



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

Patrick HEIDBREDER, Patrick KRAPF, Marit KUIJT, Beatriz PORTINHA, Michel CHAPUISAT & Jonna KULMUNI

### 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.

**Key words:** reproductive isolation, hybridization, diversification, review.

Received 20 March 2025; revision received 14 October 2025; accepted 27 October 2025

Subject Editor: Timothy A. Linksvayer

*Patrick Heidbreder (contact author), Organismal and Evolutionary Biology Research Programme, University of Helsinki, Rm 5202, Viikinkaari 1, 00790 Helsinki, Finland. E-mail: patrick.heidbreder@helsinki.fi*

*Patrick Krapf, Organismal and Evolutionary Biology Research Programme, University of Helsinki, Rm 5202, Viikinkaari 1, 00790 Helsinki, Finland; Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Postbus 94240, 1090 GE Amsterdam, Netherlands. E-mail: p.krapf@uva.nl*

*Marit Kuijt, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Postbus 94240, 1090 GE Amsterdam, Netherlands. E-mail: m.kuijt@uva.nl*

*Beatriz Portinha, Organismal and Evolutionary Biology Research Programme, University of Helsinki, Rm 5202, Viikinkaari 1, 00790 Helsinki, Finland; cE3c, Centre for Ecology, Evolution and Environmental changes & CHANGE, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal. E-mail: beatriz.portinha@helsinki.fi*

*Michel Chapuisat, Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland. E-mail: michel.chapuisat@unil.ch*

*Jonna Kulmuni (contact author), Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Postbus 94240, 1090 GE Amsterdam, Netherlands; Organismal and Evolutionary Biology Research Programme, University of Helsinki, Viikinkaari 1, 00790 Helsinki, Finland. E-mail: j.k.kulmuni@uva.nl*

## Introduction

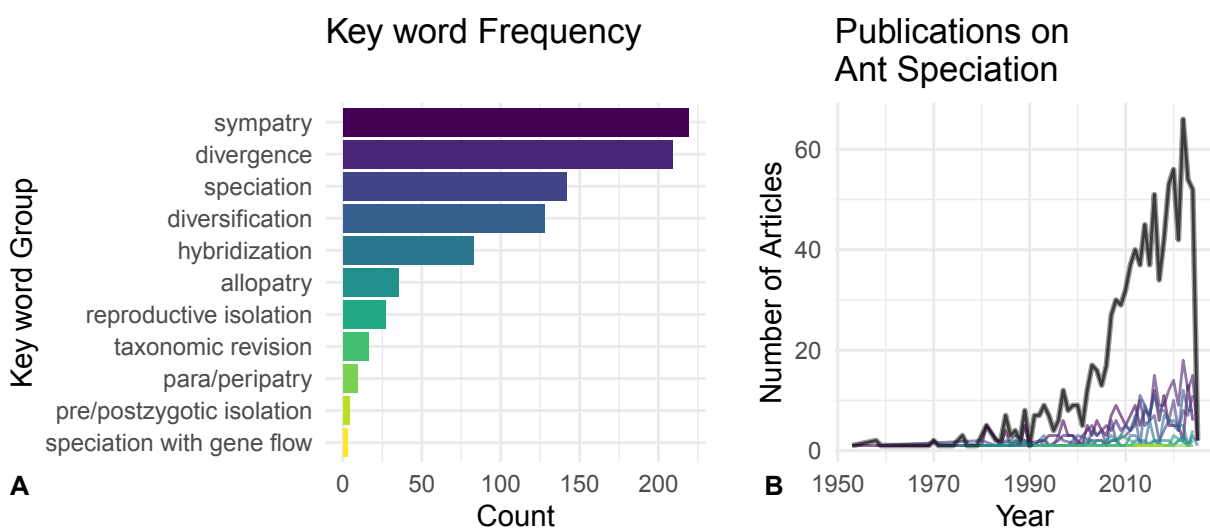
### Ants and speciation

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 million years ago (Mya), coupled with the proliferation of angiosperm forests and accompanying herbivores (MOREAU & al. 2006). Today, ants 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) and 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 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, some of their unique

features, and discuss how this group can provide answers to open questions in the field of speciation.

Speciation as a field studies the processes that generate the biological discontinuity that leads to the emergence and persistence of new species. Current open questions in speciation research include 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? We first introduce these ideas from a speciation perspective, reserving further discussion of their application to ants for later sections.

Ever since DOBZHANSKY (1937), RI has played a central role in speciation research. 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 one 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 in different environmental contexts (e.g., on new hosts in a transplant experiment as in VIA & al. 2000) or measure the reduction in gene flow between differently adapted populations, either experimentally (HENDRY 2001, NOSIL & al. 2003) or through simulations (LACKEY & BOUGHMAN 2017, ANDERSON & WEIR 2022). Similarly, if strong RI



**Fig. 1:** Speciation research on ants is not usually performed under the framework of reproductive isolation. (A) Counts of each key word together with “ant”, “ants”, or “Formicidae” in the literature search including 2665 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 key words included in the search, line colors match bar colors and labels in the bar chart, while the black line shows total number of publications.

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, as each taxon pair has a unique evolutionary history, generalizable conclusions can only be drawn when we have data from a range of divergence 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 evolutionary force that prevents the buildup of RI between taxon pairs or populations is 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. Much research has been dedicated to understanding how speciation can happen with gene flow (BOLNICK & FITZPATRICK 2007, PAPADOPULOS & al. 2011, MARTIN & al. 2013). From this research, it appears that the genomic effects of gene flow from migration, an organismal-level phenomenon, actually vary locally across the genome. That is, some genomic regions are permeable, and alleles are exchanged between populations, while other regions remain distinct, and alleles are not exchanged (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 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?” (ROLLAND & al. 2023, STANKOWSKI & al. 2024). This is exemplified by RABOSKY & 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.

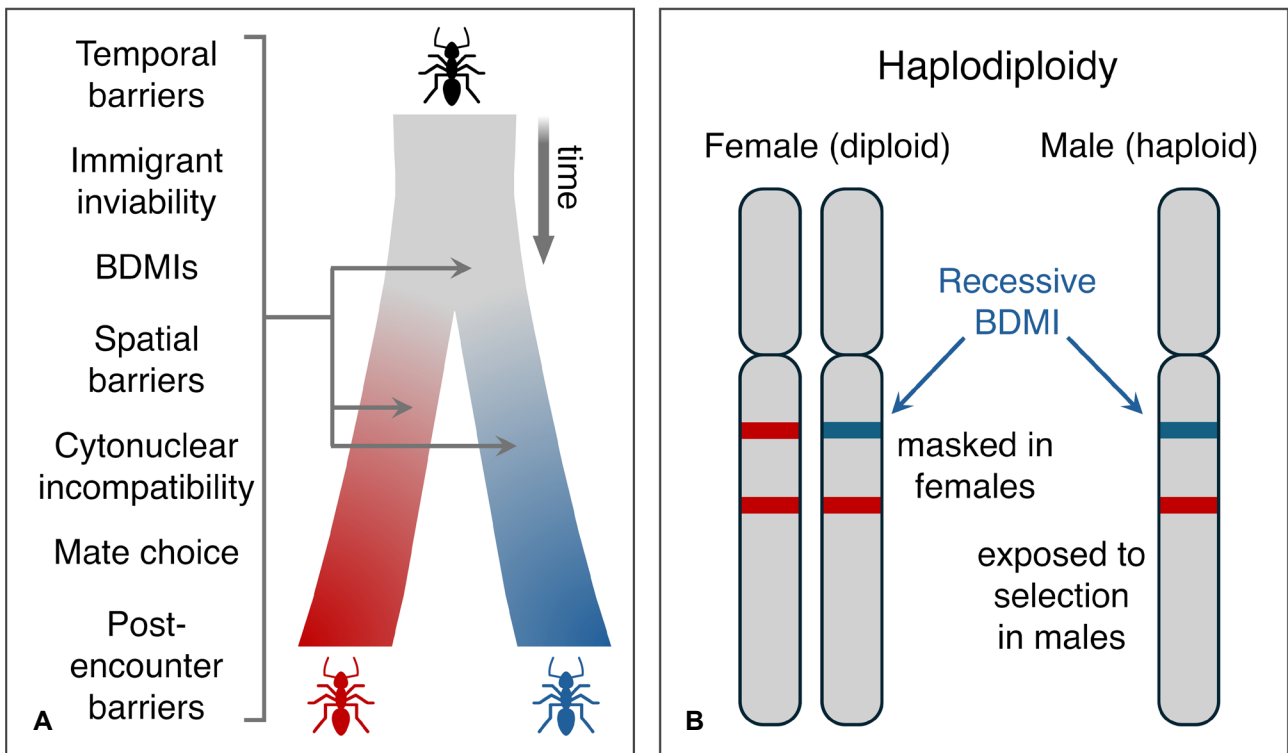
In reviewing the literature on ant speciation research, we find that there has been a wealth of research on the taxonomic distinctions of ants (i.e., species revisions, evolutionary histories, and the classification of new species; Fig. 1). However, the results of these studies are often not

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 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 increased mating



**Fig. 2:** (A) Mechanisms of reproductive isolation, listed on the left, can act at one or multiple points during species divergence (represented by an ancestral black ant diverging into two lineages represented by the red and blue ants, right). See main text section “Mechanisms of postzygotic isolation in ants” for a description of Bateson-Dobzhansky-Muller incompatibilities (BDMIs). In ants, surprisingly few studies explicitly quantify such mechanisms. (B) Sociality and haplodiploidy can alter selective processes and dynamics of speciation in ants but also in bees, wasps, and termites. For example, recessive negative epistasis between two alternative ancestries at two loci (top and bottom red and blue rectangles) is exposed to selection in haploid males (right) where no red ancestry is present at the top locus. In contrast, no selection would occur in diploid females (left) due to the presence of red ancestry at the top locus. In diploid species, no selection would occur in heterozygotes of either sex as the incompatibility is masked.

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 *Myrmica* 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 pat-

terns, 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. Two studies have looked at the *Formica* social supergene as an RI barrier. PURCELL & al. (2016) found that social structure (and therefore supergene haplotype) does not play a role in speciation between *Formica selysi* and *Formica cinerea* as hybrids were found between both single and multiple queen colonies. HEIDBREDE & al. (2025) did not find an enrichment of barrier regions

between *Formica aquilona* and *Formica polyctena* on the social chromosome, which are two species with multiple queen colonies but distinct supergene genotypes (SIGEMAN & al. 2024). With only these two studies, we still lack sufficient data on measures of RI between species with supergenes and the prevalence of social supergenes across ant taxa to draw final conclusions. 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, 2023) or *Heliconius* butterflies (MARTIN & al. 2013) and may play a role in the high rates of hybridization observed in ants.

Finally, as Hymenopterans, ants 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 haplodiploid 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 exam-

ine 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 facilitate hybridization by maintaining hybrid populations through quicker removal of incompatibilities than their diploid counterparts. Finally, two aspects of haplodiploidy may be leveraged to help identify the barrier loci that contain incompatibilities. Experimental research on mapping barrier-loci prevalence and distributions 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 with diploid females (KULMUNI & PAMILO 2014). Second, using haploid males avoids the need to phase diploid genomes (phasing is the separation of the maternally and paternally inherited copies of each chromosome into the haplotypes present in an individual, either statistically or through sequencing technology; reviewed by SUN & LI 2025) 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)). They could also be 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 they could be behavioral, for example, mismatches in courtship behavior as shown between *Drosophila* species (MARKOW 1981, SCHUG & al. 2008). It is of interest to understand which barriers currently exist in ants, what relative importance they have, 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, this occurs through the timing of sexual production 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 flight timing have been suggested to form a prezygotic barrier between species and be important to speciation (HÖLLDOBLER & WILSON 1990). Indeed, HÖLLDOBLER & WILSON (1990) present species-specific diel rhythms as separating flight timing in some *Pogonomyrmex* species, with *Pogonomyrmex maricopa* flying between 1000 and 1130 hours, *Pogonomyrmex barbatus* between 1530 and 1700, and *Pogonomyrmex rugosus* between 1630 and 1800. Similar partitioning of the day has been observed in three *Myrmica* species (KANNOWSKI 1959) and a two-hour offset between *Camponotus culeanus* and *Camponotus 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. As an example, 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 inter-specific matings. For example, *Formica aquilonia* and *Formica 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, KRAPP 2025). 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 (KRAPP & al. 2025). 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 with *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 mating if inter-specific individuals do meet.

Behavioral isolation, that is, all differences between species that reduce attraction and therefore reduce mating chance (COYNE & ORR 2004), is likely partly mediated by chemical cues (i.e., chemosensory speciation, reviewed in SMADJA & BUTLIN 2009), 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 (FIELDE 1901, 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 *Formica 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 host species 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 than 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 in 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 (REGNIER & 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 with 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, ranges 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 & ORR's (2004) definition of habitat isolation, where species exhibit habitat preferences within a heterogeneous area smaller than the dispersal range of either species. HÖLLDOBLER & WILSON (1990) suggest that related species in a genus can be separated by microspatial habitat isolation during mating flights in two ways. The first is the preference for the major habitat occupied by colonies of each species. Such spatial separation between flying queens 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 *Pogonomyrmex maricopa* gathering on bushes, while *Pogonomyrmex barbatus* and *Pogonomyrmex 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, which is home to a unique assemblage of flora. This region appears to result in increased genetic differentiation between *Mystrium barrybressleri* populations, sampled from both the littoral rainforest and other region types, compared with *Mystrium 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 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 that are potentially within dispersal range of one another (SUNDSTRÖM & al. 2005). 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 *Cataglyphis 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 *Pogonomyrmex barbatus*, which segregate at the micro-habitat scale based on soil texture and are sympatric at intermediate textures (JOHNSON 2008).

Generally, allopatry at the macro scale is defined as a reduction in gene flow to zero due to extrinsic physical factors, often a geographic barrier. However, we note that the biogeography of speciation is complex (BUTLIN & al. 2008) and allopatry (and therefore zero gene flow) could also occur through biological differences between species that lead to range differences (COYNE & ORR 2004). Here, we focus on geographic barriers as studies of ants have demonstrated such physical barriers have been important in ant diversification and yielded 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., *Leptothorax muscorum*, and *Formica 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 including Fiji and its endemic dolichoderine ant *Philidris nagasau* (WILSON & TAYLOR 1967, SARNAT & MOREAU 2011, CHOMICKI & al. 2016).

Such cases of allopatry may be interesting for studying the relative importance of drift versus selection (e.g., from adaptation or coevolution) in driving divergence 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, ideally using a standardized framework for classifying RI (WALKER & al. 2025). 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 that 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). Yet, more comparisons are needed before such general conclusions can be drawn. This also applies to habitat divergence. Quantification of prezygotic RI (as proposed by SOBEL & CHEN 2014 and WALKER & al. 2025) in ants provides opportunities for answering questions about the buildup of RI across spatial contexts. Examples for this include: 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 *Solenopsis 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). On the contrary, 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

*Heliconius 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). As a consequence of haplodiploidy, 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). This pattern is consistent with Haldane’s rule, which states that if in hybrid offspring one sex is absent, rare, or sterile, then that sex is the heterogametic (XY or ZW) sex (HALDANE 1922) or in the case of haplodiploids, the hemizygous sex (KOEVOETS & BEUKEBOOM 2009, BEUKEBOOM & al. 2015). This extended Haldane’s rule has been observed in *Formica aquilonia* × *Formica 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, RI is incomplete (SATOKANGAS & al. 2023), and gene flow between the species has occurred asymmetrically throughout their divergence (PORTINHA & al. 2022). In fire ants, hybrids between *Solenopsis invicta* and *Solenopsis richteri* show evidence on intrinsic incompatibilities at the transcriptional level, where hybrids mis-express a low number of genes associated with behavior (OMETTO & al. 2012). 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*, *Tetramorium 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”. Unexpected mitonuclear patterns have been found in *Formica* hybrids. BERESFORD & al. (2017) identified a pattern of mitonuclear mismatch in *Formica aquilonia* × *Formica polyctena* hybrids, where nuclear *F. polyctena*-like individuals tend to carry *F. aquilonia*-like mitochondrial haplotypes and vice-versa. This does not seem to negatively affect the fitness of these hybrids and therefore does not constitute an RI mechanism in this specific system. In crosses between *Formica selysi*

and *Formica cinerea*, hybrid fitness is asymmetrical with 97% of hybrids having *F. cinerea* mothers and *F. selysi* fathers, potentially indicating an incompatibility between the *F. selysi* mitochondrial genome and the *F. cinerea* nuclear genome (PURCELL & al. 2016). 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, *Pogonomyrmex barbatus* and *Pogonomyrmex 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  $A_2A_2c_2$  (where A is a nuclear locus, and c is a mitochondrial locus) 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, but this effect is environment dependent) are an exciting area of research that has attracted recent attention in well-developed model systems such as three-spine sticklebacks (KULMUNI & WESTRAM 2017, THOMPSON & al. 2022, 2024). Studies examining extrinsic incompatibilities in ants are sparse, but there is some evidence for environment dependent incompatibilities in *Formica* wood ants: 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 RI between hybrid lineages and the parent species, is more fully discussed in the section “Hybridization and its implications in ants”.

While multiple mechanisms of postzygotic isolation have been studied in ants, it is still unclear how they contribute to speciation. As in prezygotic mechanisms, further investigations and systematic measures of how each mechanism contributes to RI are required. 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 hypothesis 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 RI 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 20<sup>th</sup> 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 *Formica aquilonia* and *Formica lugubris*. More generally, hybridization provides novel combinations of ancient genetic variants, which can lead to rapid specialization and speciation (KULMUNI & al. 2024), 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 with 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 with 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, that is, 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 *Formica 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 *Pogonomyrmex rugosus* and *Pogonomyrmex 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*. They found this was due to the combined effects of asynchronous mating flights and assortative mating (both prezygotic RI mechanisms), as well as hybrid inviability where 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). These include interactions with other organisms, be them plants (NELSEN & al. 2018), fungi (DEJEAN & al. 2023), or other animals (STADLER & DIXON 2005, PARMENTIER & al. 2020) that likely promoted ant diversification and speciation and led to the evolution of highly specialized, if not unique, characteristics and lifestyles. Under the right conditions, such interactions can promote the development of prezygotic barriers and lead to speciation (WARD 1993). Below, we discuss ant-plant interactions, trophobionts (e.g., sap-sucking insects), fungus-growers, and social parasitism as case studies (Fig. 3a). 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:** 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.



**Fig. 3:** (A) Ants have a high number of interactions with other organisms, which can promote speciation. In the photographs, we see a *Polyrhachis rufipes* tending aphids (top) as well as an *Atta cephalotes* major cutting a leaf which is then used to feed fungi (bottom). (B) 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* (top) and a *Formica rufa* spraying formic acid (bottom). All photographs © Philipp Höhle.

2006, NELSEN & al. 2018). Today, more than 1700 extant ant species, spanning six subfamilies that interact with more than 11,000 plant species are known (HÖLLDOBLER & WILSON 1990, BRONSTEIN & al. 2006, NELSEN & al. 2018). Two well-known examples of ant-plant interactions are between plants of the genus *Vachellia* and acacia ants in the *Pseudomyrmex ferrugineus* group (WARD & BRANSTETTER 2017) and 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 RI in allopatry or parapatry. For example, in the *P. ferrugineus* group, RI 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 RI. This may have led to the divergence of at least ten species in the *P. ferrugineus* group that obligately use *Vachellia* plants. 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 have lost 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 (BARRERA & al. 2021). For example, *Atta colombica* is mainly found in lower-elevation regions in Central America to north-western South America, while sympatric *Atta 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 use different plants. For example, *Atta vollenweideri* and *Atta goiana* forage mainly for monocotyledonous plants, while *Atta saltensis* and *Atta 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, *Atta cubana* occurs sympatrically with *Atta 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 RI. These examples illustrate 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 and / or lead to adaptive radiations, 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 4000 aphid species worldwide 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). As myrmecophily has arisen multiple times independently (STADLER & al. 2003), it could be that a new niche opened that led to ecological speciation of a species, possibly followed by an adaptive radiation. 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: 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 RI 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 *Blochmannia* or *Wolbachia*, can induce changes in sexual reproduction and lead to RI and speciation (ROUSSET & al. 1992, DEGNAN & al. 2004). A 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 61 species across seven ant subfamilies (WENSELEERS & al. 1998, RAMALHO & al. 2021). Infection by *Wolbachia* can result in cytoplasmic incompatibility, 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 the mosquito *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. *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). 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). GOODISMANN & al. (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 *Solenopsis richteri*. These species do not hybridize in their native South American range but they do in their introduced range in North America (ROSS & ROBERTSON 1990). 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. (2008) interpreted 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 for 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) Sexu- als 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

**Tab. 1:** Subfamilies, estimated number of species, and geographic region associated with specific lifestyles discussed in the Section “Interaction with other organisms”. Note: This is a non-exhaustive list of lifestyles, subfamilies, number of species, and geographic regions.

Lifestyle	Species, genus, tribe, clade, or subfamily	No of species	Geographic region
Ant-plant interactions	six subfamilies, namely Dolichoderinae, Dorylinae, Formicinae, Myrmicinae, Ponerinae, and Pseudomyrmecinae; Myrmicinae are the most frequently interacting with plants	> 1700	globally
Ant-plant interactions	<i>Pseudomyrmex ferrugineus</i> clade (subfamily Pseudomyrmecinae)	10	Neotropics
Ant-plant interactions	species <i>Phildris nagasau</i> (subfamily Dolichoderinae)	1	Neotropics
Fungus-farming ants	tribe Attini (subfamily Myrmicinae), which are all fungus-farming ants	232 (excl. the 15 below)	Neotropics
Fungus-farming ants	genus <i>Atta</i> (subfamily Myrmicinae)	15	Neotropics
Aphid tending	four subfamilies, namely Dolichoderinae, Formicinae, Myrmicinae, and Pseudomyrmecinae; Formicinae are the most frequent aphid tenders	> 190	globally
Endosymbionts	<i>Blochmannia</i> ; tribe Camponotini (subfamily Formicinae)	> 1500	globally
Endosymbionts	<i>Wolbachia</i> ; seven extant subfamilies, namely Dolichoderinae, Dorylinae, Ectatomminae, Formicinae, Myrmeciinae, Myrmicinae, and Pseudomyrmecinae	> 60	globally
Social parasites	six extant subfamilies, namely Dolichoderinae, Ectatomminae, Formicinae, Myrmeciinae, Myrmicinae, and Pseudomyrmecinae	> 400	globally
<b>Total</b>		<b>&gt; 4108</b>	

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 worldwide (BOLTON 2025), more than 4108 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, Dorylinae, 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 earliest ant fossil record dates back to 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, PERFILEIEVA 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, PERFILEIEVA 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 conservatism 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 conservatism hypothesis, which proposes that tropic-adapted species have existed for longer since temperate environments only developed later, is the most likely. The same study found no 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 conservatism hypothesis rather than the diversification-rate hypothesis (PIE 2016). However, the tropical conservatism 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 expected.

In general, positive shifts in diversification rate are often associated with either increased ecological opportunity, for example, 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 Mya, fossils indicate a rise 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. 1; 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 (> 1700 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 & 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, that is, 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 it 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. 3B; 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 net diversification rate, that is, speciation minus extinction, for the two genera with the trap-jaw mechanism in the Ponerinae subfamily (*Anochetus* and *Odontomachus*) is estimated to be 0.064, which is twice the overall rate of diversification of the Ponerinae subfamily as a whole (LARABEE & al. 2016). This indicates that these trap-jaw lineages have accumulated species roughly twice as quickly as other ponerine ants. A higher diversification in genera with the trap-jaw mechanism has also been observed within the subfamily Myrmicinae (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. 3b), 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 abundant 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 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 micro-

evolutionary 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). Many speciation events 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 the 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 is 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 RI. 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 investiga-

tion 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 ant speciation. A standardized framework for classifying RI across the tree of life (WALKER & al. 2025) will facilitate comparisons across ants and with other taxa.

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 endeavor 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 for unlocking new knowledge on speciation.

## Acknowledgments

We thank Noora Poikela for helpful discussion on the manuscript as well as the editor-in-chief Florian Steiner, the subject editor Timothy Linksvayer, and two anonymous reviewers for helpful comments on an earlier version of the manuscript. We thank Philipp Höhle for sharing the four ant pictures displayed in Figure 3. This work was funded by a Research Council of Finland grant no. 346805 awarded to J.K., P.H. was funded by a University of Helsinki LUOVA Doctoral Programme funded Doctoral researcher position, P.K. was funded by the European Union's Horizon Europe programme under Marie Skłodowska-Curie Actions (MSCA) - Postdoctoral fellowship grant agreement no. 101204375, and B.P. was funded by FCT - Fundação para a Ciência e Tecnologia, I.P. by an individual PhD scholarship (2021.07182.BD, <https://doi.org/10.54499/2021.07182.BD>), and M.C. by a grant from the Swiss National Science Foundation (310030-207642).

## Declaration on use of generative artificial intelligence tools

The authors declare that they did not utilize generative artificial intelligence tools in any part of the composition of this manuscript except that the artificial-intelligence tool ChatGPT was used to produce code for data processing for Figure 1. The authors take full responsibility of the content of the manuscript in every detail.

## References

- ANDERSON, K.E., GADAU, J., MOTT, B.M., JOHNSON, R.A., ALTAMIRANO, A., STREHL, C. & FEWELL, J.H. 2006: Distribution and evolution of genetic caste determination in *Pogonomyrmex* seed-harvester ants. – *Ecology* 87: 2171-2184.
- ANDERSON, S.A.S. & WEIR, J.T. 2022: The role of divergent ecological adaptation during allopatric speciation in vertebrates. – *Science* 378: 1214-1218.
- ARON, S., PASSERA, L. & KELLER, L. 2004: Evolution of miniaturisation in inquiline parasitic ants: timing of male elimination in *Plagiolepis pygmaea*, the host of *Plagiolepis xene*. – *Insectes Sociaux* 51: 395-399.
- EVERY, P.J. 1984: The population genetics of haplo-diploids and X-linked genes. – *Genetics Research* 44: 321-341.
- AYALA-LOPEZ, J.A. & BANK, C. 2024: What can we gain from modeling complex hybrid incompatibilities? – *Evolutionary Journal of the Linnean Society* 4: art. kzae034.
- BALDRIDGE, R.S., RETTENMEYER, C.W. & WATKINS, J.F. 1980: Seasonal, nocturnal and diurnal flight periodicities of nearctic army ant males (Hymenoptera: Formicidae). – *Journal of the Kansas Entomological Society* 53: 189-204.
- BARCELLOS, M.S., MARTINS, L.C.B., COSSOLIN, J.F.S., SERRÃO, J.E., DELABIE, J.H.C. & LINO-NETO, J. 2015: Testes and spermatozoa as characters for distinguishing two ant species of the genus *Neoponera* (Hymenoptera: Formicidae). – *Florida Entomologist* 98: 1254-1256.
- BARDEN, P. & ENGEL, M.S. 2020: Fossil social insects. In: STARR, C. (Ed.): *Encyclopedia of social insects*. – Springer International Publishing, Cham, pp. 1-21.
- BARNARD-KUBOW, K.B., SO, N. & GALLOWAY, L.F. 2016: Cytonuclear incompatibility contributes to the early stages of speciation. – *Evolution* 70: 2752-2766.
- BARRERA, C.A., SOSA-CALVO, J., SCHULTZ, T.R., RABELING, C. & BACCI Jr, M. 2021: Phylogenomic reconstruction reveals new insights into the evolution and biogeography of *Atta* leaf-cutting ants (Hymenoptera: Formicidae). – *Systematic Entomology* 47: 13-35.
- BARTH, M.B., MORITZ, R.F.A. & KRAUS, F.B. 2014: The evolution of extreme polyandry in social insects: insights from army ants. – *Public Library of Science One* 9: art. e105621.
- BATESON, W. 1909: Heredity and variation in modern lights. In: SEWARD, A.C. (Ed.): *Darwin and modern science: Essays in commemoration of the centenary of the birth of Charles Darwin and of the fiftieth anniversary of the publication of the Origin of Species*. – Cambridge University Press, Cambridge, UK, pp. 85-101.
- BEIBL, J., BUSCHINGER, A., FOITZIK, S. & HEINZE, J. 2007: Phylogeny and phylogeography of the Mediterranean species of the parasitic ant genus *Chalepoxenus* and its *Temnothorax* hosts. – *Insectes Sociaux* 54: 189-199.
- BELL-ROBERTS, L., TURNER, J.F., WERNER, G.D., DOWNING, P.A., ROSS, L. & WEST, S.A. 2024: Larger colony sizes favoured the evolution of more worker castes in ants. – *Nature Ecology and Evolution* 8: 1959-1971.
- BERESFORD, J. 2021: The role of hybrids in the process of speciation: a study of naturally occurring *Formica* wood ant hybrids. – Ph.D. thesis, University of Helsinki, Helsinki, 44pp.
- BERESFORD, J., ELIAS, M., PLUCKROSE, L., SUNDSTRÖM, L., BUTLIN, R.K., PAMILO, P. & KULMUNI, J. 2017: Widespread hybridization within mound-building wood ants in southern Finland results in cytonuclear mismatches and potential for sex-specific hybrid breakdown. – *Molecular Ecology* 26: 4013-4026.

- BEUKEBOOM, L.W., KOEVOETS, T., MORALES, H.E., FERBER, S. & VAN DE ZANDE, L. 2015: Hybrid incompatibilities are affected by dominance and dosage in the haplodiploid wasp *Nasonia*. – *Frontiers in Genetics* 6: art. 140.
- BLACHER, P., ZAHND, S., PURCELL, J., AVRIL, A., HONORATO, T.O., BAILAT-ROSSET, G., STAEDLER, D., BRELSFORD, A. & CHAPUISAT, M. 2022: Species recognition limits mating between hybridizing ant species. – *Evolution* 76: 2105-2115.
- BLAIMER, B.B., SANTOS, B.F., CRUAUD, A., GATES, M.W., KULA, R.R., MIKÓ, I., RASPLUS, J.-Y., SMITH, D.R., TALAMAS, E.J., BRADY, S.G. & BUFFINGTON, M.L. 2023: Key innovations and the diversification of Hymenoptera. – *Nature Communications* 14: art. 1212.
- BOLNICK, D.I. & FITZPATRICK, B.M. 2007: Sympatric speciation: models and empirical evidence. – *Annual Review of Ecology, Evolution, and Systematics* 38: 459-487.
- BOLTON, B. 2025: An online catalog of the ants of the world. – <<https://antcat.org>>, retrieved on 10 July 2025.
- BOOHER, D.B., GIBSON, J.C., LIU, C., LONGINO, J.T., FISHER, B.L., JANDA, M., NARULA, N., TOULKERIDOU, E., MIKHEYEV, A.S., SUAREZ, A.V. & ECONOMO, E.P. 2021: Functional innovation promotes diversification of form in the evolution of an ultrafast trap-jaw mechanism in ants. – *Public Library of Science Biology* 19: art. e3001031.
- BOOMSMA, J.J. 2022: Domains and major transitions of social evolution. – Oxford University Press, Oxford, UK, 315 pp.
- BOOMSMA, J.J. & GAWNE, R. 2018: Superorganismality and caste differentiation as points of no return: how the major evolutionary transitions were lost in translation. – *Biological Reviews* 93: 28-54.
- BOOMSMA, J.J. & LEUSINK, A. 1981: Weather conditions during nuptial flights of four European ant species. – *Oecologia* 50: 236-241.
- BOOMSMA, J.J. & VAN DER HAVE, T.M. 2002: Queen mating and paternity variation in the ant *Lasius niger*. – *Molecular Ecology* 7: 1709-1718.
- BOROWIEC, M.L., COVER, S.P. & RABELING, C. 2021: The evolution of social parasitism in *Formica* ants revealed by a global phylogeny. – *Proceedings of the National Academy of Sciences of the United States of America* 118: art. e2026029118.
- BOUDINOT, B.E., BOROWIEC, M.L. & PREBUS, M.M. 2022a: Phylogeny, evolution, and classification of the ant genus *Lasius*, the tribe Lasini and the subfamily Formicinae (Hymenoptera: Formicidae). – *Systematic Entomology* 47: 113-151.
- BOUDINOT, B.E., RICHTER, A., KATZKE, J., CHAUL, J.C.M., KELLER, R.A., ECONOMO, E.P., BEUTEL, R.G. & YAMAMOTO, S. 2022b: Evidence for the evolution of eusociality in stem ants and a systematic revision of †*Gerontofornica* (Hymenoptera: Formicidae). – *Zoological Journal of the Linnean Society* 195: 1355-1389.
- BOURKE, A.F.G. & FRANKS, N.R. 1991: Alternative adaptations, sympatric speciation and the evolution of parasitic, inquiline ants. – *Biological Journal of the Linnean Society* 43: 157-178.
- BOURTZIS, K. & O'NEILL, S. 1998: *Wolbachia* infections and arthropod reproduction: *Wolbachia* can cause cytoplasmic incompatibility, parthenogenesis, and feminization in many arthropods. – *BioScience* 48: 287-293.
- BRANSTETTER, M.G., JEŠOVNIK, A., SOSA-CALVO, J., LLOYD, M.W., FAIRCLOTH, B.C., BRADY, S.G. & SCHULTZ, T.R. 2017: Dry habitats were crucibles of domestication in the evolution of agriculture in ants. – *Proceedings of the Royal Society B-Biological Sciences* 284: art. 20170095.
- BRONSTEIN, J.L., ALARCÓN, R. & GEBER, M. 2006: The evolution of plant-insect mutualisms. – *New Phytologist* 172: 412-428.
- BROWER, A.V.Z. 2013: Introgression of wing pattern alleles and speciation via homoploid hybridization in *Heliconius* butterflies: a review of evidence from the genome. – *Proceedings of the Royal Society B-Biological Sciences* 280: art. 20122302.
- BUSCHINGER, A. 2009: Social parasitism among ants: a review (Hymenoptera: Formicidae). – *Myrmecological News* 12: 219-235.
- BUTLIN, R.K., GALINDO, J. & GRAHAME, J.W. 2008: Sympatric, parapatric or allopatric: the most important way to classify speciation? – *Philosophical Transactions of the Royal Society B-Biological Sciences* 363: 2997-3007.
- CAHAN, S.H., PARKER, J.D., RISSING, S.W., JOHNSON, R.A., POLONY, T.S., WEISER, M.D. & SMITH, D.R. 2002: Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants. – *Proceedings of the Royal Society B-Biological Sciences* 269: 1871-1877.
- CHAPUISAT, M. 2023a: Evolution: a social parasite was born from a virgin. – *Current Biology* 33: 225-228.
- CHAPUISAT, M. 2023b: Supergenes as drivers of ant evolution. – *Myrmecological News* 33: 1-18.
- CHAPUISAT, M., GOUDET, J. & KELLER, L. 1997: Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. – *Evolution* 51: 475-482.
- CHARLESWORTH, B., COYNE, J.A. & BARTON, N.H. 1987: The relative rates of evolution of sex chromosomes and autosomes. – *The American Naturalist* 130: 113-146.
- CHENG, D., CHEN, S., HUANG, Y., PIERCE, N.E., RIEGLER, M., YANG, F., ZENG, L., LU, Y., LIANG, G. & XU, Y. 2019: Symbiotic microbiota may reflect host adaptation by resident to invasive ant species. – *Public Library of Science Pathogens* 15: art. e1007942.
- CHOMICKI, G. & RENNER, S.S. 2016: Obligate plant farming by a specialized ant. – *Nature Plants* 2: art. 16181.
- CHOMICKI, G., STAEDLER, Y.M., SCHÖNENBERGER, J. & RENNER, S.S. 2016: Partner choice through concealed floral sugar rewards evolved with the specialization of ant-plant mutualisms. – *New Phytologist* 211: 1358-1370.
- CHRISTIE, K., FRASER, L.S. & LOWRY, D.B. 2022: The strength of reproductive isolating barriers in seed plants: insights from studies quantifying premating and postmating reproductive barriers over the past 15 years. – *Evolution* 76: 2228-2243.
- COHEN, P. & PRIVMAN, E. 2019: Speciation and hybridization in invasive fire ants. – *BioMed Central Evolutionary Biology* 19: art. 111.
- CORDONNIER, M., ESCARGUEL, G., DUMET, A. & KAUFMANN, B. 2020: Multiple mating in the context of interspecific hybridization between two *Tetramorium* ant species. – *Heredity* 124: 675-684.
- COUGHLAN, J.M. & MATUTE, D.R. 2020: The importance of intrinsic postzygotic barriers throughout the speciation process. – *Philosophical Transactions of the Royal Society B-Biological Sciences* 375: art. 20190533.
- COYNE, J.A. & ORR, H.A. 2004: Speciation. – Sinauer Associates Inc., Sunderland, MA, 545 pp.
- DEGNAN, P.H., LAZARUS, A.B., BROCK, C.D. & WERNEGREN, J.J. 2004: Host-symbiont stability and fast evolutionary rates in an ant-bacterium association: cospeciation of *Camponotus* species and their endosymbionts, *Candidatus* Blochmannia. – *Systematic Biology* 53: 95-110.
- DEJEAN, A., AZÉMAR, F., NASKRECKI, P., TINDO, M., ROSSI, V., FAUCHER, C. & GRUYA, H. 2023: Mutualistic interactions between ants and fungi: a review. – *Ecology and Evolution* 13: art. e10386.

- DELABIE, J.H.C. 2001: Trophobiosis between Formicidae and Hemiptera (Sternorrhyncha and Auchenorrhyncha): an overview. – *Neotropical Entomology* 30: 501-516.
- DE LA FILIA, A.G., BAIN, S.A. & ROSS, L. 2015: Haplodiploidy and the reproductive ecology of arthropods. – *Current Opinion in Insect Science* 9: 36-43.
- DESHMUKH, R., BARAL, S., KUWALEKAR, M., KIZHAKKE, A.G. & KUNTE, K. 2025: Reproductive barriers and genomic hotspots of adaptation during allopatric species divergence. – *Molecular Ecology* 34: art. e17703.
- DIETRICH, B. & WEHNER, R. 2003: Sympatry and allopatry in two desert ant sister species: How do *Cataglyphis bicolor* and *C. savignyi* coexist? – *Oecologia* 136: 63-72.
- DOBZHANSKY, T. 1936: Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. – *Genetics* 21: 113-135.
- DOBZHANSKY, T.G. 1937: Genetics and the origin of species. – Columbia University Press, New York, NY, 364 pp.
- DOUGLAS, A. & BROWN, W.L. 1959: *Myrmecia inquilina* new species: the first parasite among the lower ants. – *Insectes Sociaux* 6: 13-19.
- DOUWES, P. 2012: Formicinae - bitmyror. In: Nationalnyckeln till Sveriges flora och fauna. Steklar: Myror-getingar. Hymenoptera: Formicidae-Vespidae. – ArtDatabanken, SLU, Uppsala, pp. 128-199.
- ECONOMO, E.P., HUANG, J.-P., FISCHER, G., SARNAT, E.M., NARULA, N., JANDA, M., GUÉNARD, B., LONGINO, J.T. & KNOWLES, L.L. 2019: Evolution of the latitudinal diversity gradient in the hyperdiverse ant genus *Pheidole*. – *Global Ecology and Biogeography* 28: 456-470.
- ECONOMO, E.P., NARULA, N., FRIEDMAN, N.R., WEISER, M.D. & GUÉNARD, B. 2018: Macroecology and macroevolution of the latitudinal diversity gradient in ants. – *Nature Communications* 9: art. 1778.
- EMERY, C. 1909: Über den Ursprung der dulotischen, parasitischen und myrmekophilen Ameisen. – *Biologisches Centralblatt* 29: 352-362.
- FELDHAAR, H., FOITZIK, S. & HEINZE, J. 2008: Lifelong commitment to the wrong partner: hybridization in ants. – *Philosophical Transactions of the Royal Society B-Biological Sciences* 363: 2891-2899.
- FIELDE, A.M. 1901: Further study of an ant. – *Proceedings of the Academy of Natural Sciences of Philadelphia* 53: 521-544.
- FIELDE, A.M. 1903: Supplementary notes on an ant. – *Proceedings of the Academy of Natural Sciences of Philadelphia* 55: 491-495.
- FISCHER, G., FRIEDMAN, N.R., HUANG, J.-P., NARULA, N., KNOWLES, L.L., FISHER, B.L., MIKHEYEV, A.S. & ECONOMO, E.P. 2020: Socially parasitic ants evolve a mosaic of host-matching and parasitic morphological traits. – *Current Biology* 30: 3639-3646.e4.
- FISHER, B.L. & GIRMAN, D.J. 2000: Biogeography of ants in eastern Madagascar. In: LOURENÇO, W. & GOODMAN, S. (Eds.): *Diversité et endémisme à Madagascar*. – Mémoires de la Société de Biogéographie, Paris, pp. 331-344.
- FONTENLA, J.L. 1995: New species of *Atta* (Hymenoptera: Formicidae) from Cuban archipelago. – *Avicennia* 3: 77-86.
- GARLOVSKY, M.D. & SNOOK, R.R. 2018: Persistent postmating, prezygotic reproductive isolation between populations. – *Ecology and Evolution* 8: 9062-9073.
- GOMPERT, Z., FEDER, J.L., PARCHMAN, T.L., PLANIDIN, N.P., WHITING, F.J.H. & NOSIL, P. 2025: Adaptation repeatedly uses complex structural genomic variation. – *Science* 388: art. eadp3745.
- GOODISMAN, M.A.D., SHOEMAKER, D.D. & ASMUSSEN, M.A. 1998: Cytonuclear theory for haplodiploid species and X-linked genes. II. Stepping-stone models of gene flow and application to a fire ant hybrid zone. – *Evolution* 52: 1423-1440.
- GOROPASHNAYA, A.V., FEDOROV, V.B. & PAMILO, P. 2004: Recent speciation in the *Formica rufa* group ants (Hymenoptera, Formicidae): inference from mitochondrial DNA phylogeny. – *Molecular Phylogenetics and Evolution* 32: 198-206.
- GOROPASHNAYA, A.V., FEDOROV, V.B., SEIFERT, B. & PAMILO, P. 2012: Phylogenetic relationships of palaeartic *Formica* species (Hymenoptera, Formicidae) based on mitochondrial cytochrome b sequences. – *Public Library of Science One* 7: art. e41697.
- GRAHAM, N.R., FISHER, B.L. & GIRMAN, D.J. 2016: Phylogeography in response to reproductive strategies and ecogeographic isolation in ant species on Madagascar: genus *Mystrium* (Formicidae: Amblyoponinae). – *Public Library of Science One* 11: art. e0146170.
- GREENBERG, L., JOHNSON, C.A., TRAGER, J.C., MCELFFRESH, J.S., RODSTEIN, J. & MILLAR, J.G. 2018: Sex attractant pheromones of virgin queens of sympatric slave-making ant species in the genus *Polyergus*, and their possible roles in reproductive isolation. – *Journal of Chemical Ecology* 44: 547-555.
- GREENBERG, L., TRÖGER, A.G., FRANCKE, W., MCELFFRESH, J.S., TOPOFF, H., ALIABADI, A. & MILLAR, J.G. 2007: Queen sex pheromone of the slave-making ant *Polyergus breviceps*. – *Journal of Chemical Ecology* 33: 935-945.
- GRIMALDI, D. & AGOSTI, D. 2000: A formicine in New Jersey Cretaceous amber (Hymenoptera: Formicidae) and early evolution of the ants. – *Proceedings of the National Academy of Sciences of the United States of America* 97: 13678-13683.
- GULL-E-FAREEN, A., BODLAH, I., RASHEED, M.T., NIAZ, Y., BODLAH, M.A., ASIF, M. & KHOKHAR N.M. 2021: Trophic associations of ants with aphid partners and new distribution records of some ants in Pothwar region of Pakistan. – *Pakistan Journal of Zoology* 53: 101-110.
- HABE, S., MATSUYAMA, S., KANZAKI, N., HAMAGUCHI, K., OZAKI, M. & AKINO, T. 2024: Male-specific substances possibly contributing to nuptial flight of the Japanese carpenter ant *Camponotus japonicus* (Hymenoptera: Formicidae). – *Journal of Chemical Ecology* 50: 884-893.
- HALDANE, J.B.S. 1922: Sex ratio and unisexual sterility in hybrid animals. – *Journal of Genetics* 12: 101-109.
- HANDEL, S.N. & BEATTIE, A.J. 1990: Seed dispersal by ants. – *Scientific American* 263: 76-83B.
- HARTL, D.L. 1971: Some aspects of natural selection in arrhenotokous populations. – *American Zoologist* 11: 309-325.
- HAYNES, K.F. & BIRCH, M.C. 1986: Temporal reproductive isolation between two species of plume moths (Lepidoptera: Pterophoridae). – *Annals of the Entomological Society of America* 79: 210-215.
- HEIDBREDER, P., POIKELA, N., NOUHAUD, P., PUUKKO, T., LOHSE, K. & KULMUNI, J. 2025: Genomic incompatibilities are persistent barriers when speciation happens with gene flow in *Formica* ants. – *BioRxiv*; doi: 10.1101/2025.03.27.645773.
- HELANTERÄ, H. 2016: An organismal perspective on the evolution of insect societies. – *Frontiers in Ecology and Evolution* 4: art. 6.
- HELANTERÄ, H. 2022: Supercolonies of ants (Hymenoptera: Formicidae): ecological patterns, behavioural processes and their implications for social evolution. – *Myrmecological News* 32: 1-22.
- HELMS, K.R. & HELMS CAHAN, S. 2010: Divergence in mating-flight patterns of the seed-harvester ant *Pogonomyrmex rugosus* (Hymenoptera: Formicidae) in the western Mojave Desert. – *Myrmecological News* 13: 15-17.

- HELMS CAHAN, S. & KELLER, L. 2003: Complex hybrid origin of genetic caste determination in harvester ants. – *Nature* 424: 306-309.
- HENDRY, A.P. 2001: Adaptive divergence and the evolution of reproductive isolation in the wild: an empirical demonstration using introduced sockeye salmon. – *Genetica* 112: 515-534.
- HODGES, S.A. & ARNOLD, M.L. 1995: Spurring plant diversification: Are floral nectar spurs a key innovation? – *Proceedings of the Royal Society B-Biological Sciences* 262: 343-348.
- HÖLLDOBLER, B. 1971: Sex pheromone in the ant *Xenomyrmex floridanus*. – *Journal of Insect Physiology* 17: 1497-1499.
- HÖLLDOBLER, B. 1976: Recruitment behavior, home range orientation and territoriality in harvester ants, *pogonomyrmex*. – *Behavioral Ecology and Sociobiology* 1: 3-44.
- HÖLLDOBLER, B. & BARTZ, S.H. 1985: Sociobiology of reproduction in ants. – *Fortschritte der Zoologie* 31: 237-257.
- HÖLLDOBLER, B. & WILSON, E.O. 1990: *The ants*. – Springer-Verlag, Berlin, 784 pp.
- JEAN, P. & JEAN-CHRISTOPHE, S. 2010: The pea aphid complex as a model of ecological speciation. – *Ecological Entomology* 35: 119-130.
- JOHNSON, R.A. 2008: Habitat segregation based on soil texture and body size in the seed-harvester ants *Pogonomyrmex rugosus* and *P. barbatus*. – *Ecological Entomology* 25: 403-412.
- JULIAN, G.E., FEWELL, J.H., GADAU, J., JOHNSON, R.A. & LARABEE, D. 2002: Genetic determination of the queen caste in an ant hybrid zone. – *Proceedings of the National Academy of Sciences of the United States of America* 99: 8157-8160.
- KANNOVSKI, P.B. 1959: The flight activities and colony-founding behavior of bog ants in southeastern Michigan. – *Insectes Sociaux* 6: 115-162.
- KASPARI, M., WARD, P.S. & YUAN, M. 2004: Energy gradients and the geographic distribution of local ant diversity. – *Oecologia* 140: 407-413.
- KOCH, E.L., MORALES, H.E., LARSSON, J., WESTRAM, A.M., FARIA, R., LEMMON, A.R., LEMMON, E.M., JOHANNESSEN, K. & BUTLIN, R.K. 2021: Genetic variation for adaptive traits is associated with polymorphic inversions in *Littorina saxatilis*. – *Evolution Letters* 5: 196-213.
- KOEVOETS, T. & BEUKEBOOM, L.W. 2009: Genetics of postzygotic isolation and Haldane's rule in haplodiploids. – *Heredity* 102: 16-23.
- KRAPF, P., HEIDBREDER, P., BRUIJNING, M., MEIRMANS, P.G., STANKOWSKI, S. & KULMUNI, J. 2025: Evidence for climate-mediated range expansion of hybrid wood ants. – *BioRxiv*; doi: 10.1101/2025.09.25.678544
- KRONAUER, D.J.C., O'DONNELL, S., BOOMSMA, J.J. & PIERCE, N.E. 2011: Strict monandry in the ponerine army ant genus *Simopelta* suggests that colony size and complexity drive mating system evolution in social insects. – *Molecular Ecology* 20: 420-428.
- KULMUNI, J., BUTLIN, R.K., LUCEK, K., SAVOLAINEN, V. & WESTRAM, A.M. 2020a: Towards the completion of speciation: the evolution of reproductive isolation beyond the first barriers. – *Philosophical Transactions of the Royal Society B-Biological Sciences* 375: art. 20190528.
- KULMUNI, J., NOUHAUD, P., PLUCKROSE, L., SATOKANGAS, I., DHAYGUDE, K. & BUTLIN, R.K. 2020b: Instability of natural selection at candidate barrier loci underlying speciation in wood ants. – *Molecular Ecology* 29: 3988-3999.
- KULMUNI, J. & PAMILO, P. 2014: Introgression in hybrid ants is favored in females but selected against in males. – *Proceedings of the National Academy of Sciences of the United States of America* 111: 12805-12810.
- KULMUNI, J., SEIFERT, B. & PAMILO, P. 2010: Segregation distortion causes large-scale differences between male and female genomes in hybrid ants. – *Proceedings of the National Academy of Sciences of the United States of America* 107: 7371-7376.
- KULMUNI, J. & WESTRAM, A.M. 2017: Intrinsic incompatibilities evolving as a by-product of divergent ecological selection: considering them in empirical studies on divergence with gene flow. – *Molecular Ecology* 26: 3093-3103.
- KULMUNI, J., WILEY, B. & OTTO, S.P. 2024: On the fast track: Hybrids adapt more rapidly than parental populations in a novel environment. – *Evolution Letters* 8: 128-136.
- LACKEY, A.C.R. & BOUGHMAN, J.W. 2017: Evolution of reproductive isolation in stickleback fish. – *Evolution* 71: 357-372.
- LAPOLLA, J.S. 2005: Ancient trophophoresy: a fossil *Acropyga* (Hymenoptera: Formicidae) from dominican amber. – *Transactions of the American Entomological Society* 131: 21-28.
- LAPOLLA, J.S., COVER, S.P. & MUELLER, U.G. 2002: Natural history of the mealybug-tending ant, *Acropyga epedana*, with descriptions of the male and queen castes. – *Transactions of the American Entomological Society* 128: 367-376.
- LAPOLLA, J.S., DLUSKY, G.M. & PERRICHOT, V. 2013: Ants and the fossil record. – *Annual Review of Entomology* 58: 609-630.
- LARABEE, F.J., FISHER, B.L., SCHMIDT, C.A., MATOS-MARAVÍ, P., JANDA, M. & SUAREZ, A.V. 2016: Molecular phylogenetics and diversification of trap-jaw ants in the genera *Anochetus* and *Odontomachus* (Hymenoptera: Formicidae). – *Molecular Phylogenetics and Evolution* 103: 143-154.
- LARABEE, F.J. & SUAREZ, A.V. 2014: The evolution and functional morphology of trap-jaw ants (Hymenoptera: Formicidae). – *Myrmecological News* 20: 25-36.
- LAVANCHY, G. & SCHWANDER, T. 2019: Hybridogenesis. – *Current Biology* 29: 9-11.
- LAVEN, H. 1967: Speciation and evolution in *Culex pipiens*. In: WRIGHT, J.W. & PAL, R. (Eds.): *Genetics of insect vectors of disease*. – Elsevier, Amsterdam, pp. 251-275.
- LENIAUD, L., DARRAS, H., BOULAY, R. & ARON, S. 2012: Social hybridogenesis in the clonal ant *Cataglyphis hispanica*. – *Current Biology* 22: 1188-1193.
- LEPPÄNEN, J., SEPPÄ, P., VEPSÄLÄINEN, K. & SAVOLAINEN, R. 2015: Genetic divergence between the sympatric queen morphs of the ant *Myrmica rubra*. – *Molecular Ecology* 24: 2463-2476.
- LI, J., SCHUMER, M. & BANK, C. 2022: Imbalanced segregation of recombinant haplotypes in hybrid populations reveals inter- and intrachromosomal Dobzhansky-Muller incompatibilities. – *Public Library of Science Genetics* 18: art. e1010120.
- LINKSVAYER, T.A., WADE, M.J. & GORDON, D.M. 2006: Genetic caste determination in harvester ants: possible origin and maintenance by cyto-nuclear epistasis. – *Ecology* 87: 2185-2193.
- LÖFSTEDT, C., HERREBOUT, W.M. & MENKEN, S.B.J. 1991: Sex pheromones and their potential role in the evolution of reproductive isolation in small ermine moths (Yponomeutidae). – *Chemoecology* 2: 20-28.
- LUCKY, A., TRAUTWEIN, M.D., GUÉNARD, B.S., WEISER, M.D. & DUNN, R.R. 2013: Tracing the rise of ants - out of the ground. – *Public Library of Science One* 8: art. e84012.
- MANTHEY, J.D., GIRÓN, J.C. & HRUSKA, J.P. 2022: Impact of host demography and evolutionary history on endosymbiont molecular evolution: a test in carpenter ants (genus *Camponotus*) and their *Blochmannia* endosymbionts. – *Ecology and Evolution* 12: art. e9026.

- MARKOW, T.A. 1981: Courtship behavior and control of reproductive isolation between *Drosophila mojavensis* and *Drosophila arizonensis*. – *Evolution* 35: 1022-1026.
- MARQUES, D.A., MEIER, J.I. & SEEHAUSEN, O. 2019: A combinatorial view on speciation and adaptive radiation. – *Trends in Ecology & Evolution* 34: 531-544.
- MARTIN, S. & DRIJFHOUT, F. 2009: A review of ant cuticular hydrocarbons. – *Journal of Chemical Ecology* 35: 1151-1161.
- MARTIN, S.H., DASMAHAPATRA, K.K., NADEAU, N.J., SALAZAR, C., WALTERS, J.R., SIMPSON, F., BLAXTER, M., MANICA, A., MALLETT, J. & JIGGINS, C.D. 2013: Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. – *Genome Research* 23: 1817-1828.
- MARTIN, S.J., HELANTERÄ, H. & DRIJFHOUT, F.P. 2008: Evolution of species-specific cuticular hydrocarbon patterns in *Formica* ants. – *Biological Journal of the Linnean Society* 95: 131-140.
- MARTIN-ROY, R., NYGÅRD, E., NOUHAUD, P. & KULMUNI, J. 2021: Differences in thermal tolerance between parental species could fuel thermal adaptation in hybrid wood ants. – *The American Naturalist* 198: 278-294.
- MATTE, A. & LEBOEUF, A.C. 2025: Innovation in ant larval feeding facilitated queen-worker divergence and social complexity. – *Proceedings of the National Academy of Sciences of the United States of America* 122: art. e2413742122.
- MCCUSKEY, E.S. 1992: Periodicity and diversity in ant mating flights. – *Comparative Biochemistry and Physiology Part A: Physiology* 103: 241-243.
- MEER, R.K.V., LOFGREN, C.S. & ALVAREZ, F.M. 1985: Biochemical evidence for hybridization in fire ants. – *The Florida Entomologist* 68: 501-506.
- MEHDIABADI, N.J. & SCHULTZ, T.R. 2009: Natural history and phylogeny of the fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini). – *Myrmecological News* 13: 37-55.
- MEIER, J.I., MARQUES, D.A., MWAIKO, S., WAGNER, C.E., EXCOFFIER, L. & SEEHAUSEN, O. 2017: Ancient hybridization fuels rapid cichlid fish adaptive radiations. – *Nature Communications* 8: art. 14363.
- MEIER, J.I., MCGEE, M.D., MARQUES, D.A., MWAIKO, S., KISHE, M., WANDERA, S., NEUMANN, D., MROSSO, H., CHAPMAN, L.J., CHAPMAN, C.A., KAUFMAN, L., TAABU-MUNYAHU, A., WAGNER, C.E., BRUGGMANN, R., EXCOFFIER, L. & SEEHAUSEN, O. 2023: Cycles of fusion and fission enabled rapid parallel adaptive radiations in African cichlids. – *Science* 381: art. eade2833.
- MEISEL, R.P. & CONNALLON, T. 2013: The faster-X effect: integrating theory and data. – *Trends in Genetics* 29: 537-544.
- MERA-RODRÍGUEZ, D., JOURDAN, H., WARD, P.S., SHATTUCK, S., COVER, S.P., WILSON, E.O. & RABELING, C. 2023: Biogeography and evolution of social parasitism in Australian *Myrmecia* bulldog ants revealed by phylogenomics. – *Molecular Phylogenetics and Evolution* 186: art. 107825.
- MERRILL, R.M., WALLBANK, R.W.R., BULL, V., SALAZAR, P.C.A., MALLETT, J., STEVENS, M. & JIGGINS, C.D. 2012: Disruptive ecological selection on a mating cue. – *Proceedings of the Royal Society B-Biological Sciences* 279: 4907-4913.
- MESSER, S., COVER, S. & LAPOLLA, J. 2016: *Nylanderia deceptrix* sp. n., a new species of obligately socially parasitic formicine ant (Hymenoptera, Formicidae). – *ZooKeys* 552: 49-65.
- MEUNIER, J., WEST, S.A. & CHAPUISAT, M. 2008: Split sex ratios in the social Hymenoptera: a meta-analysis. – *Behavioral Ecology* 19: 382-390.
- MIKHEYEV, A.S., MUELLER, U.G. & ABBOT, P. 2006: Cryptic sex and many-to-one coevolution in the fungus-growing ant symbiosis. – *Proceedings of the National Academy of Sciences of the United States of America* 103: 10702-10706.
- MILLER, S.E. & SHEEHAN, M.J. 2023: Sex differences in deleterious genetic variants in a haplodiploid social insect. – *Molecular Ecology* 32: 4546-4556.
- MOREAU, C.S. & BELL, C.D. 2013: Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. – *Evolution* 67: 2240-2257.
- MOREAU, C.S., BELL, C.D., VILA, R., ARCHIBALD, S.B. & PIERCE, N.E. 2006: Phylogeny of the ants: diversification in the age of angiosperms. – *Science* 312: 101-104.
- MORRISON III, W.R. & WITTE, V. 2011: Strong differences in chemical recognition cues between two closely related species of ants from the genus *Lasius* (Hymenoptera: Formicidae). – *Journal of Evolutionary Biology* 24: 2389-2397.
- MUELLER, U.G., GERARDO, N.M., AANEN, D.K., SIX, D.L. & SCHULTZ, T.R. 2005: The evolution of agriculture in insects. – *Annual Review of Ecology, Evolution, and Systematics* 36: 563-595.
- MUELLER, U.G., SCHULTZ, T.R., CURRIE, C.R., ADAMS, R.M.M. & MALLOCH, D. 2001: The origin of the *Attine* ant-fungus mutualism. – *The Quarterly Review of Biology* 76: 169-197.
- MULLER, H.J. 1942: Isolation mechanisms, evolution and temperature. – *Biological Symposia* 6: 71-125.
- NELSEN, M.P., REE, R.H. & MOREAU, C.S. 2018: Ant-plant interactions evolved through increasing interdependence. – *Proceedings of the National Academy of Sciences of the United States of America* 115: 12253-12258.
- NETTEL-HERNANZ, A., LACHAUD, J.-P., FRESNEAU, D., LÓPEZ-MUÑOZ, R.A. & POTEAUX, C. 2015: Biogeography, cryptic diversity, and queen dimorphism evolution of the Neotropical ant genus *Ectatomma* SMITH, 1958 (Formicidae, Ectatomminae). – *Organisms Diversity & Evolution* 15: 543-553.
- NOSIL, P., CRESPI, B.J. & SANDOVAL, C.P. 2003: Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. – *Proceedings of the Royal Society B-Biological Sciences* 270: 1911-1918.
- NOSIL, P. & FEDER, J.L. 2012: Genomic divergence during speciation: causes and consequences. – *Philosophical Transactions of the Royal Society B-Biological Sciences* 367: 332-342.
- NOSIL, P., FUNK, D.J. & ORTIZ-BARRIENTOS, D. 2009: Divergent selection and heterogeneous genomic divergence. – *Molecular Ecology* 18: 375-402.
- NOSIL, P., VINES, T.H. & FUNK, D.J. 2005: Reproductive isolation caused by natural selection against immigrants from divergent habitats. – *Evolution* 59: 705-719.
- NOUHAUD, P., BLANCKAERT, A., BANK, C. & KULMUNI, J. 2020: Understanding admixture: haplodiploidy to the rescue. – *Trends in Ecology & Evolution* 35: 34-42.
- NYGAARD, S., HU, H., LI, C., SCHIÖTT, M., CHEN, Z., YANG, Z., XIE, Q., MA, C., DENG, Y., DIKOW, R.B., RABELING, C., NASH, D.R., WCISLO, W.T., BRADY, S.G., SCHULTZ, T.R., ZHANG, G. & BOOMSMA, J.J. 2016: Reciprocal genomic evolution in the ant-fungus agricultural symbiosis. – *Nature Communications* 7: art. 12233.
- OMETTO, L., ROSS, K.G., SHOEMAKER, D. & KELLER, L. 2012: Disruption of gene expression in hybrids of the fire ants *Solenopsis invicta* and *Solenopsis richteri*. – *Molecular Ecology* 21: 2488-2501.
- PAMILO, P. 1993: Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. – *Heredity* 70: 472-480.
- PAPADOPULOS, A.S.T., BAKER, W.J., CRAYN, D., BUTLIN, R.K., KYNAST, R.G., HUTTON, I. & SAVOLAINEN, V. 2011: Speciation with gene flow on Lord Howe Island. – *Proceedings of the National Academy of Sciences of the United States of America* 108: 13188-13193.

- PARKER, J. & KRONAUER, D.J.C. 2021: How ants shape biodiversity. – *Current Biology* 31: 1208-1214.
- PARMENTIER, T., DE LAENDER, F. & BONTE, D. 2020: The topology and drivers of ant-symbiont networks across Europe. – *Biological Reviews* 95: 1664-1688.
- PARR, C.L., EGGLETON, P., DAVIES, A.B., EVANS, T.A. & HOLDSWORTH, S. 2016: Suppression of savanna ants alters invertebrate composition and influences key ecosystem processes. – *Ecology* 97: 1611-1617.
- PATTEN, M.M., CARIOSCIA, S.A. & LINNEN, C.R. 2015: Biased introgression of mitochondrial and nuclear genes: a comparison of diploid and haplodiploid systems. – *Molecular Ecology* 24: 5200-5210.
- PERFILIEVA, K.S. 2023: Cretaceous-Burmese-amber ants: morphological features and community structure. – *Biology Bulletin Reviews* 13: 38-54.
- PERKOVSKY, E.E. 2009: Differences in ant (Hymenoptera, Formicidae) species composition between weight fractions of Rovno amber. – *Paleontological Journal* 43: 1087-1091.
- PERRICHOT, V., LACAU, S., NÉRAUDEAU, D. & NEL, A. 2008: Fossil evidence for the early ant evolution. – *Naturwissenschaften* 95: 85-90.
- PIANKA, E.R. 1966: Latitudinal gradients in species diversity: a review of concepts. – *The American Naturalist* 100: 33-46.
- PIE, M.R. 2016: The macroevolution of climatic niches and its role in ant diversification. – *Ecological Entomology* 41: 301-307.
- PORTINHA, B., AVRIL, A., BERNASCONI, C., HELANTERÄ, H., MONAGHAN, J., SEIFERT, B., SOUSA, V.C., KULMUNI, J. & NOUHAUD, P. 2022: Whole-genome analysis of multiple wood ant population pairs supports similar speciation histories, but different degrees of gene flow, across their European ranges. – *Molecular Ecology* 31: 3416-3431.
- PREBUS, M. 2017: Insights into the evolution, biogeography and natural history of the acorn ants, genus *Temnothorax* MAYR (Hymenoptera: Formicidae). – *BioMed Central Evolutionary Biology* 17: art. 250.
- PRESGRAVES, D.C. 2003: A fine-scale genetic analysis of hybrid incompatibilities in *Drosophila*. – *Genetics* 163: 955-972.
- PRICE, S.L., POWELL, S., KRONAUER, D.J.C., TRAN, L.A.P., PIERCE, N.E. & WAYNE, R.K. 2014: Renewed diversification is associated with new ecological opportunity in the Neotropical turtle ants. – *Journal of Evolutionary Biology* 27: 242-258.
- PURCELL, J., BRELSFORD, A., WURM, Y., PERRIN, N. & CHAPUISAT, M. 2014: Convergent genetic architecture underlies social organization in ants. – *Current Biology* 24: 2728-2732.
- PURCELL, J., ZAHND, S., ATHANASIADIS, A., TÜRLER, R., CHAPUISAT, M. & BRELSFORD, A. 2016: Ants exhibit asymmetric hybridization in a mosaic hybrid zone. – *Molecular Ecology* 25: 4866-4874.
- QUELLER, D.C. & STRASSMANN, J.E. 2002: The many selves of social insects. – *Science* 296: 311-313.
- RABELING, C. 2021: Social parasitism. In: STARR, C.K. (Ed.): *Encyclopedia of social insects*. – Springer International Publishing, Cham, pp. 836-858.
- RABELING, C., SCHULTZ, T.R., PIERCE, N.E. & BACCI, M. 2014: A social parasite evolved reproductive isolation from its fungus-growing ant host in sympatry. – *Current Biology* 24: 2047-2052.
- RABOSKY, D.L. & MATUTE, D.R. 2013: Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in *Drosophila* and birds. – *Proceedings of the National Academy of Sciences of the United States of America* 110: 15354-15359.
- RAMALHO, M. DE O., KIM, Z., WANG, S. & MOREAU, C.S. 2021: *Wolbachia* across social insects: patterns and implications. – *Annals of the Entomological Society of America* 114: 206-218.
- RAVINET, M., FARIA, R., BUTLIN, R.K., GALINDO, J., BIERNE, N., RAFAJLOVIĆ, M., NOOR, M.A.F., MEHLIG, B. & WESTRAM, A.M. 2017: Interpreting the genomic landscape of speciation: a road map for finding barriers to gene flow. – *Journal of Evolutionary Biology* 30: 1450-1477.
- REGNIER, F.E. & LAW, J.H. 1968: Insect pheromones. – *Journal of Lipid Research* 9: 541-551.
- REIFOVÁ, R., AMENT-VELÁSQUEZ, S.L., BOURGEOIS, Y., COUGHLAN, J., KULMUNI, J., LIPINSKA, A.P., OKUDE, G., STEVISON, L., YOSHIDA, K. & KITANO, J. 2023: Mechanisms of intrinsic postzygotic isolation: from traditional genic and chromosomal views to genomic and epigenetic perspectives. – *Cold Spring Harbor Perspectives in Biology* 15: art. a041607.
- RIESEBERG, L.H. 2001: Chromosomal rearrangements and speciation. – *Trends in Ecology & Evolution* 16: 351-358.
- ROLLAND, J., HENAO-DIAZ, L.F., DOEBELI, M., GERMAIN, R., HARMON, L.J., KNOWLES, L.L., LIOW, L.H., MANK, J.E., MACHAC, A., OTTO, S.P., PENNELL, M., SALAMIN, N., SILVESTRO, D., SUGAWARA, M., UYEDA, J., WAGNER, C.E. & SCHLUTER, D. 2023: Conceptual and empirical bridges between micro- and macroevolution. – *Nature Ecology and Evolution* 7: 1181-1193.
- ROSS, K.G. & ROBERTSON, J.L. 1990: Developmental stability, heterozygosity, and fitness in two introduced fire ants (*Solenopsis invicta* and *S. richteri*) and their hybrid. – *Heredity* 64: 93-103.
- ROUSSET, F., BOUCHON, D., PINTUREAU, B., JUCHAULT, P. & SOLIGNAC, M. 1992: *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. – *Proceedings of the Royal Society B-Biological Sciences* 250: 91-98.
- RUSSELL, J.A. 2012: The ants (Hymenoptera: Formicidae) are unique and enigmatic hosts of prevalent *Wolbachia* (Alphaproteobacteria) symbionts. – *Myrmecological News* 16: 7-23.
- SARNAT, E.M. & MOREAU, C.S. 2011: Biogeography and morphological evolution in a Pacific island ant radiation. – *Molecular Ecology* 20: 114-130.
- SATOKANGAS, I., NOUHAUD, P., SEIFERT, B., PUNTTILA, P., SCHULTZ, R., JONES, M.M., SIRÉN, J., HELANTERÄ, H. & KULMUNI, J. 2023: Semipermeable species boundaries create opportunities for gene flow and adaptive potential. – *Molecular Ecology* 32: 4329-4347.
- SAVOLAINEN, R. & VEPSÄLÄINEN, K. 2003: Sympatric speciation through intraspecific social parasitism. – *Proceedings of the National Academy of Sciences of the United States of America* 100: 7169-7174.
- SCHÄR, S., TALAVERA, G., ESPADALER, X., RANA, J.D., ANDERSEN ANDERSEN, A., COVER, S.P. & VILA, R. 2018: Do Holarctic ant species exist? Trans-Beringian dispersal and homoplasy in the Formicidae. – *Journal of Biogeography* 45: 1917-1928.
- SCHMID-HEMPEL, P. & CROZIER, R.H. 1999: Polyandry versus polygyny versus parasites. – *Philosophical Transactions of the Royal Society B-Biological Sciences* 354: 507-515.
- SCHUG, M.D., BAINES, J.F., KILLON-ATWOOD, A., MOHANTY, S., DAS, A., GRATH, S., SMITH, S.G., ZARGHAM, S., MCEVEY, S.F. & STEPHAN, W. 2008: Evolution of mating isolation between populations of *Drosophila ananassae*. – *Molecular Ecology* 17: 2706-2721.
- SCHULTHEISS, P., NOOTEN, S.S., WANG, R., WONG, M.K.L., BRASSARD, F. & GUÉNARD, B. 2022: The abundance, biomass, and distribution of ants on Earth. – *Proceedings of the National Academy of Sciences of the United States of America* 119: art. e2201550119.

- SCHULTZ, T.R., BEKKEVOLD, D. & BOOMSMA, J.J. 1998: *Acromyrmex insinuator* new species: an incipient social parasite of fungus-growing ants. – *Insectes Sociaux* 45: 457-471.
- SCHULTZ, T.R. & BRADY, S.G. 2008: Major evolutionary transitions in ant agriculture. – *Proceedings of the National Academy of Sciences of the United States of America* 105: 5435-5440.
- SCHULTZ, T.R., SOSA-CALVO, J., KWESKIN, M.P., LLOYD, M.W., DENTINGER, B., KOOLJ, P.W., VELLINGA, E.C., REHNER, S.A., RODRIGUES, A., MONTOYA, Q.V., FERNÁNDEZ-MARÍN, H., JEŠOVNIK, A., NISKANEN, T., LIIMATAINEN, K., LEAL-DUTRA, C.A., SOLOMON, S.E., GERARDO, N.M., CURRIE, C.R., BACCI, M., VASCONCELOS, H.L., RABELING, C., FAIRCLOTH, B.C. & DOYLE, V.P. 2024: The coevolution of fungus-ant agriculture. – *Science* 386: 105-110.
- SCHWANDER, T., SUNI, S.S., CAHAN, S.H. & KELLER, L. 2008: Mechanisms of reproductive isolation between an ant species of hybrid origin and one of its parents. – *Evolution* 62: 1635-1643.
- SEEHAUSEN, O., BUTLIN, R.K., KELLER, I., WAGNER, C.E., BOUGHMAN, J.W., HOHENLOHE, P.A., PEICHEL, C.L., SAETRE, G.-P., BANK, C., BRÄNNSTRÖM, Å., BRELSFORD, A., CLARKSON, C.S., EROUKHMANOFF, F., FEDER, J.L., FISCHER, M.C., FOOTE, A.D., FRANCHINI, P., JIGGINS, C.D., JONES, F.C., LINDHOLM, A.K., LUCEK, K., MAAN, M.E., MARQUES, D.A., MARTIN, S.H., MATTHEWS, B., MEIER, J.I., MÖST, M., NACHMAN, M.W., NONAKA, E., RENNISON, D.J., SCHWARZER, J., WATSON, E.T., WESTRAM, A.M. & WIDMER, A. 2014: Genomics and the origin of species. – *Nature Reviews Genetics* 15: 176-192.
- SEIFERT, B. 1999: Interspecific hybridisations in natural populations of ants by example of a regional fauna (Hymenoptera, Formicidae). – *Insectes Sociaux* 46: 45-52.
- SEIFERT, B. 2009: Cryptic species in ants (Hymenoptera: Formicidae) revisited: we need a change in the alpha-taxonomic approach. – *Myrmecological News* 12: 149-166.
- SEIFERT, B. 2010: Intranidal mating, gyne polymorphism, polygyny, and supercoloniality as factors for sympatric and parapatric speciation in ants. – *Ecological Entomology* 35: 33-40.
- SEIFERT, B. 2018: The ants of central and northern Europe. – *Lutra*, Tauer, 408 pp.
- SEIFERT, B. 2021: A taxonomic revision of the Palaearctic members of the *Formica rufa* group (Hymenoptera: Formicidae) – the famous mound-building red wood ants. – *Myrmecological News* 31: 133-179.
- SHOEMAKER, D.D., AHRENS, M.E. & ROSS, K.G. 2006: Molecular phylogeny of fire ants of the *Solenopsis saevissima* species-group based on mtDNA sequences. – *Molecular Phylogenetics and Evolution* 38: 200-215.
- SIDDIQUI, J.A. 2019: Meta-analysis of the global diversity and spatial patterns of aphid-ant mutualistic relationships. – *Applied Ecology and Environmental Research* 17: 5471-5524.
- SIGEMAN, H., SATOKANGAS, I., DE LAMARRE, M., DESHMUKH, R., NOUHAUD, P., HELANTERÄ, H., CHAPUISAT, M., KULMUNI, J. & VILJAKAINEN, L. 2024: The loss of a supergene in obligately polygynous *Formica* wood ant species. – *bioRxiv*; doi: 10.1101/2024.09.20.613865.
- SIMPSON, E.H. 1949: Measurement of diversity. – *Nature* 163: 688-688.
- SMADJA, C. & BUTLIN, R.K. 2008: On the scent of speciation: the chemosensory system and its role in premating isolation. – *Heredity* 102: 77-97.
- SOBEL, J.M. & CHEN, G.F. 2014: Unification of methods for estimating the strength of reproductive isolation. – *Evolution* 68: 1511-1522.
- SOCIAS-MARTÍNEZ, L. & PECKRE, L.R. 2023: Does sociality affect evolutionary speed? – *Peer Community Journal* 3: art. e118.
- SPRENGER, P.P. & MENZEL, F. 2020: Cuticular hydrocarbons in ants (Hymenoptera: Formicidae) and other insects: how and why they differ among individuals, colonies, and species. – *Myrmecological News* 30: 1-26.
- STADLER, B. & DIXON, A.F.G. 2005: Ecology and evolution of aphid-ant interactions. – *Annual Review of Ecology, Evolution, and Systematics* 36: 345-372.
- STADLER, B., KINDLMANN, P., ŠMILAUER, P. & FIEDLER, K. 2003: A comparative analysis of morphological and ecological characters of European aphids and lycaenids in relation to ant attendance. – *Oecologia* 135: 422-430.
- STANKOWSKI, S., CUTTER, A.D., SATOKANGAS, I., LERCH, B.A., ROLLAND, J., SMADJA, C.M., SEGAMI MARZAL, J.C., COONEY, C.R., FEULNER, P.G.D., DOMINGOS, F.M.C.B., NORTH, H.L., YAMAGUCHI, R., BUTLIN, R.K., WOLF, J.B.W., COUGHLAN, J., HEIDBREDER, P., HERNÁNDEZ-GUTIÉRREZ, R., BARNARD-KUBOW, K.B., PEDE, D., RANCILHAC, L., SALVADOR, R.B., THOMPSON, K.A., STACY, E.A., MOYLE, L.C., GARLOVSKY, M.D., MAULANA, A., KANTELINEN, A., CACHO, N.I., SCHNEEMANN, H., DOMÍNGUEZ, M., DOPMAN, E.B., LOHSE, K., ROMETSCH, S.J., COMEAULT, A.A., MERRILL, R.M., SCORDATO, E.S.C., SINGHAL, S., PÄRSSINEN, V., LACKEY, A.C.R., KUMAR, S., MEIER, J.I., BARTON, N., FRAÏSSE, C., RAVINET, M. & KULMUNI, J. 2024: Toward the integration of speciation research. – *Evolutionary Journal of the Linnean Society* 3: art. kzae001.
- STANKOWSKI, S. & RAVINET, M. 2021: Defining the speciation continuum. – *Evolution* 75: 1256-1273.
- STEINER, F.M., SCHLICK-STEINER, B.C., KONRAD, H., MODER, K., CHRISTIAN, E., SEIFERT, B., CROZIER, R.H., STAUFFER, C. & BUSCHINGER, A. 2006: No sympatric speciation here: Multiple data sources show that the ant *Myrmica microrubra* is not a separate species but an alternate reproductive morph of *Myrmica rubra*. – *Journal of Evolutionary Biology* 19: 777-787.
- STOCKAN, J.A. & ROBINSON, E.J.H. (Eds.) 2016: Wood ant ecology and conservation. – Cambridge University Press, Cambridge, UK, 321 pp.
- SUN, Q. & LI, Y. 2025: Advances in haplotype phasing and genotype imputation. – *Nature Reviews Genetics*; doi: 10.1038/s41576-025-00895-2.
- SUNDSTRÖM, L., SEPPÄ, P. & PAMILO, P. 2005: Genetic population structure and dispersal patterns in *Formica* ants – a review. – *Annales Zoologici Fennici* 42: 163-177.
- TALBOT, M. 1976: The natural history of the workerless ant parasite *Formica talbotae*. – *Psyche: A Journal of Entomology* 83: 282-288.
- THOMPSON, K.A., BRANDVAIN, Y., COUGHLAN, J.M., DELMORE, K.E., JUSTEN, H., LINNEN, C.R., ORTIZ-BARRIENTOS, D., RUSHWORTH, C.A., SCHNEEMANN, H., SCHUMER, M. & STELKENS, R. 2024: The ecology of hybrid incompatibilities. – *Cold Spring Harbor Perspectives in Biology* 16: art. a041440.
- THOMPSON, K.A., PEICHEL, C.L., RENNISON, D.J., MCGEE, M.D., ALBERT, A.Y.K., VINES, T.H., GREENWOOD, A.K., WARK, A.R., BRANDVAIN, Y., SCHUMER, M. & SCHLUTER, D. 2022: Analysis of ancestry heterozygosity suggests that hybrid incompatibilities in threespine stickleback are environment dependent. – *Public Library of Science Biology* 20: art. e3001469.
- TINAUT, A. & RUANO, F. 2021: Biogeography of Iberian ants (Hymenoptera: Formicidae). – *Diversity* 13: art. 88.
- TORRES, J.A., SNELLING, R.R., BLUM, M.S., FLOURNOY, R.C., JONES, T.H. & DUFFIELD, R.M. 2001: Mandibular gland chemistry of four Caribbean species of *Camponotus* (Hymenoptera: Formicidae). – *Biochemical Systematics and Ecology* 29: 673-680.

- TRIBLE, W., CHANDRA, V., LACY, K.D., LIMÓN, G., MCKENZIE, S.K., OLIVOS-CISNEROS, L., ARSENAULT, S.V. & KRONAUER, D.J.C. 2023: A caste differentiation mutant elucidates the evolution of socially parasitic ants. – *Current Biology* 33: 1047-1058.e4.
- TURNER, T.L., HAHN, M.W. & NUZHIDIN, S.V. 2005: Genomic islands of speciation in *Anopheles gambiae*. – *Public Library of Science Biology* 3: art. e285.
- ÜN, Ç., SCHULTNER, E., MANZANO-MARÍN, A., FLÓREZ, L.V., SEIFERT, B., HEINZE, J. & OETTLER, J. 2021: Cytoplasmic incompatibility between Old and New World populations of a tramp ant. – *Evolution* 75: 1775-1791.
- VENETI, Z., ZABALOU, S., PAPAFOIOU, G., PARASKEVOPOULOS, C., PATTAS, S., LIVADARAS, I., MARKAKIS, G., HERREN, J.K., JAENIKE, J. & BOURTZIS, K. 2012: Loss of reproductive parasitism following transfer of male-killing *Wolbachia* to *Drosophila melanogaster* and *Drosophila simulans*. – *Heredity* 109: 306-312.
- VIA, S., BOUCK, A.C. & SKILLMAN, S. 2000: Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. – *Evolution* 54: 1626-1637.
- VIZUETA, J., XIONG, Z., DING, G., LARSEN, R.S., RAN, H., GAO, Q., STILLER, J., DAI, W., JIANG, W., ZHAO, J., GUO, C., ZHANG, X., ZUO, D., ZHONG, W., SCHIÖTT, M., LIU, C., ZHANG, H., DAI, X., ANDREU, I., SHI, Y., TRETTER, S., HE, D., GAUTAM, S., LI, Z., HICKEY, G., IVENS, A.B.F., MEURVILLE, M.-P., HITA-GARCIA, F., KASS, J.M., GUÉNARD, B., MOREAU, C., PATEN, B., LEBOEUF, A.C., ECONOMO, E.P., CHAPUISAT, M., SHIK, J.Z., WARD, P.S., HEINZE, J., SCHULTZ, T.R., LI, Q., DUNN, R.R., SANDERS, N.J., LIU, W., SCHRADER, L., BOOMSMA, J.J. & ZHANG, G. 2025: Adaptive radiation and social evolution of the ants. – *Cell* 188: 4828-4848.
- WALKER, J., COONEY, C., KULMUNI, J., LOHSE, K., MEIER, J., MERRILL, R., SCORDATO, E., SMADJA, C.M., SINGHAL, S., BUTLIN, R. & STANKOWSKI, S. 2025: A standardised framework for classifying estimates of reproductive isolation across the Tree of Life. – *EcoEvoRxiv*; doi: 10.32942/X2P92P.
- WANG, J., WURM, Y., NIPITWATTANAPHON, M., RIBA-GROGNUM, O., HUANG, Y.-C., SHOEMAKER, D. & KELLER, L. 2013: A Y-like social chromosome causes alternative colony organization in fire ants. – *Nature* 493: 664-668.
- WARD, P.S. 1989: Genetic and social changes associated with ant speciation. In: BREED, M.D. & PAGE, R.E. (Eds.): *The genetics of social evolution*. – Westview, Boulder, CO, pp. 261-270.
- WARD, P.S. 1993: Systematic studies on *Pseudomyrmex* acacia-ants (Hymenoptera: Formicidae: Pseudomyrmecinae). – *Journal of Hymenoptera Research* 2: 117-168.
- WARD, P.S. & BRADY, S.G. 2003: Phylogeny and biogeography of the ant subfamily Myrmeciinae (Hymenoptera: Formicidae). – *Invertebrate Systematics* 17: 361-386.
- WARD, P.S., BRADY, S.G., FISHER, B.L. & SCHULTZ, T.R. 2015: The evolution of Myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). – *Systematic Entomology* 40: 61-81.
- WARD, P.S. & BRANSTETTER, M.G. 2017: The acacia ants revisited: convergent evolution and biogeographic context in an iconic ant/plant mutualism. – *Proceedings of the Royal Society B-Biological Sciences* 284: art. 20162569.
- WARD, P.S. & BRANSTETTER, M.G. 2022: Species paraphyly and social parasitism: Phylogenomics, morphology, and geography clarify the evolution of the *Pseudomyrmex elongatulus* group (Hymenoptera: Formicidae), a Mesoamerican ant clade. – *Insect Systematics and Diversity* 6: art. 4.
- WEIBLEN, G.D. & BUSH, G.L. 2002: Speciation in fig pollinators and parasites. – *Molecular Ecology* 11: 1573-1578.
- WENSELEERS, T., ITO, F., VAN BORM, S., HUYBRECHTS, R., VOLCKAERT, F. & BILLEN, J. 1998: Widespread occurrence of the microorganism *Wolbachia* in ants. – *Proceedings of the Royal Society B-Biological Sciences* 265: 1447-1452.
- WERNEGREEN, J.J., KAUPPINEN, S.N., BRADY, S.G. & WARD, P.S. 2009: One nutritional symbiosis begat another: phylogenetic evidence that the ant tribe Camponotini acquired *Blochmannia* by tending sap-feeding insects. – *BioMed Central Evolutionary Biology* 9: art. 292.
- WERREN, J.H. 1993: The evolution of inbreeding in haplodiploid organisms. In: THORNHILL, N.W. (Ed.): *The natural history of inbreeding and outbreeding: theoretical and empirical perspectives*. – The University of Chicago Press, Chicago, IL, pp. 42-59.
- WERREN, J.H. 1997: *Biology of Wolbachia*. – *Annual Review of Entomology* 42: 587-609.
- WESTRAM, A.M., STANKOWSKI, S., SURENDRANADH, P. & BARTON, N. 2022: What is reproductive isolation? – *Journal of Evolutionary Biology* 35: 1143-1164.
- WEYNA, A., BOUROUINA, L., GALTIER, N. & ROMIGUIER, J. 2022: Detection of F1 hybrids from single-genome data reveals frequent hybridization in Hymenoptera and particularly ants. – *Molecular Biology and Evolution* 39: art. msac071.
- WHEELER, D.E. 1986: Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. – *The American Naturalist* 128: 13-34.
- WIENS, J.J. & DONOGHUE, M.J. 2004: Historical biogeography, ecology and species richness. – *Trends in Ecology & Evolution* 19: 639-644.
- WILSON, E.O. 1971: *The insect societies*. – Belknap Press of Harvard University Press, Cambridge, MA, 548 pp.
- WILSON, E.O. & HÖLLDOBLER, B. 2005: The rise of the ants: a phylogenetic and ecological explanation. – *Proceedings of the National Academy of Sciences of the United States of America* 102: 7411-7414.
- WILSON, E.O. & NOWAK, M.A. 2014: Natural selection drives the evolution of ant life cycles. – *Proceedings of the National Academy of Sciences of the United States of America* 111: 12585-12590.
- WILSON, E.O. & TAYLOR, R. 1967: *The ants of Polynesia* (Hymenoptera: Formicidae). – *Pacific Insects Monographs* 14: 1-109.