review what is known about prezygotic and postzygotic isolating mechanisms in ants (Fig. 2a).

Mechanisms of prezygotic isolation in ants

What are prezygotic isolating mechanisms? Prezygotic RI refers to mechanisms that operate before zygote formation and impede the transfer of gametes to individuals of the other species (COYNE & ORR 2004). These could be temporal, where gametes are released at different times of the year or individuals of the two species mate or call at different times of the day (e.g., time segregated release of the same sex pheromone in *Platyptilia* moths HAYNES & BIRCH 1986); spatial, where individuals of the two species live in different habitats, on different host plants or in different geographical regions (e.g., pea aphid host separation VIA & al. 2000); or behavioral, for example mismatches in courtship behavior as shown between *Drosophila* species (MARKOW 1981, SCHUG & al. 2008). It is of interest as to which barriers currently exist in ants, what is their relative importance, and how they could be involved either in initial species divergence and or persistence. Two more forms of prezygotic isolation have been explored in other systems: immigrant inviability and mechanical or gametic incompatibility. Immigrant inviability is RI through a reduction in encounters due to mortality of maladapted immigrants (NOSIL & al. 2005). Mechanical isolation prevents fertilization due to physical incompatibilities between reproductive structures, while gametic incompatibility involves a failure of gametes to fuse, possibly due to sperm being inviable in the reproductive tract of the other species or problems fertilizing the egg (GARLOVSKY & SNOOK 2018). Neither of these has been systematically explored in ants (though sperm differences are known to distinguish ant species BARCELLOS & al. 2015). On the other hand, inquilinism (social parasitism) acts as a prezygotic barrier similar or equivalent to another RI mechanism: divergence in host specialization, which is well studied in other taxa such as pea aphids (JEAN & JEAN-CHRISTOPHE 2010) or fig wasps (WEIBLEN & BUSH 2002). We further discuss social parasitism and speciation in the section “Interactions with other organisms as drivers of speciation”.

Temporal or allochronic isolation is the result of differing breeding times between species, reducing gene flow (COYNE & ORR 2004). In ants, temporal isolation occurs through the timing of the production of sexuals and when they leave the nest to find a mate, either through nuptial flights, female calling, or male aggregation. In ants which perform nuptial flights, differences in the timing of these flights has been suggested to form a prezygotic barrier between species and be important to speciation (Hölldobler & Wilson 1990). Indeed, Hölldober and Wilson (1990) present species-specific diel rhythms as separation flight timing in some *Pogonomyrmex*, with *P. maricopa* flying between 1000 and 1130 hours, *P. barbatus* between 1530 and 1700, and *P. rugosus* between 1630 and 1800. Similar partitioning of the day has been observed in three *Myrmica* spp. (Kannowski 1959) and a two hour offset between *Camponotus culeanus* and *C. ligniperda* (Seifert 2018). In addition to within-day partitioning, related ant species can differ in when their nuptial flights occur from a few days to over two months (Baldridge & al. 1980, McCluskey 1992). So, the timing of nuptial flights is a strong candidate for a prezygotic RI mechanism in ants. However, we still lack an understanding of how divergence of flight times may initially diverge. Within ant species, coordination of flights between days is often based on weather conditions (Boomsma & Leusink 1981), such as rainfall as in *Lasius neoniger* and *Atta texana*. The result is that nuptial flight timings in ants are generally consistent within species (McCluskey 1992). However, drivers of divergent flight timing within species may be informative to understand if and how it can evolve as a barrier to gene flow between species. Differences in flight times have been observed within the geographic range of a single species, *P. rugosus*, based on differing environmental cues across the range (Helms & Helms Cahan 2010).

Flight timing, however, is not a perfect barrier as overlaps between species can still result in successful interspecific matings. For example, *F. aquilonia* and *F. polyctena* have diverged in mating flight times in Finland (Douwes 2012), yet *F. polyctena* hybridizes extensively with *F. aquilonia* and forms stable hybrid populations (Beresford & al. 2017, Satokangas & al. 2023, Krapf 2025 unpublished data). An additional complexity arises when considering flight times of hybrids. Further temporal isolation is observed between hybrids and *F. aquilonia*, as the emergence and sexuals differ significantly and thus likely also the flight times, indicating a temporal prezygotic barrier that could eventually lead to speciation between the hybrids and one of their parental species (Krapf & al. 2025 unpublished data). Similar findings have been made for *Pogonomyrmex rugosus* and two independent lineages arising via hybridization. The two hybrid lineages not only fly at different times compared to *P. rugosus,* but they also have strong preference to mate within and between hybrid lineages, rather than with *P. rugosus* (Schwander & al. 2008). However flight timing varies, there are other interacting barriers that may still prevent matings if interspecific individuals do meet.

Behavioral isolation, i.e., all differences between species that reduce attraction and therefore reduce mating chance (Coyne & Orr 2004), is likely partly mediated by chemical cues, including cuticular hydrocarbon (CHC) profiles and sex pheromones in ants. CHCs are a complex blend of non-volatile chemicals present on the ant cuticle (Martin & Drijfhout 2009). They are important for preventing desiccation (Sprenger & Menzel 2020) but are also used for nestmate recognition, following antennal recognition of CHC profiles (Fielde 1901, Fielde 1903). Thus, CHCs could create a basis for mate recognition and mate choice in ants, providing mechanisms which could act as a starting point for prezygotic RI. CHC profiles are species-specific and stable across geography in *Formica* (Martin & al. 2008) but may also be acquired or modified by the environment (Beibl & al. 2007), potentially allowing for divergence of recognition signals and, eventually, speciation driven by the environment. There is consistent evidence for mating preferences accompanying differences in CHC profiles (Morrison Iii & Witte 2011). Blacher & al. (2022) found a strong preference for conspecifics in mate choice experiments between *Formica selysi* and *F. cinerea*, coinciding with distinct CHC profiles. Beibl & al. (2007) found that sexuals of the dulotic (i.e., parasitic "slave-making" ants) ant *Chalepoxenus muellerianus* reared in their natural host's nests versus an alternative species host discriminated against each other. This suggests that mate choice cues in dulotic ants could diverge following host switching. The processes of mate choice and CHC recognition are complex and associated with errors. This is highlighted by hybridization, which may asymmetrically impact mate preference of hybrids for either of the parent species (Beresford 2021). In addition to CHC profiles for recognition, some ant species release sex pheromones to attract mates either during nuptial flights, as in *Xenomyrmex floridanus* (Hölldobler 1971), or as part of female-calling syndrome (Hölldobler & Bartz 1985). Sex pheromones are generally larger and more volatile compounds in contrast to those found in CHCs, which are more important in contact recognition. The exact role of sex pheromones varies by mating type, with sex pheromone release by males playing a role in male aggregation some *Camponotus* species (Torres & al. 2001, Habe & al. 2024), while release by virgin queens of *Polyergus breviceps* attracts males (Greenberg & al. 2007). Regardless of mating type however, sex pheromones are species specific and likely serve as a prezygotic RI mechanism in ants (Reginier & Law 1968, Greenberg & al. 2018). As both sex pheromones and CHC profiles play roles in assortative mating through mate attraction and recognition, the relative importance of each may be predicted by their order during mating; the earlier acting barrier (sex pheromones) should reduce gene flow more than the later barrier (CHC profiles) (Coyne & Orr 2004). Therefore, investigations into the evolution of CHCs and sex pheromones as RI barriers may be interested in the order of their divergence between species. Additionally, comparisons to the role of sex pheromones as an RI mechanism in other taxa, such as moths (Löfstedt & al. 1991) may prove insightful.

Spatial isolation in ants, as in other organisms, takes place at scales ranging from micro to macro scales. Starting at the micro scale, species with overlapping ranges may exhibit habitat preferences either generally or during mating that reduce interspecific encounters. Such preferences match Coyne and Orr's (2004) definition of habitat isolation where species exhibit habitat preferences within a heterogeneous area smaller than the dispersal range of either species. This spatial isolation reduces gene flow by reducing opportunities for mating. Hölldobler and Wilson (1990) suggest that related species in a genus can be separated by microspatial habitat isolation during mating flights. This separation may occur in two ways. The first being the preference for the major habitat occupied by colonies of each species. Such spatial separation between flying queens serves to reduce interspecific hybridization and has been observed in a range of genera including *Myrmecia*, *Pheidole*, *Solenopsis*, and *Lasius* (Hölldobler & Wilson 1990). Further spatial isolation within major habitat types can occur as mating site preferences, such as *Pogonomyrmex desertorum* and *P. maricopa* gathering on bushes, while *P. barbatus* and *P. rugosus* gather on ground sites (Hölldobler 1976).

The role of habitat preference in the diversification of ant species from common ancestors remains unclear. Differentiation resulting from habitat isolation (prezygotic ecological isolation) is often the result of adaptation to different environments (Coyne & Orr 2004). Such adaptation is likely closely linked to species interactions (see section "Interactions with plants, fungi, and other organisms"). One example for habitat isolation occurs within the genus *Mystrium*: Two species are present in the southern Madagascar littoral rainforest region which is home to a unique assemblage of flora. This region appears to result in increased genetic differentiation between *M. barrybressleri* populations, which inhabit both littoral rainforest and other region types, in contrast to *M. rogeri* which was not sampled from the littoral rainforest region (Graham & al. 2016). This pattern was also suggested for *Strumigenys*, *Pyramica*, and *Mystrium* species which inhabit both the littoral rainforest and other regions (Fisher & Girman 2000). However, the causes of this regional difference on differentiation are still under investigation. Differences in habitat preferences have also been shown between related species in the *Formica rufa* group. In this case species ranges overlap, but colony separation follows climatic variables resulting in a mosaic pattern of colonies, but possibly within dispersal range of one another (Sundström & al. 2005). In this case however, habitat preference is an incomplete barrier to gene flow for many species pairs as inferred by introgression between the species (Satokangas & al. 2023). Yet even in the *Formica* system, a formal calculation of RI due to habitat isolation is not available. Finally, micro habitat preferences may contribute to keeping species separate after divergence. Where the sister species *Cataglyphis bicolor* and *C. savignyi* exist in sympatry, they exhibit nest site segregation determined by preferences for either wet or dry vegetation, respectively (Dietrich & Wehner 2003). This preference, however, disappears in allopatric areas of the species' ranges. A similar pattern was found in the sister species *Pogonomyrmex rugosus* and *P. barbatus*, which segregate at the micro habitat scale based on soil texture and are sympatric at intermediate textures (Johnson 2008).

Allopatry at the macro scale of non-overlapping ranges can separate species in two ways, through 1) biological differences between species which lead to range differences or 2) allopatry resulting not from genetic differences, but geographic barriers. The result of both however is reduction of gene flow to zero, allowing for genetic divergence. Studies of ants have demonstrated that this second case has been important in ant diversification and yielded useful insights into the rate of speciation in ants. Schär & al. (2018) suggest that ants speciate in 2-5 Mya of allopatry as evidenced by divergence between Nearctic (North American) and Palearctic (North African and Northern Eurasian) *Lasius* spp., *Le. muscorum*, *and F. fusca* populations. Allopatry or allopatric periods may have also been important in the divergence of *Formica* species in Europe (Goropashnaya & al. 2004) and multiple Iberian ant species (Tinaut & Ruano 2021) through refugia, as well as across Pacific islands (Wilson & Taylor 1967, Sarnat & Moreau 2011). Such cases of allopatry may be interesting to study the relative importance of drift versus selection in driving divergence in allopatry as in Deshmukh & al. (2025), or in the effects of secondary contact after periods of allopatry.

In summary, there is a wealth of literature relevant for prezygotic RI mechanisms in ants including published flight timings, ecology, habitats, and ranges, while other mechanisms such as immigrant or gametic inviability have been understudied. We suggest that there is a need for more studies integrating this information and quantifying the types of prezygotic isolation between related, ideally sister, ant species. This would allow for an understanding of the importance and evolution of each barrier type. For example, the findings of Blacher & al. (2022) that nuptial flight timing was not an important barrier between sister species, but CHC profiles were, suggest that divergence in flight timing may evolve later, or that CHCs are easier to change as a trait. Additionally, the role of the mating system in the species, whether 'male aggregation syndrome' or female calling, may affect the importance and evolution of barrier types (Feldhaar & al. 2008). However, more comparisons are needed before any such general conclusions can be drawn. This is true as well for habitat divergence. Quantification of prezygotic RI (as proposed by Sobel and Chen 2014) in ants provides opportunities for answering questions about the buildup of RI across spatial contexts. For example: What is the importance of allopatric periods to establish permanent RI? Finally, premating barriers may also shift or disappear with range changes, offering opportunities to understand which barriers are currently acting. An example is provided by the fire ants *Solenopsis invicta* and *S. richteri*, which experience no gene flow in their native ranges in southern South America yet readily hybridize in the contact zone of their introduced ranges in North America (Cohen & Privman 2019).

Mechanisms of postzygotic isolation in ants

Postzygotic isolating mechanisms operate after zygote formation and can be either intrinsic or extrinsic. The best known intrinsic postzygotic isolating mechanisms are BDMIs (Bateson 1909, Dobzhansky 1936, Muller 1942). BDMIs are heterospecific allele combinations that cause reduced fitness in hybrids, that is, allele combinations from the two parental species are incompatible in hybrids, which reduces their viability or causes sterility independent of the environment. Intrinsic incompatibilities may take many forms however, including mismatches in ploidy, chromosomal rearrangements, genetic or allelic incompatibilities (e.g. BDMIs), and endosymbiont infections (COYNE & ORR 2004, REIFOVÁ & al. 2023). Meanwhile, extrinsic postzygotic isolating mechanisms reduce the fitness of hybrid individuals in specific environments. Hybrid fitness being lower than the fitness of the parental species could be due to hybrids having intermediate morphological or behavioral traits. For example, in mimetic *Heliconius* butterflies, hybrids between *Heliconius melpomene* and *H. cydno* are fully viable, but they have novel wing patterns, so that the predators do not recognize them as butterflies they have learned to avoid and subsequently predate them (MERRILL & al. 2012, BROWER 2013).

Nuclear-nuclear incompatibilities as postzygotic isolating mechanisms are only recently starting to be explored in ants (KULMUNI & al. 2020b). Recessive intrinsic incompatibilities are selected against in haploid hybrid males during development but masked in diploid females, leading to inviability of hybrid males (Fig. 2b), a hallmark of Haldane's rule. Haldanes's rule posits that in the F1 offspring of intraspecific crosses, the heterogametic sex is more likely to be absent, inviable, or infertile than the homogametic sex (HALDANE 1922). These patterns have been observed in *Formica aquilonia* × *F. polyctena* hybrids, where selection during development removed males with specific introgressed alleles, but these alleles were present in females as heterozygotes (KULMUNI & al. 2010, KULMUNI & PAMILO 2014). Later studies using the BDMI model, have shown that intrinsic incompatibilities between these species are genome-wide (KULMUNI & al. 2020b, HEIDBREDER & al. 2025), yet reproductive isolation is incomplete (SATOKANGAS & al. 2023) and gene flow between the species has occurred asymmetrically throughout their divergence (PORTINHA & al. 2022). Although ants are an ideal study system to map the genomic location and extent of recessive incompatibilities, genomic studies of barrier loci in ants are surprisingly rare. Instead, some studies relied on behavioral experiments and viability analysis to study postzygotic isolation in ants. Indirect evidence of intrinsic incompatibility leading to inviability of males is also found in *Tetramorium*, where *Tetramorium immigrans*, *T. caespitum* and hybrid colonies are found in the field, but based on microsatellite analysis, no hybrid males were inferred from the worker genotypes (CORDONNIER & al. 2020).

Incompatibilities could also be induced by endosymbionts or by the co-evolution between the mitochondria and nucleus (BARNARD-KUBOW & al. 2016). Below, we focus on cytonuclear interactions between the mitochondrial and nuclear genomes (mitonuclear incompatibilities), and we discuss endosymbiont and ant speciation further in the section "Interactions with plants, fungi, and other organisms as drivers of speciation". For example, unexpected mitonuclear patterns have been found in *Formica* hybrids. Beresford & al. (2017) identified a pattern of mitonuclear mismatch in *F. aquilonia* × *F. polyctena* hybrids, where nuclear *F. polyctena*-like individuals tend to carry *F. aquilonia*-like mitochondrial haplotypes, and vice-versa. This mitonuclear mismatch does not seem to negatively affect the fitness of these hybrids and therefore does not constitute a reproductive isolation mechanism in this specific system. Additionally, while not a barrier between species but rather hybrid lineages, there is an interesting case of mitonuclear interactions impacting caste determination in two *Pogonomyrmex* species, *P. barbatus* and *P. rugosus* (Linksvayer & al. 2006). In this system, a model of mitonuclear mismatch determines what caste, worker or gyne, diploid females develop into. There are two derived lineages $A_1A_1c_1$ and $A_2A_2c_2$ which can produce both workers and gynes. However, crosses containing a mismatch in nuclear and mitochondrial genotypes ($A_1A_2c_1$ or $A_1A_2c_2$) do not produce gynes. This results in two independent lineages that cannot cross to produce reproductive offspring.

In addition to intrinsic incompatibilities, extrinsic hybrid incompatibilities (i.e., hybrids are less fit than either parental species, perhaps due to intermediate phenotypes) are an exciting area of research that has attracted recent attention (Kulmuni & Westram 2017, Thompson & al. 2022, Thompson & al. 2024). Incompatibilities that depend on the environment have been detected for example in three-spine sticklebacks (Thompson & al. 2022). There is also some evidence for environment dependent incompatibilities in *Formica* wood ants, since males with heterospecific introgressed alleles are selected against in cold Springs but favored in warm springs (Martin-Roy & al. 2021).

Lastly, one incredibly unique and interesting mechanism related to postzygotic RI is lineage-caste shunting as described in various forms of social hybridogenesis. This system, which results in reproductive isolation between hybrid lineages and the parent species, is more fully discussed in the section “Hybridization and its implications in ants”.

While overall, multiple mechanisms of postzygotic isolation have been studied in ants, how they contribute to speciation is still unclear. As in prezygotic mechanisms, further investigations and systematic measures of how each mechanism contributes to RI are warranted. We suggest that ants are particularly well suited for studies looking to investigate intrinsic genomic incompatibilities (see section “Key factors affecting speciation in ants”). The end goal of studies on both pre- and postzygotic RI mechanisms is to have comprehensive, comparative datasets with which speciation questions could be tested. The handful of comparative studies that exist in other taxa have provided novel insights to speciation. For example, in contrast to the dominant hypotheses that intrinsic postzygotic RI is important primarily in the final stages of speciation, Coughlan & Matute (2020) showed that it can effectively act already early in the speciation process. Yet, in a comparative study of 89 plant taxa, the strength of prezygotic isolation was twice as much as that of postzygotic isolation (Christie & al. 2022). A necessary component for such comparisons is consistent and standardized measures and reporting of RI, that facilitates comparisons across studies and taxa. We suggest following Sobel & Chen's (2014) reporting recommendations, which will facilitate understanding complex and interesting interplay between ant sociality, genetics, ecological interactions (see section "Interactions with other organisms as drivers of speciation") and pre- and postzygotic RI.

Hybridization and its implications in ants

Studies of hybridization are constitutive to the research on reproductive isolation and speciation in ants. Hybridization occurs when different species, subspecies or genetically distinct lineages mate and produce offspring that carry genetic material from both parents. Hybridization in ants has been a topic of interest throughout the late 20th century, with studies of hybridization between fire ant species introduced to North America (MEER & al. 1985) and between *Formica* species in Central Europe (SEIFERT 1999). Furthermore, a recent comparative study developed an approach to detect F1 hybrids from single genome data and found that hybridization is especially prevalent in ants (WEYNA & al. 2022). High rates of hybridization could further lead to hybrid speciation, as several studies have documented mechanisms that isolate hybrids from one or both of their parental species (SCHWANDER & al. 2008, BLACHER & al. 2022, SATOKANGAS & al. 2023). There is, at least, one potential case of hybrid speciation in ants: Seifert (2021) found evidence that the red wood ant *Formica paralugubris* is a hybrid species between *F. aquilonia* and *F. lugubris*. More generally, hybridization provides novel combinations of ancient genetic variants, which can lead to rapid specialization and speciation, as demonstrated in the adaptive radiation of Lake Victoria cichlid fishes, for example (MARQUES & al. 2019, MEIER & al. 2023). While not necessarily focused on speciation itself, the growing research on hybridizing ant species has expanded our understanding of RI mechanisms between ant species and revealed interesting evolutionary consequences of hybridization in ants.

As described above, ants are social organisms and haplodiploid, so that males are produced asexually. These factors modulate the way ants are affected by hybridization. When a gyne mates with a male from another species, this leads to asymmetry between sexes; all the females (queens and workers) are F1 hybrids, but as males are produced asexually by the mother (i.e., pure parental queen), they are not hybrids. Hybrid males occur only in the F2 generation, and these hybrid males are the sons of F1 queens (KOEVOETS & BEUKEBOOM 2009), which means that hybrid males trail behind hybrid females by one generation. The delayed appearance of hybrid males after an interspecific crossing means that there are reduced opportunities for introgression between the nuclear genome of the hybridizing species in haplodiploids compared to diploids, while the mitochondrial genome is always available for introgression in hybrid diploid females. Therefore, there is potential for biased mitochondrial introgression, relative to nuclear introgression, in haplodiploid hybrid organisms compared to diploid organisms, as found by Patten & al. (2015) through mathematical modelling.

To produce F2 and further generation hybrids, the sexual hybrid individuals must be fertile. If sexual hybrids are sterile, it is not possible to establish a stable, self-sustaining, hybrid lineage, i.e., a hybrid lineage that can produce further generation hybrids autonomously, with no need for further input from the parental species. If hybrids are sterile, they have to be recurrently produced by repeated interspecific matings. This is a severe limitation in ants, as queens mate after their emergence and store the sperm in their spermatheca for later use, meaning that they are "stuck" with the same genetic material for the entirety of their reproductive lives. Multiple matings can lessen this problem and there is evidence for frequent, but usually low level, polyandry (i.e., queens mate with multiple males) in ants (SCHMID-HEMPEL & CROZIER 1999). For example, Pamilo (1993) found that about 60% of *F. aquilonia* queens mate with multiple males, but that the number of matings is low (1-6). Although low in most ant species, the number of male mates per queen can go up to 25 in some species, such as army ants (BOOMSMA & VAN DER HAVE 2002, KRONAUER & al. 2011, BARTH & al. 2014). If a pure species queen mated with a heterospecific male can produce viable hybrid workers, but no hybrid sexuals, she can rescue her fitness by producing pure males asexually, and/or by mating multiply to secure intra-specific partners (FELDHAAR & al. 2008).

Various forms of social hybridogenesis have been described in ants, with hybrid workers resulting from inter-lineage matings, while males are produced asexually, and queens either asexually or from intra-lineage crosses (LENIAUD & al. 2012, LAVANCHY & SCHWANDER 2019). The case of *Pogonomyrmex* harvester ants presents an interesting example of this. The distributions of the species *P. rugosus* and *P. barbatus* overlap in southwest New Mexico, North America, where hybrid populations show strong genetic caste determination (JULIAN & al. 2002). Helms Cahan & Keller (2003) identified four genetic lineages (H1, H2, J1, J2) and showed that inter-lineage matings produce hybrid workers, while intra-lineage matings produce queens. These lineages are essential to the caste-functionality of the colonies in these populations. Anderson & al. (2006) found evidence that this genetic caste determination was obtained by introgression from *P. barbatus* into *P. rugosus*, following interspecific hybridization. Later, Schwander & al. (2008) found that the H1 and H2 lineages are almost completely reproductively isolated from one of their parental species, *P. rugosus*, due to the combined effects of asynchronous mating flights as well as assortative mating (both prezygotic reproductive isolation mechanisms), and hybrid inviability, as the majority of eggs produced in backcrosses with *P. rugosus* die before reaching the larval stage (post-zygotic mechanism). This hybrid *Pogonomyrmex* system highlights how sociality provides original ways by which hybrid lineages can circumvent post-zygotic reproductive barriers.

If the hurdles of incompatibilities are buffered by social conditions, a population of hybrids could persist through time. In the case of highly polygynous and supercolonial mound-building wood ants (STOCKAN & ROBINSON 2016), the presence of multiple reproductive queens coupled with nest budding could facilitate the maintenance of hybrid queens despite strong hybrid incompatibilities and low hatching rate of hybrid eggs (MARTIN-ROY & al. 2021). Even if the hatching rate of hybrid queens is low, hundreds of reproductive queens can produce enough offspring to sustain the colony. Furthermore, hybrid queens with low egg hatching rates or high larval mortality are buffered from possible detrimental environmental conditions within the stable nest environment. Such low-fitness hybrid queens would be quickly eliminated in solitary species, or in social monogynous species with independent colony founding.

The interplay between sociality and haplodiploidy fundamentally shapes how hybridization and interspecific gene flow unfold in ants, with complex outcomes that often differ from expectations derived from diploids. Hybridization is prevalent in ants (WEYNA & al. 2022) with several cases documented either at the genomic level (e.g., KULMUNI & al. 2010, COHEN & PRIVMAN 2019, CORDONNIER & al. 2020) or inferred in taxonomic studies (SEIFERT 2009). These high rates of hybridization coupled with the excellent genomic resources for ants (VIZUETA & al. 2025) offer good opportunities to study the genomic patterns of speciation with gene flow. By investigating barrier loci between genomes of hybridizing taxa, it will be possible to infer if barriers accumulate in certain genomic regions or if social supergenes (see section "Key factors affecting speciation in ants") or other rearrangements play a disproportionate role in promoting divergence.

Interactions with other organisms as drivers of speciation

Ants display a diverse set of lifestyles and behaviors (HÖLLDOBLER & WILSON 1990). In addition to the various social behaviors briefly mentioned above, they can cultivate plants, tend fungi or other animals as food sources, and parasitize other species (HÖLLDOBLER & WILSON 1990). These interactions with other organisms, be them plants (NELSEN & al. 2018), fungi (DEJEAN & al. 2023), or other animals (STADLER & DIXON 2005, PARMENTIER & al. 2020), likely promoted ant diversification and speciation, and led to the evolution of highly specialized, if not unique characteristics and lifestyles. Much research exists on the evolution, taxonomy and distribution of these interactions and resulting lifestyles, but fewer studies specifically investigate RI mechanisms that could have led to speciation in these cases. Below, we will discuss ant-plant interactions, trophobionts (e.g., sap-sucking insects), fungus-growers, and social parasitism as case studies (Fig. 2c). We highlight which RI mechanisms might act in each case and how they could be investigated in future research.

Interactions with plants, fungi, and other organisms

Ants can interact with plants, fungi, and other organisms on various intensity levels ranging from facultative and generalized, to obligatory and highly specialized (Delabie 2001, Nelsen & al. 2018, Parmentier & al. 2020, Dejean & al. 2023). Under the right conditions, such interactions can promote the development of prezygotic barriers and lead to speciation (Ward 1993).

Ant-plant co-evolution has led to the evolution of specialized resources provided by plants for ants, such as nest sites or food, and reciprocally of nutrients and protection against herbivores provided by the ants to the plants (Hölldobler & Wilson 1990, Bronstein & al. 2006, Nelsen & al. 2018). Today, more than 1,700 extant ant species spanning six subfamilies are known that interact with more than 11,000 plant species (Hölldobler & Wilson 1990, Bronstein & al. 2006, Nelsen & al. 2018). One well-known example of ant-plant interactions is between plants of the genus *Vachellia* and acacia ants in the *Pseudomyrmex ferrugineus* group (Ward & Branstetter 2017), or the dolichoderine ant *Philidris nagasau* attending six *Squamellaria* epiphyte plant species (Chomicki & Renner 2016). After such interactions have formed, they may allow dispersal and persistence in slightly different habitats than the ancestral lineage, promoting reproductive isolation in allopatry or parapatry. For example, in the *P. ferrugineus* group reproductive isolation due to geographical isolation is assumed to be the driving speciation force (Ward 1993): With the shift of ants to open habitats ~6 Mya (Ward & Branstetter 2017), fewer ant colonies may have been in the nearby surroundings, thus providing fewer possibilities to mate, promoting reproductive isolation. This may have led to the divergence of, at least, ten species in the *P. ferrugineus* group obligately using *Vachellia* plants. Similarly, the dolichoderine ant *P. nagasau* seems to be endemic to the Fiji Islands, which likely has promoted reproductive isolation in allopatry after arriving there (Chomicki & al. 2016). Notably, *Philidris* species have received little attention so far, so that other obligate interactions may be found to further elucidate the speciation event in this genus.

Ants have also evolved symbiotic associations with fungi (Mueller & al. 2005). Attini ants are the most famous examples of obligatory mutualism in which ants nourish various fungus cultivars with leaves and use the fungi bodies as food (Schultz & Brady 2008). Today, at least 247 extant and largely Neotropical obligate fungus-farming ant species are known, which use several fungal species (Mikheyev & al. 2006, Mehdiabadi & Schultz 2009, Schultz & al. 2024). Notably, leaf-cutter ants of the genus *Atta* have obligate mutualistic interactions with a single fungus species, *Leucoagaricus gongylophorus* (Mikheyev & al. 2006, Schultz & al. 2024). This co-evolution occurred via several changes in both partners and promoted RI and speciation in the *Atta* genus: ants and fungus lose the ability to produce arginine (Branstetter & al. 2017) and to break down lignin (Nygaard & al. 2016), respectively, thus being dependent on the partner, or the obligate vertical transmission of the fungus by dispersing queens (Mueller & al. 2001).

As in ant-plant interactions, geographic isolation but also ecological and behavioral differences have likely driven speciation in *Atta* species, which have spread from Central America to the southern parts of South America and led to the divergence of 15 species alone in the genus *Atta* (BARRERA & al. 2021). For example, *A. colombica* is mainly found in lower-elevation regions in Central America to north-western South America, while sympatric *A. cephalotes* is present in higher-elevation regions across South America (BARRERA & al. 2021), which suggests that geographic and ecological barriers may have promoted RI and speciation. Additionally, some species are separated geographically and also use different plants. For example, *A. vollenweideri* and *A. goiana* forage mainly for monocotyledonous plants, while *A. saltensis* and *A. opaciceps* only use dicotyledonous plants (BARRERA & al. 2021), indicating that they could have speciated geographically and behaviorally. Indeed, behavioral differences may further drive speciation. For example, *A. cubana* occurs sympatrically with *A. insularis* on the island of Cuba and uses coastal regions and sandy soils as nesting sites, while *A. insularis* prefers forest and grassland habitats (FONTENLA 1995, BARRERA & al. 2021). These differences in habitat preferences may have led to a change in the CHC bouquet and thus to reproductive isolation. However, there is a clear interplay between behavior (selecting nest substrate) and ecology (habitat) in this case. The potential barriers and reproductive isolating mechanisms discussed above await further testing.

Ants also interact with other organisms that can promote speciation, such as trophobionts (e.g., caterpillars and sap-sucking insects; (STADLER & DIXON 2005, PARMENTIER & al. 2020) or endosymbionts such as *Wolbachia* or *Blochmannia* (ROUSSET & al. 1992, DEGNAN & al. 2004). Ants tend trophobionts and protect them against predators. In turn, trophobionts provide food by excreting a sugary solution called honeydew (DELABIE 2001, STADLER & DIXON 2005). Such interactions can range from facultative to obligate (STADLER & DIXON 2005) and approximately 25% of more than 4,000 aphid species are tended by ants (PARKER & KRONAUER 2021). Overall, studies suggest that over 190 ant species belonging to 4 subfamilies (Dolichoderinae, Pseudomyrmecinae, Formicinae, Myrmicinae) tend aphids with Formicinae being the most frequent aphid tenders followed by Myrmicinae (ROUSSET & al. 1992, DEGNAN & al. 2004, SIDDIQUI 2019, GULL-E-FAREEN & al. 2021). For example, ant species of the genus *Acropyga* have an obligate trophobiotic interaction with mealybugs of the genera *Eumyrmococcus, Neochavesia,* and *Xenococcus* (all subfamily Rhizoecinae). This interaction formed 15-20 Mya (LAPOLLA 2005) and seems to have driven speciation. For example, mealybugs have only been found inside *Acropyga* nests, ants only feed on honeydew provided by the mealybugs, and *Acropyga* gynes carry mealybugs in their mandibles during mating flights to start a new “herd” in the new colony (a behavior termed “trophophoresy”; (LAPOLLA & al. 2002). Additionally, these ants and mealybugs only live belowground and are found in Arizona (USA) and Mexico (LAPOLLA & al. 2002). These close interactions between the partners suggest that behavioral and geographical barriers could have led to reproductive isolation and speciation. However, to elucidate whether these interactions are species-specific, several ant species in the genus as well as their mealybugs should be collected, and mealybugs should be switched among ant species. If the interactions between ants and mealybugs are species-specific, this would provide support for ecological speciation.

Interactions with endosymbionts, such as *Wolbachia* or *Blochmannia,* can induce changes in sexual reproduction and lead to RI and speciation (ROUSSET & al. 1992, DEGNAN & al. 2004). Today, 61 species across seven subfamilies are known to be infected with *Wolbachia* (RAMALHO & al. 2021). Another well-known example of endosymbiont infection are *Camponotus* ants and their endosymbionts *Blochmannia* (DEGNAN & al. 2004, WERNEGREEN & al. 2009), with each of the more than 1500 *Camponotus* species and subspecies having its own vertically transmitted endosymbiont (MANTHEY & al. 2022). Notably, *Blochmannia* genes are evolving about 30 times faster than *Camponotus* genes, and both rates of molecular evolution are positively correlated (MANTHEY & al. 2022). This rapid gene evolution may have led to *Blochmannia* diversification and thus to *Camponotus* co-speciation and can, in the future, promote RI and speciation.

Wolbachia are a genus of bacteria found in the ovaries and testes of various invertebrate species, including many species of ants (WENSELEERS & al. 1998). Infection by *Wolbachia* can result in cytoplasmic incompatibility (CI), parthenogenesis, and feminization in insects (reviewed in WERREN 1997). Due to these effects, *Wolbachia* has been put forth as a possible factor causing RI between infected and non-infected populations (DEGNAN & al. 2004). While good evidence of isolation due to *Wolbachia* has been found for example in mosquitos, *Culex pipiens* (LAVEN 1967), and multiple *Drosophila* species (BOURTZIS & O'NEILL 1998, VENETI & al. 2012), the effects of *Wolbachia* in *Formicidae* are still relatively unknown (RUSSELL 2012, RAMALHO & al. 2021), but do include cytoplasmic incompatibility and potential for male killing. For example, *Wolbachia*-induced unidirectional cytoplasmic incompatibility was recently discovered between Old and New World populations of *Cardiocondyla obscurior* (ÜN & al. 2021), preventing hybridization between populations of these species that carry different strains of *Wolbachia* (DEGNAN & al. 2004). This mechanism could eventually lead to speciation. In addition to speciation through direct RI, coevolution between *Wolbachia* and the host can lead to interdependencies which, if disrupted by hybridization, could lead to incompatibilities. For example, *Wolbachia* provides nutrients by supplementing vitamin B in *Tapinoma melanocephalum* (CHENG & al. 2019). Goodisman and colleagues (1998) developed a theoretical framework for cytonuclear interactions between haplodiploid species and applied it to a North American hybrid zone between the imported red fire ant *Solenopsis invicta* and the black imported fire ant *S. richteri*. These species do not hybridize in their native South American range, but they do in their introduced range (ROSS & ROBERTSON 1990) in North America. Shoemaker & al. (2006) show, through mtDNA based phylogenies, that the introduced populations lost their *Wolbachia* endosymbionts during their invasion of North America, which Feldhaar & al. (FELDHAAR & al. 2008) interpret as the possible loss of a postzygotic barrier to hybridization driven by cytoplasmic incompatibility.

Social parasitism as a driver of speciation

Social parasitism is the 'parasitic dependence of a social insect species on one or several free-living social species' (BUSCHINGER 2009). It has evolved at least 60 times independently in six different, distantly related ant subfamilies and can be found in over 400 species (RABELING 2021). Four types of social parasitism are distinguished, namely ant guests (xenobiosis), temporary social parasitism, permanent social parasitism with dulosis ('slave-making'), and permanent social parasitism without dulosis ('inquilinism'; BOURKE & FRANKS 1991, BUSCHINGER 2009, RABELING 2021). All temporary and permanent parasites are closely related to their respective host species (EMERY 1909; later termed 'Emery's rule'; WILSON 1971). Today, a *strict form* of Emery's rule (i.e., parasites are the closest relatives to their host (WARD 1989) is distinguished from a *loose form* (i.e., parasites and their hosts are closely related). Two hypotheses following Emery's rule have been postulated to explain how social parasitism evolved (BOURKE & FRANKS 1991): The *intraspecific* hypothesis suggests that a social parasite evolves directly from its host in sympatry (strict Emery's rule), while the *interspecific* hypothesis suggests that a non-parasitic species evolves a parasitic behavior allopatrically and starts parasitizing another non-parasitic species (loose Emery's rule). For both hypotheses, polygyny and polydomy seem to be a prerequisite (BOURKE & FRANKS 1991, BUSCHINGER 2009, RABELING 2021): Polygyny allows the adoption of non-nestmate queens, and polydomy to easily find other host colonies because many colonies are in the near surroundings. Both traits are common in genera containing socially parasitic species, such as *Acromyrmex* (RABELING & al. 2014), *Formica* (BOROWIEC & al. 2021), *Lasius* (BOUDINOT & al. 2022a), *Myrmecia* (DOUGLAS & BROWN 1959, MERA-RODRÍGUEZ & al. 2023), and *Myrmica* (SAVOLAINEN & VEPSÄLÄINEN 2003).

Whether social parasites evolved in sympatry or allopatry has implications on how RI builds up and thus on the process of speciation. For example, inquilines seem to speciate more frequently in sympatry (intraspecific hypothesis), while temporary and dulotic social parasites seem to speciate in allopatry (interspecific hypothesis; RABELING 2021, MERA-RODRÍGUEZ & al. 2023, but see also TALBOT 1976 and FISCHER & al. 2020 for allopatric speciation of inquilines). In one case, a socially parasitic lineage evolved quickly and intraspecifically, within lab-bred colonies of asexual workers of clonal raider ants (TRIBLE & al. 2023). Winged, queen-like individuals appeared suddenly, and these clonal "social parasites" had lost heterozygosity at a large genomic region, suggesting that a pre-existing genetic module, or supergene, equipped them with the necessary behavioral, morphological, and physiological specialization (CHAPUISAT 2023a, TRIBLE & al. 2023).

In non-clonal ants, RI can also occur intraspecifically and in sympatry, via several non mutually-exclusive ways frequently observed in inquilines (BUSCHINGER 2009, RABELING 2021): (i) Socially parasitic offspring are smaller (i.e., microgynes) and have a shorter developmental time than regular-size sexual offspring of the host, which can lead to assortative mating and temporal RI. A smaller body size of social parasites has been observed in *Acromyrmex* (SCHULTZ & al. 1998, MERA-RODRÍGUEZ & al. 2023), *Ectatomma* (NETTEL-HERNANZ & al. 2015), *Myrmica* (SAVOLAINEN & VEPSÄLÄINEN 2003, LEPPÄNEN & al. 2015 but see also STEINER & al. 2006), *Nylanderia* (MESSER & al. 2016), *Plagiolepis* (ARON & al. 2004), and *Pseudomyrmex* (WARD & BRANSTETTER 2022). (ii) Sexuals of social parasites mate inside or close to the colony, which also allows assortative mating and promotes spatial RI. Such a shift in the mating system has been observed in *Mycocepurus* (RABELING & al. 2014) and *Myrmica* (SAVOLAINEN & VEPSÄLÄINEN 2003), and is suspected for *Nylanderia* (MESSER & al. 2016). (iii) At later stages in the speciation continuum, gene flow and hybridization between host and social parasite cease (MERA-RODRÍGUEZ & al. 2023) and their male genitalia differ largely, leading to complete RI, as observed in *Mycocepurus* (RABELING & al. 2014). (iv) Parasitic queens may also suppress the production of host sexual offspring, also observed in *Mycocepurus* (RABELING & al. 2014).

For allopatric speciation of social parasites, evidence for RI is indirect (BUSCHINGER 2009) and has been found in the genera *Formica* (BOROWIEC & al. 2021), *Pheidole* (FISCHER & al. 2020), and *Temnothorax* (PREBUS 2017), based on phylogenomic reconstructions. Comparisons mainly comprise temporary and dulotic social parasites, but also examples of inquiline species are known (RABELING 2021). A non-parasitic species can evolve some parasitic behavior in allopatry and start parasitizing other, free-living species (RABELING 2021). For example, *Formica* temporary social parasites seem to have evolved after the loss of independent colony foundation (BOROWIEC & al. 2021). In theory, if a species splits into two allopatric daughter species A and B, queens of species A may start to "cheat" and rely on nearby colonies of other species for colony foundation. After secondary contact, species A may start parasitizing species B, while remaining reproductively isolated, as postulated by (BOURKE & FRANKS 1991). Dulosis seems to have evolved in *Formica* ants from temporary social parasitism (BOROWIEC & al. 2021). Three scenarios for its development have been proposed (BUSCHINGER 2009, BOROWIEC & al. 2021, and references in both): (1) A species is highly territorial and competitive, allowing it to invade weaker or smaller colonies and annihilate them, (2) A species may predate on brood and food of other species, or (3) A species transports brood over longer distances. Socially parasitic ants and their hosts thus provide excellent systems to test models of sympatric and allopatric speciation, and examine how behavioral, social, ecological, and genetic changes jointly lead to and promote RI.

Of the extant 14,343 ant species (Bolton, 2025), more than 4,108 species (29%) show interactions with other organisms spanning across various geographic regions and several subfamilies. While these interactions can be found globally, they are mainly concentrated in seven subfamilies, namely Dolichoderinae, Dorylinea, Ectatomminae, Formicinae, Myrmeciinae, Myrmicinae, and Pseudomyrmecinae (Tab. 1), which are also the most species-rich subfamilies totaling 13,836 extant species (Tab. S1). We emphasize that this is a non-exhaustive list which could serve as a starting point for a more detailed study. Additionally, ant-plant interactions, endosymbionts, specifically *Blochmannia*, and social parasitism are good candidates as drivers of diversification and speciation. Interactions with other organisms may have played a role in the divergence of more ant species, but further research needs to test this idea.

More broadly, being ecosystem engineers and keystone species in several habitats, ants have a multitude of interactions with other species. Many of these ecological interactions have been investigated and provide a solid basis to study how interactions could establish and enhance the buildup of RI. An additional interesting question linking to macroevolution is "Do species interactions promote persistence of species in changing environments or over macroevolutionary time scales, and if so, what kinds?".

Patterns of macroevolutionary diversification in ants

Early ants and modern ant development

One of the current challenges of speciation research is to connect microevolutionary processes to patterns observed at macroevolutionary time scales. Due to their well resolved phylogeny and wealth of ecological and taxonomic studies, ants offer interesting opportunities to connect these two scales.

The ant fossil record begins around 99 million years ago, during the mid-Cretaceous period. This fossil record, which mainly consists of specimens preserved in amber, includes representatives of extinct ant subfamilies, like the Sphecomyrminae, and extant ones, like the Formicinae, Aneuretinae, Dolichoderinae, and Ponerinae (LAPOLLA & al. 2013, PERFILIEVA 2023). These fossils are mainly found in the Neotropics, but specimens occur more globally, pointing to an earlier initial divergence of the Formicidae family (BOUDINOT & al. 2022b, PERFILIEVA 2023). This is supported by molecular data: studies estimating divergence times using molecular clocks suggest that ants began diversifying between 139 and 158 million years ago, stemming from a wasp-like ancestor (MOREAU & al. 2006, PERRICHOT & al. 2008, MOREAU & BELL 2013). These early ants likely lived in soil environments as predators, hunting small invertebrates (LUCKY & al. 2013). Furthermore, they had already evolved sociality, based on differences in body sizes found in fossils (GRIMALDI & AGOSTI 2000, BOUDINOT & al. 2022b).

A distinct pattern of species richness in modern ants shows a concentration in tropical regions, particularly in the Neotropics and Asia (KASPARI & al. 2004). Different hypotheses have been tested for this phenomenon, including the tropical conservation hypothesis (WIENS & DONOGHUE 2004), the diversification-rate hypothesis (PIANKA 1966), and the ecological regulation hypothesis (PIANKA 1966). A study by Economo and colleagues (ECONOMO & al. 2018) found that the tropical conservation hypothesis, which proposes that tropic-adapted species have existed for longer, since temperate environments only developed later, is the most likely. The same study did not find support for the diversification-rate hypothesis, which proposes that the tropical environment causes a higher diversification rate. The Neotropics, where ant lineages likely originated, have the highest diversity, whereas other ecological regions with similar climates have lower diversity, supporting the tropical conservation hypothesis rather than the diversification-rate hypothesis (PIE 2016). However, the tropical conservation hypothesis does not explain the observed diversification patterns by itself, since some very old lineages do not have as much diversity as would be expected under this hypothesis (ECONOMO & al. 2019). Therefore, other causes for shifts in ant diversification rates, for example interactions with other organisms as potential drivers of speciation, are undeniable.

In general, positive shifts in diversification rate are often associated with either increased ecological opportunity, e.g., dispersal to, and colonization of, a new environment, or with the evolution of a key innovation, such as a novel trait that offers a competitive advantage to an organism or allows it to expand into a previously inaccessible environmental niche space (SIMPSON 1949). Below, we discuss both mechanisms as drivers of ant diversification.

Increased ecological opportunity as a driver of ant diversification

Increased ecological opportunity allows species to diversify by adapting to new environments and inhabiting different parts of a new niche, driving adaptive radiation and diversification. In ants, ecological opportunities in part promoted the diversity we see now. Around 50 million years ago, fossils indicate an increase in ant diversity. The most accepted hypothesis of this spike in divergence is that ants evolved and speciated alongside the divergence of angiosperms (MOREAU & al. 2006). Angiosperm forest floors are much more diverse than gymnosperm forests, which allowed ants to diverge across terrestrial habitats and live in canopies (MOREAU & al. 2006). Predatory ants also benefited from the increased insect abundance that accompanied angiosperm expansion. This shift in food availability may have driven the evolution of specialized foraging behaviors, such as honeydew-feeding in Formicinae and Dolichoderinae (with >190 ant species tending aphids; Tab. XY; (ROUSSET & al. 1992, DEGNAN & al. 2004, SIDDIQUI 2019). The ability to exploit liquid food sources likely led to the evolution of repletes and semi-replete major workers in some species, enhancing their survival in resource-scarce environments. Increased ecological opportunities have also been inferred as a driver of diversification for some lineages of *Cephalotes* turtle ants. Diversification occurred alongside the emergence of novel biomes and flora in the Chacoan region of the Amazon (PRICE & al. 2014).

A non-mutually exclusive hypothesis that might explain the Formicidae diversification is the dynastic succession hypothesis (WILSON & HÖLLDOBLER 2005). It suggests that the early establishment of ants in ecosystems provided them with opportunities to diversify and fill ecological niches with limited competition. Fossil evidence from Eocene amber deposits demonstrates that Formicinae ants were among the first to exploit tree canopies, taking advantage of the expanding angiosperm-dominated forests of the time (PERKOVSKY 2009, WARD & al. 2015). Additionally, mutualistic ant-plant interactions (>1,700 ant species; NELSEN & al. 2018), such as protecting plants from herbivores in exchange for shelter or nectar, further cement their ecological roles. This mutualism is supported by fossil and modern evidence, showing that Formicinae ants have long been integral to arboreal ecosystems (WARD & BRADY 2003).

Key innovations in the diversification of ants

Hodges and Arnold (HODGES & ARNOLD 1995) defined key innovations as “biological traits that promote lineage diversification via mechanisms that increase the rate of speciation and/or decrease the rate of extinction”. In Hymenoptera, several key innovations (like wasp-waists, stingers, and parasitoidism) have been studied. However, secondary phytophagy, i.e., the secondary transitions to plant feeding, was found to be the most important key innovation in the diversification of the order (BLAIMER & al. 2023). This is in line with the evolution of angiosperms alongside the Formicidae, as mentioned before. Despite suggestions on the role of sociality in ant diversification, it is not considered a key innovation, as both termites and ants were social for tens of millions of years before independent evolutionary events led to radiations within specific subgroups (BARDEN & ENGEL 2020).

One key innovation that has specifically influenced ant diversification is claustral colony founding. In species that show this trait, queens rely on stored energy reserves to establish new colonies without needing to leave the nest to forage. This adaptation reduces early mortality risks from predation and competition, providing a significant survival advantage. Claustrality has independently evolved in three of the four most species-rich lineages: Formicinae, Myrmicinae, and Dolichoderinae, and is thought to contribute to their high diversification rates by allowing them to establish colonies that are protected from predators and enemy ant species present in the surrounding environment (WILSON & NOWAK 2014).

A predation adaptation found in some genera of the subfamilies Ponerinae, Myrmicinae, and Formicinae, is the spring-loaded trap-jaw mechanism (Fig. 2d; MOREAU & BELL 2013, LARABEE &

Suarez 2014). This adaptation enhances the hunting efficiency of their bearers, allowing them to secure niches that require precision and fast predation (Larabee & al. 2016). This innovation has thus caused an ecological niche expansion and allowed these genera to diversify. Indeed, the diversification rate in ant lineages with trap-jaw mechanism is 0.064, which is twice the overall rate of diversification in their Ponerinae subfamily (Larabee & al. 2016). A higher diversification in genera with the trap-jaw mechanism has also been observed within the subfamily Myrmicinae (A*nochetus* + *Odontomachus* and *Pyramica* + *Strumigenys*; (Moreau & Bell 2013). The convergent pattern in two separate subfamilies strongly suggests that the trap-jaw mechanism is an innovation promoting species diversification. Booher & al. (2021) further support this with the finding that diversification in *Strumigenys* came after the evolution of the trap jaw mechanism. However, as noted by Larabee & Suarez (2014), while the phylogenetic evidence of the trap jaw being a key innovation is mounting, it remains to be demonstrated that the trap jaw has allowed ants to enter new adaptive zones and improved the ecological performance versus non-trap jaw species.

Key innovations can also be defensive. The Formicinae subfamily famously produces formic acid (Fig. 2d), which is a unique chemical defense (Wilson & Hölldobler 2005). Especially in the tropics, formic acid is suggested to be an advantage because there are many interspecific interactions. The evolution of formic acid is associated with an increase in speciation rate, likely because these ants were able to thrive in environments where they normally would not, due to many natural enemies. Similarly, in the Dolichoderinae subfamily, the evolutionary success of this group can be attributed to their sophisticated chemical communication strategies (Ward & Branstetter 2017). This increases the efficiency of both foraging and colony expansion, saving energy for reproduction.

Aforementioned key innovations and increased ecological opportunities thus appear to be important drivers of diversification across ant lineages, and further research is needed to link the specific microevolutionary reproductive isolating mechanisms to these drivers. Additionally, interspecific interactions, such as mutualisms or symbioses with plants or fungi, also drive diversification by niche expansion, as seen in subfamilies like *Pseudomyrmecinae* and *Attina,* but what is the extent of RI generated by these interactions remains to be studied. One of the major challenges in speciation research is bridging the gap between microevolutionary studies of RI and macroevolutionary studies of species diversification and persistence (Stankowski & al. 2024). One obstacle in this is the fact that the rate of initial species divergence does not necessarily correspond to persistence of species at longer evolutionary time scales (Rabosky & Matute 2013). Much speciation may be ephemeral, with lineages disappearing before they contribute to macroevolutionary patterns of speciation. However, the wealth of ecological, taxonomic, behavioral, and geographical studies in ants coupled with evenly sequenced and well resolved phylogeny present opportunities to tackle these challenges. This was recently demonstrated by Vizueta & al. (2025) who showed that rate of genome rearrangements correlated with species richness across the ant phylogeny. Similarly, ants provide further opportunities to test if high rates of hybridization correlate with species richness and what kinds of species interactions might promote species persistence.

Conclusions and outlook

Thanks to their ubiquity, geographic spread, and ecological diversity, ants provide an ideal system to study open questions in speciation, with many complementary areas in which we lack an understanding of ant speciation. First, studies quantifying RI are concentrated on certain animal and plant taxa, with RI estimates lacking in many taxa, including in ants. There are a wealth of ant species with known ecology and life histories serving as a good basis for answering general speciation questions of how and when components of RI evolve. Ants provide a tractable system to investigate these questions at both the organismal and genomic levels. At the organismal level, prezygotic barriers such as the timing of emergence of sexuals and mate choice can be measured and quantified. The numerous interactions with other taxa also allow us to address questions of how species interactions could drive RI and eco-evolutionary interactions could promote speciation. An important aspect to these questions in ants is that they can more easily be investigated at the genomic level than ever before. The genomic data on ants is accumulating and the ease of analyses on non-model organisms is only increasing. The particular feature of haplodiploidy allows for the possibility of accessing population samples of haploid male genomes without the need for phasing. This will facilitate the mapping of barrier loci, allowing for answers about the distribution and buildup of barrier loci in the genome. The interplay of sociality and genomics also has potential to help in addressing the importance of chromosomal inversions and supergenes in speciation and reproductive isolation. Furthermore, hybridization is known to be frequent in ants. Yet, there are open questions as to whether hybridization is even more common in ants than other taxa, and whether their unique features of sociality and haplodiploidy allow them to minimize the costs of interspecific matings, increasing the frequency of hybridization and its success. Either way, frequent hybridization in ants provides opportunities for studying the impact of gene flow on genomic divergence, a key area of current speciation research Further investigation would allow us to address questions about the relative importance, timing, and evolution of pre- and postzygotic barriers, and the role of intrinsic and extrinsic factors in the ant speciation.

A better understanding of RI in ants would open the door to addressing one of the main challenges of speciation research: bridging the gap between microevolutionary processes and macroevolutionary patterns. Ants may help with this as well-resolved phylogenies would allow for comparative analyses correlating current diversity with different drivers of diversification. In ants, ecological release was potentially important for diversification on some Pacific Islands, in the Amazon basin, and Australia. Furthermore, across ant subfamilies the numerous interactions with other organisms may promote speciation. Large comparative studies can begin to link broad diversification patterns in ants, and the processes driving them allowing for a complete picture of which components of RI have been important drivers of diversity in ants, and how their frequency and importance vary across ants or within tribes. This will in turn benefit the speciation community's understanding of these same questions. Untangling the complex interactions of adaptation, sociality, genomics, geography, and their roles in divergence will always be daunting tasks. However, there are opportunities to utilize ants for research programs in these areas which will be fruitful to unlocking new knowledge of speciation.

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Table and figure captions

Fig. 1: Speciation research on ants is not usually performed under the framework of reproductive isolation. A) Counts of each keyword together with “ant”, “ants”, or “Formicidae” in the literature search including 2,665 published articles found from Web of Science, Google Scholar, and Scopus. B) Time series by year for the number of articles containing any of the keywords included in the search, line colors match bar colors and labels in the bar chart, while the black line shows total publications.

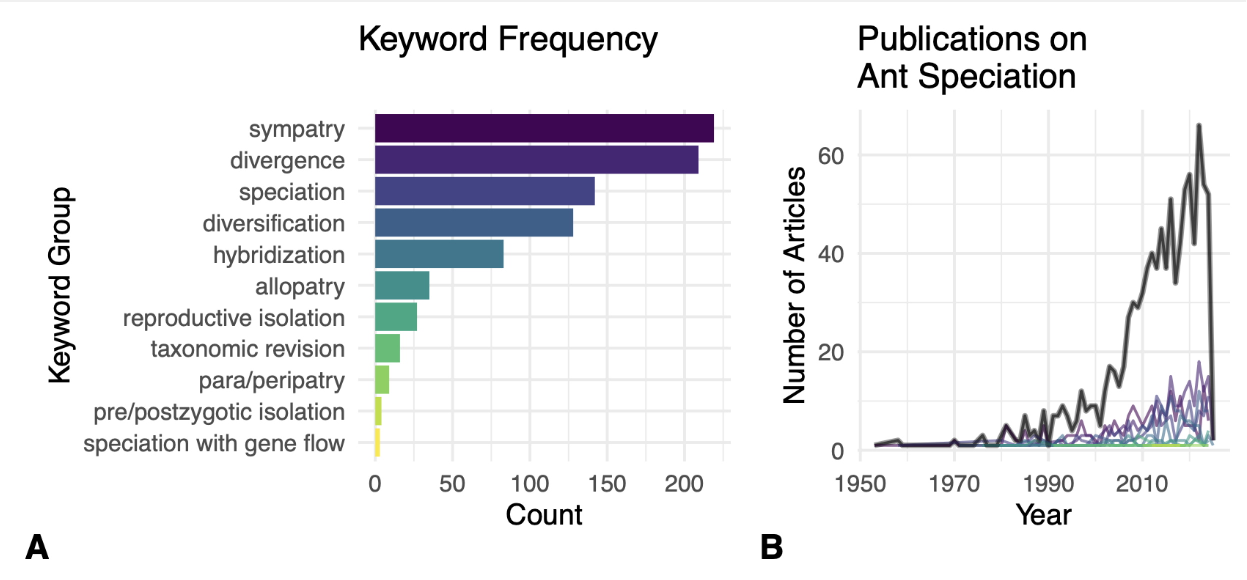
Keyword Frequency
Keyword Group
sympatry
divergence
speciation
diversification
hybridization
allopatry
reproductive isolation
taxonomic revision
para/peripatry
pre/postzygotic isolation
speciation with gene flow
0
50
100
150
200
Count
A
Publications on Ant Speciation
Number of Articles
60
40
20
0
1950
1970
1990
2010
Year
B

Fig. 2: A) In ants, surprisingly few studies quantify mechanisms of reproductive isolation (RI). B) Sociality and haplodiploidy can alter selective processes and dynamics of speciation in ants, but also in bees, wasps, and termites. C) Ants have a high number of interactions with other organisms, which can promote speciation. In the photographs, we see a *Polyrhachis rufipes* tending aphids as well as an *Atta cepahlotes* major cutting a leaf which is then used to feed fungi. D) Ant diversification was likely promoted by ecological opportunities and key innovations, such as the trap-jaw mechanism and formic acid. In the photograph, we see examples of two key innovations: a trap-jaw ant *Odontomachus tyrannicus* and a *Formica rufa* spraying formic acid. All photographs are © Philipp Hönle.

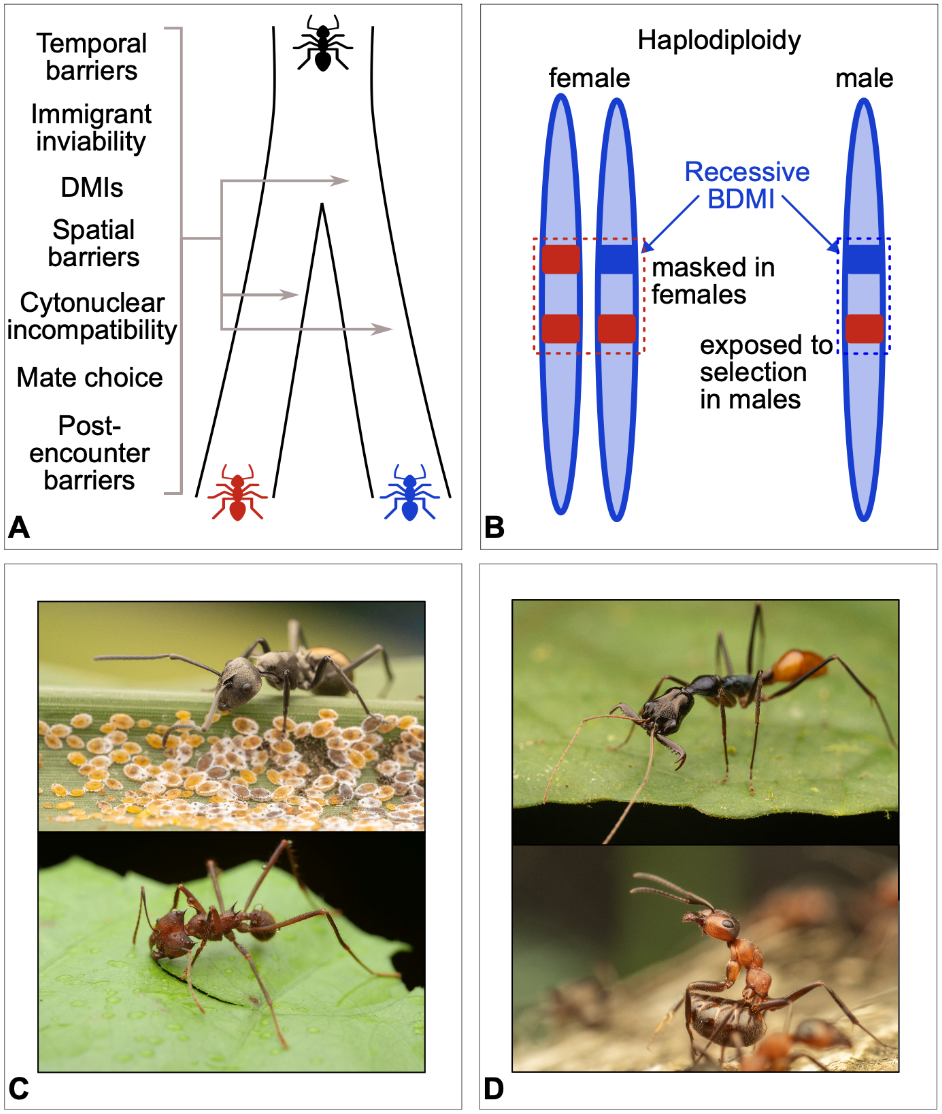
Temporal barriers
Immigrant inviability
DMIs
Spatial barriers
Cytonuclear incompatibility
Mate choice
Post-encounter barriers
A
Haplodiploidy
female
male
Recessive BDMI
masked in females
exposed to selection in males
B
C
D

Table 1: Subfamilies, estimated number of species, and geographic region associated with specific lifestyles discussed in the Section "Interaction with other organisms"

Lifestyle	Species, genus, tribe, clade, or subfamily	No. of species	Geographic region
Ant-plant interactions	Six subfamilies, namely Dolichoderinae, Dorylinea, Formicinae, Myrmicinae, Ponerinae, and Pseudomyrmecinae; Myrmicinae the most frequently interacting with plants	>1700	Globally
Ant-plant interactions	*Pseudomyrmex ferruginues* clade (subfamily Pseudomyrmecinae)	10	Neotropics
Ant-plant interactions	Species *Philidris nagasau* (subfamily Dolichoderinae)	1	Neotropics
Fungus-farming ants	Tribe Attini (subfamily Myrmicinae) which are all fungus-farming ants	232 (excluding the 15 below)	Neotropics
Fungus-farming ants	Genus Atta (subfamily Myrmicinae	15	Neotropics
Aphid tending	Four subfamilies, namely Dolichoderinae, Formicinae, Myrmicinae, and Pseudomyrmecinae; Formicinae the most frequent aphid tenders	>190	Globally
Endosymbionts	*Blochmannia*; tribe Camponittini (subfamily Formicinae)	>1500	Globally
Endosymbionts	*Wolbachia*; seven extant subfamilies, namely Dolichoderinae, Dorylinea, Ectatomminae, Formicinae, Myrmeciinae, Myrmicinae, and Pseudomyrmecinae	>60	Globally
Social parasites	Six extant subfamilies, namely Dolichoderinae, Ectatomminae, Formicinae, Myrmeciinae, Myrmicinae, and Pseudomyrmecinae	>400	Globally
Total		**>4108**	**14343**

Note: This is a non-exhaustive list of lifestyles, subfamilies, number of species, and geographic regions

Supplementary Table. 1. Subfamilies and number of species for each subfamily based on Bolton, 2025

Subfamily	No of species	Known interactions with other species
Myrmicinae	7,219	x
Formicinae	3,287	x
Ponerinae	1,290	x
Dorylinae	775	x
Dolichoderinae	725	x
Ectatomminae	305	x
Pseudomyrmecinae	235	x
Proceratiinae	166	
Amblyoponinae	147	
Myrmeciinae	94	
Leptanillinae	88	
Agroecomyrmecinae	2	
Aneuretinae	1	
Apomyrminae	1	
Martialinae	1	
Paraponerinae	1	