



The life history of *Formica exsecta* (Hymenoptera: Formicidae) from an ecological and evolutionary perspective

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Abstract

With their sedentary colonies, long-lived ant colonies lend themselves to long-term studies where fitness effects of life-history traits can be investigated in the wild, a task that is challenging in any organism and particularly rarely feasible in insects. Here, we summarize and examine the insights we have gained from a 28-year study on monogyne colonies of the narrow-headed ant *Formica exsecta* NYLANDER, 1846, and discuss the ecological and genetic repercussions that emanate from ecological realities and conflicting selection pressures in a fragmented landscape matrix. These entail the effects of habitat structuration on genetic diversity, the effects of reduced genetic diversity on the fitness of individuals and colonies, and the impact of the opposing selection pressures on short- versus long-range dispersal.

Key words: *Formica*, narrow-headed ant, monogyny, dispersal, inbreeding, mating frequency, sex-ratio evolution, long-term study, review.

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Introduction

Life-history strategies pervade evolutionary ecology as these define how organisms distribute their available resources across their lifetime, ultimately determining the evolutionary fitness of that organism (STEARNS 1992). Life-history parameters, such as age at first reproduction, number of reproductive events during an individual's lifetime, number of offspring produced per reproductive event, and the longevity of an individual (STEARNS 1992, ROFF 2002), define the typical life trajectory of members of a species. Those combinations of these parameters which produce the highest fitness returns in terms of genes transferred to the next generation will come to dominate in populations and ultimately in the species. Yet, individuals of a species can express alternative strategies, which produce different trade-offs between current and future reproduction and / or reproduction and survival (ROFF 2002). As a result, multiple strategies may prevail in a species. Social life, as seen in many birds, mammals, and especially social insects, adds further complexity to these patterns as some individuals may forego reproduction

partially or even completely (HAMILTON 1972). Added complexity emerges as different categories of individuals may exhibit different life-history strategies within a single species, especially in species in which individuals mostly abstain from reproduction during their entire lifetime (e.g., NEGRONI & al. 2016).

Life-history strategies form the core of evolutionary ecology, and the value of long-term studies has been recognized (CLUTTON-BROCK & al. 2010). Laboratory experiments may bring insight to single traits and physiological trade-offs, but in order to understand how life-history strategies evolve, data of animals exposed to the multitude of simultaneous selection pressures they face in the wild – for example, competitors, predators and prey, pathogens, and environmental fluctuations – and their fitness consequences are required. Indeed, an increasing number of studies have focused on the ecological and evolutionary forces that shape life-history patterns in wild populations. Most studies have addressed individual aspects of life-history components (e.g., LEMAITRE & al.

2020). However, with the advent of new tracking tools and genetic profiling, an increasing number of studies have simultaneously addressed ever more comprehensive sets of these components across the entire lifetime of individuals (e.g., HUISMAN & al. 2016, VITIKAINEN & al. 2019, FROY & al. 2021). To date, most long-term studies that focus on the ecological and evolutionary forces that shape life-history patterns have been conducted on birds and mammals (e.g., BJÖRKLUND & GUSTAFSSON 2013, CLUTTON-BROCK 2016, BJÖRKLUND & GUSTAFSSON 2017, EVANS & GUSTAFSSON 2017, GERMAIN & al. 2017, WOLAK & al. 2018, HAMMERS & al. 2019, REID & al. 2019, BERG & al. 2020, TOLVANEN & al. 2020, HAMMERS & al. 2021, NADACHOWSKA-BRZYSKA & al. 2021, REID & al. 2021). The ecological importance of invertebrates and the rising concerns regarding the decline of insect populations (e.g., HALLMAN & al. 2017) call for detailed studies also on these taxa. Yet, studies that combine individual and population level processes have only been conducted in a handful of insect systems, such as *Melitaea cinxia* (e.g., SAASTAMOINEN & RANTALA 2013, DUPLOUY & al. 2017) and *Gryllus campestris* (e.g., RODRÍGUEZ-MUÑOZ & al. 2019). Even fewer studies have been conducted on plants (FRIEDMAN 2020). Notably, several of the long-term studies on birds and mammals concern social species, thus highlighting the intricacies that sociality confers.

Long-term field studies on ants have been conducted for a considerable time, mostly focusing on the general ecology and behaviour (BRIAN 1952, BRIAN & BRIAN 1955, BRIAN & al. 1965, HORSTMANN 1970, ROSENGREN 1971, HORSTMANN 1972, BRIAN 1973, HORSTMANN 1974, BRIAN 1980, ROSENGREN & FORTELIUS 1986, ELMES 1987a, b, GÖSSWALD 1989, ELMES & PETAL 1990, GÖSSWALD 1990, ROSENGREN & SUNDSTRÖM 1991, SALO & ROSENGREN 2001, RADCHENKO & ELMES 2010, STOCKAN & ROBINSON 2016). The development of genetic markers such as allozymes and microsatellites started a new era of marker-based studies that permitted the assessment of colony kin structure (e.g., PAMILO & CROZIER 1996, PAMILO & al. 1997). More recently, also comprehensive population studies have been conducted on colony kin structure, dispersal patterns, population genetics, and genomics (e.g., SUNI & GORDON 2010, INGRAM & al. 2013 on *Pogonomyrmex barbatus*; SUNDSTRÖM 1995a, b, 1996 on *Formica*; SEPPÄ & WALIN 1996, PAMILO & al. 1997, SEPPÄ & al. 2004, 2009 on *Formica* and *Myrmica*; CHAPUISAT & al. 2004, AVRIL & al. 2019, 2020, ZAHND & al. 2021 on *Formica selysi*). Conversely, long-term studies on life-history strategies, genetics, and behaviour have been conducted on individual species in the lab, most notably on *Cardiocondyla* (see HEINZE 2017). A major constraint on long-term studies on the same colonies is that sampling by necessity often is destructive, or that colonies readily change nest sites. Notably, however, very few, if any, have comprehensively investigated the association between sociality, life-history traits, and population biology in social insects, although this could shed light on the interrelationships between proximate mechanisms related to

fecundity and longevity and ultimate factors that shape social evolution.

Social insects are good candidates for long-term studies that combine ecological and life-history analyses, given that colonies usually are stationary, and that many species have just one queen (e.g., KELLER & REEVE 1994, HUGHES & al. 2008, BOOMSMA 2009, BOOMSMA & al. 2014), which enables following the same individuals over several seasons in changing environment. Thus, charting the life cycle of individual colonies, in conjunction with measurement of various life-history parameters that contribute key fitness components and assessment of family relationships, is often feasible, also in the field. In this sense, social insects portray traits also found in perennial plants (FRIEDMAN 2020), such as trees, in that the location of an individual or colony remains unchanged (HÖLLDOBLER & WILSON 1990). As a result, one can chart the life-history “decisions” that reproductive individuals make over mating and dispersal and determine the sociogenetic structure of sedentary colonies, sometimes for decades to come. This in turn may have consequences for the local population persistence, through effects on colony sex ratio, productivity, or inbreeding. Indeed, the extraordinary life span of the queens of some species, for example up to 30 years in *Formica exsecta* (see PAMILO 1991), allows the investigation of individual reproductive strategies over extended time periods under changing conditions. More importantly, given that colonies are sedentary, and that haplodiploidy allows non-destructive inference of queen and paternal genotypes, a pedigree can be constructed and dispersal patterns assessed. Combined with information on longevity, reproductive output, and sex ratios of colonies, one can thus assess colony founding success and lifetime reproductive success. To our knowledge, only three studies have reported an analysis of at least some of these factors (SUNI & GORDON 2010, INGRAM & al. 2013, VITIKAINEN & al. 2015).

In this review, we collate life-history data from 28 years of study on a population of *Formica exsecta* NYLANDER, 1846, located in the archipelago in South West Finland, near Tvärminne zoological station. We examine how queen mating strategy and dispersal tie in with the sociogenetic structure of the colonies, how this in turn affects the incidence of inbreeding and the population structure, and what individual, colony, and population level consequences ensue. In addition to the long term monitoring, we discuss an array of short-term experiments conducted on individuals collected non-destructively from these colonies (e.g., MARTIN & al. 2008, VITIKAINEN & SUNDSTRÖM 2011, VITIKAINEN & al. 2011, MARTIN & al. 2013, FREITAK & al. 2014, BOS & al. 2016, STUCKI & al. 2017a, b, PULLIAINEN & al. 2018, PEIGNIER & al. 2019, PULLIAINEN & al. 2021).

The general biology of *Formica exsecta*

The narrow-headed ant, *Formica exsecta*, is common throughout the Palaearctic region (CZECHOWSKI & al. 2002, SEIFERT 2007; Fig. 1), where it inhabits meadows and



Fig. 1: Female and male *Formica exsecta* during the nuptial flight. © Liselotte Sundström.

open woodlands (COLLINGWOOD 1979, SEIFERT 2007). The habitats are often ephemeral, such as early-stage meadows after forest fires or forest clearings (CZECHOWSKI & al. 2002, MAGGINI & al. 2002), but if the habitat remains open, and *Formica* s.str. species that require forest habitats remain absent, the competitive environment may become more amenable for long term persistence of the species. Indeed, long-term persistence of the species has been reported from the European alpine regions, where the species also thrives on meadows and grazed areas (WIEZIK & al. 2017). A similar situation probably prevails elsewhere within the distribution area of the species, where record-breaking population densities have been reported (MARKÓ & al. 2012).

The breeding biology follows much the same pattern as seen in other boreal ant species, with onset of reproduction in early May after hibernation. The first cohort of eggs give rise to a new generation of sexuals and gradually transition into eggs that eventually will develop into the first worker generation of the season (SEIFERT 2007). Sexuals emerge from mid-June onwards throughout the distribution range, with late sexual brood being intermixed with the first worker cohort. A second worker cohort is initiated after the sexuals have emerged, and new worker brood is regularly produced until early autumn (SEIFERT 2007). Workers usually hibernate once, but some may survive to hibernate twice. Hibernation starts earlier than in the *Formica* s.str., that is, in late September / early October, at least in Scandinavia (SEIFERT 2007, L. Sundström & E. Vitikainen, unpubl.).

As is the case in all mound-building *Formica* ants, also *Formica exsecta* founds new colonies via temporary social parasitism on *Serviformica* species (CZECHOWSKI & al. 2002, SEIFERT 2007), and thus requires the presence of these species, especially *Formica fusca* (see CZECHOWSKI & al. 2002, SEIFERT 2007, CHERNENKO & al. 2013), for establishment in an area. Both monogyne and polygyne colonies occur, the latter often characterised by dense polydomous societies (e.g., BROWN & KELLER 2000, LIAUTARD & KELLER 2001, BROWN & KELLER 2002, SEPPÄ & al. 2004, SUNDSTRÖM & al. 2005, KÜMMERLI & KELLER 2008, SEPPÄ & al. 2012, WIEZIK & al. 2017). Polygyny most likely arises through the adoption of daughter queens (CZECHOWSKI & al. 2002, SEIFERT 2007), and indeed colony queens are related also in polydomous populations (KÜMMERLI & KELLER 2007a). Genetic studies have shown that Scandinavian populations comprise a patchwork of either mainly monogyne or mainly polygyne populations, though always intermixed with some colonies of the other type (SEPPÄ & al. 2004, 2012). By contrast, the populations studied more in detail in the alpine regions of Central Europe are mainly polygyne and polydomous (BROWN & KELLER 2000, 2002, WIEZIK & al. 2017).

Both the Scandinavian and the Swiss populations in the Jura mountains have been extensively studied with respect to sociogenetics (SUNDSTRÖM & al. 1996, BROWN & KELLER 2000, LIAUTARD & KELLER 2001, BROWN & KELLER 2002, BROWN & al. 2002, LIAUTARD & al. 2003, SUNDSTRÖM & al. 2003, SEPPÄ & al. 2004, KÜMMERLI & al. 2005, KÜMMERLI & KELLER 2007a, b, c, 2008, HAAG-LIAUTARD & al. 2008, 2009, VITIKAINEN & al. 2011, SEPPÄ & al. 2012, VITIKAINEN & al. 2015). The social form (monogyne and monodomy vs. polygyny and polydomy) differs fundamentally among the populations, which inevitably has led to fundamental differences also in the questions addressed. Hence, the studies in the mainly monogyne population in Tvärminne have focused on dispersal patterns and fitness-associated traits at the level of individual colonies (SUNDSTRÖM & al. 1996, CHAPUISAT & al. 1997, SUNDSTRÖM & al. 2003, HAAG-LIAUTARD & al. 2009, VITIKAINEN & al. 2011, 2015). Conversely, the studies on the highly polygyne populations in the Jura mountains have focused on a variety of traits associated with reproductive sharing among resident queens and genetic population structure (BROWN & KELLER 2000, LIAUTARD & KELLER 2001, BROWN & KELLER 2002, BROWN & al. 2002, 2003, LIAUTARD & al. 2003, KÜMMERLI & al. 2005, KÜMMERLI & KELLER 2007a, b, c, HAAG-LIAUTARD & al. 2008). One avenue of research is, nonetheless, shared between the studies on the Tvärminne and the Jura populations, namely the selective principles that underpin sex allocation, in particular the role of relatedness asymmetry (BOOMSMA & GRAFEN 1991) as a determinant of sex ratio. Interestingly, the two social types differ in that sex allocation is mainly driven by the relatedness asymmetry in the monogyne Tvärminne population (SUNDSTRÖM & al. 1996), whereas other factors, such as queen number (BROWN & KELLER 2000, KÜMMERLI & al. 2005) and queen replenishment

(BROWN & KELLER 2002, BROWN & al. 2002) govern sex ratio specialization in the polygyne, polydomous social form in the Jura mountains. The remaining Scandinavian studies have focused on the genetic structure within and across populations (SEPPÄ & al. 2004, 2012) as well as issues related to species delimitation and behavioural interactions within polydomous populations (HAKALA & al. 2018, 2019, 2020).

The long reach of ecological genetics and the Tvärminne population of *Formica exsecta*

All organisms live in a landscape matrix which can take different forms, from large continuous areas of similar habitat to highly fragmented landscapes with interstitial sections of uninhabitable areas. Whether a landscape appears fragmented also depends on what distance ranges individuals of a species can cover on a regular basis, which in turn will dictate dispersal rates and eventually the genetic structure of such populations, should they come to persist long enough (Fig. 2; HARTL & CLARK 2007). Thus, the same landscape matrix may appear either continuous or fragmented to different species, depending on their range of operation during daily activities and their ability to cover distances during dispersal. For example, an area of 10km² may appear continuous to a bird, whereas the same area may appear highly fragmented to an insect. For ants that depend on winged sexuals for dispersal and the

colonization of new areas, distances of even a few hundred meters may constitute a dispersal barrier if intermittent areas comprise water. Furthermore, wind directions may dictate the main dispersal directions and so constrain the colonization of otherwise inhabitable areas.

The study area on the SW coast of Finland, close to the Tvärminne zoological station (59° 84' 196" N, 23° 20' 182" E) (Fig. 3), encompasses an archipelago area of 8.3km² and 17 islands. The landscape matrix in Tvärminne can thus be considered highly fragmented for most ants, given that the distances between islands often exceed the longest distance within any of the islands (Fig. 4). The area has a land rise of about 0.5 m / 100 years, and most of the islands were void of trees until the early 20th century (Fig. 5). With the establishment of the Tvärminne zoological station in 1902 and the transfer of ownership of most of the study islands to the station, the area has been a nature reserve since. However, the area was appropriated by Russia during the Second World War and the islands cleared of trees. We thus estimate that the environment has become habitable for *Formica exsecta* less than 60 years ago.

The biotope on the islands today comprises pine and spruce thickets intermixed with granite cliffs, dry meadows, and some lush patches of grove. In addition to pine and spruce, the vegetation consists mainly of junipers and ericoid shrubs. The immediate surroundings of the nests encompass plant communities that vary both spatially and temporally. The soil type consists mainly of thin layers of

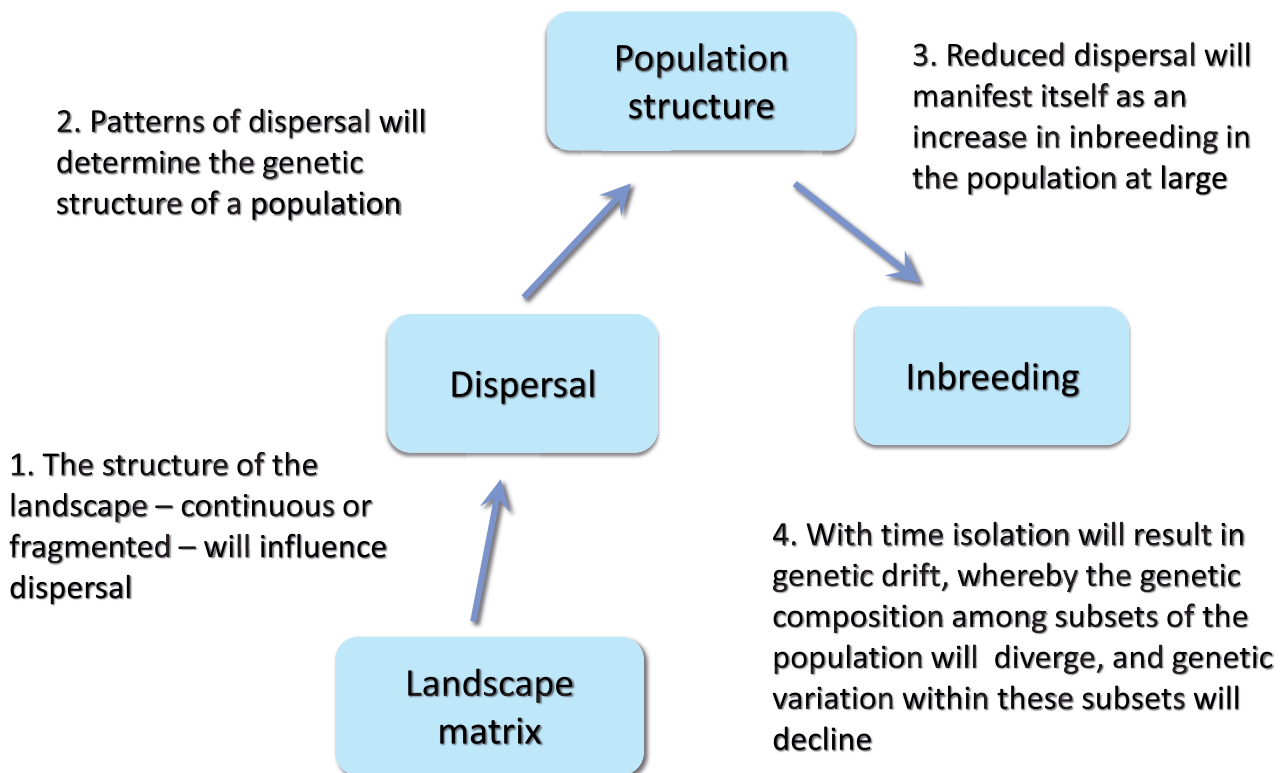


Fig. 2: The long reach of ecological genetics. The chart illustrates the general flow from a fragmented landscape matrix through dispersal and population structure to inbreeding.



Fig. 3: The Tvärminne archipelago, including some of the study islands, in 2020. The zoological station is in the foreground, and the distance to the island furthest away is ca 2.6 km. © Alf Norkko, used with permission.



Fig. 4: Map of the study area and the location of the study islands. The red stars indicate islands on which colonies have been monitored throughout the study, the blue stars indicate three islands on each of which only one colony was found; these three colonies were not monitored (map location <<https://mapcarta.com/14077134>>). Map credits: © OpenStreetMap.

topsoil on bedrock (leptosol intermixed with stratified podzol, LINDSTRÖM & al. 2018). The nests are located in open and dry areas and constructed from spruce and pine needles, some excised plant material (mainly various grasses), leaves of *Calluna vulgaris* and other ericoids, and, more rarely, mosses and lichen.

Given the destruction of vegetation on the islands during the Second World war, *Formica exsecta*, being a mid-successional species (SEPPÄ & al. 1995, CZECHOWSKI & al. 2002), could have colonized the first locations in the late 1950's or early 1960's at the earliest, although most likely present on the mainland nearby. The main host species for colony founding, *Formica fusca*, is a typical pioneer species that rapidly colonizes open areas and would therefore have been present on the islands (SEPPÄ & al. 1995). Hence, competition-free space was available as most *Formica* species, except *Formica truncorum* and

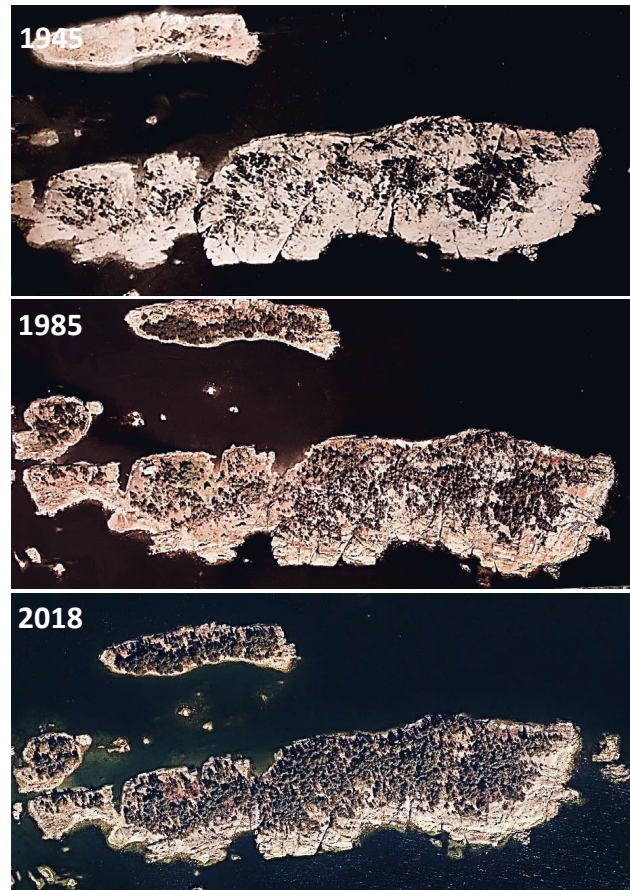


Fig. 5: Aerial photographs of one of the study islands, taken in 1945, 1985, and 2018, illustrating the revegetation of this and the other islands in the area. The vegetation in 1945 would mostly have been juniper shrubs, with very few pine trees, whereas the vegetation today is dominated by pine trees. Picture courtesy: 1945 and 1985 archives of Tvärminne zoological station, 2018 © Maanmittauslaitos CC BY 4.0.

Formica sanguinea, require more mature forest (SEPPÄ & al. 1995). Indeed, the advent of immigration by *Formica* s.str. wood ants has led to a clear decline of *F. exsecta* on one of the study islands (Kalvholmen) (L. Sundström & E. Vitikainen, unpubl.). The presence of *Formica* s.str. species on the islands is very fragmentary and seems contingent on renewal from existing colonies, rather than a continuous flow of new immigrants from the mainland (L. SUNDSTRÖM & E.VITIKAINEN, unpubl.). Thus, *Formica lugubris*, *F. truncorum*, and *F. sanguinea* are present only on a few islands, and *F. truncorum* has all but disappeared – two of an original ca. 30 colonies (SUNDSTRÖM 1994, 1995a, b) remain on two islands out of the original seven. *Formica aquilonia* and *Formica polyctena* apparently never colonized the islands.

The entire area of 8.3 km² has been monitored for the presence of this species since 1993. *Formica exsecta* has been recorded on 10 out of the 17 islands. Seven of the islands have been continuously surveyed (Fig. 4), and currently, four islands are inhabited by the species (L. Sundström & E. Vitikainen, unpubl.). The wind directions

Tab. 1: Life-history traits and genetic population structure, inbreeding (HL and F_{is}), neighbourhood size, and population differentiation (F_{ST}) of Tvärminne population of *Formica exsecta*. ¹Inferred from dispersal distances (N^e , mean number within median dispersal distance). See also Figures 2, 6, and 7.

Statistics		References
Colony size	2300 ± 200 (mean ± SE, N = 52 colonies)	VITIKAINEN & al. (2011)
Mating frequency	1.27 ± 0.03 (N = 192)	VITIKAINEN & al. (2015)
Colony lifespan (= queen lifespan; data only available from monogyne colonies)	Average 6.5 years; updated to 10 years (N = 177, excluding colonies alive in 2021)	HAAG-LIAUTARD & al. (2009); this study / E. VITIKAINEN & L. SUNDRÖM, unpubl.
Colony maximum lifespan	30 years (estimated)	PAMILO (1991)
Among colony workers	R = 0.71 ± 0.03 (N = 70 colonies)	SUNDRÖM & al. (2003)
Between mating partners	R = 0.08 ± 0.008 (N = 310)	VITIKAINEN & al. (2015)
Between multiple mates of polyandrous queens	R = 0.31 ± 0.03 (N = 92)	VITIKAINEN & al. (2015)
F_{is} (95% CI)	Workers: 0.142 (0.114 - 0.221) Queens: 0.002 (-0.042 - 0.043)	SUNDRÖM & al. (2003)
Aparicio's HL (range) APARICIO & al. (2006)	Workers: Polygyne colonies 0.37 (0.18 - 0.58)	this study / E. VITIKAINEN & L. SUNDRÖM, unpubl.
	Monogyne colonies: 0.28 (0.02 - 0.66)	
	Workers: HL = 0.258 ± 0.008	VITIKAINEN & al. (2015)
	Queens: HL = 0.219 ± 0.001	VITIKAINEN & al. (2015)
Genetic neighbourhood size (ROUSSET 1997)	22 queens, 50 males	SUNDRÖM & al. (2003)
Observed neighbourhood size ¹	17.8 queens, 42.3 males	VITIKAINEN & al. (2015)
Average dispersal distance	Queens: 59 m; quartiles 25 m, 125 m, N = 65 Males: 140 m; quartiles 58 m, 344 m, N = 68	VITIKAINEN & al. (2015)
F_{ST} (95% CI) Within islands	Queens: 0.061 (0.042 - 0.101) Workers: 0.046 (0.023 - 0.084) Males: 0.027 (-0.051 - 0.122)	SUNDRÖM & al. (2003)
F_{ST} (95% CI) Between islands	Queens: -0.005 (-0.032 - 0.022) Workers: 0.001 (-0.025 - 0.025) Males: 0.023 (0.016 - 0.048)	SUNDRÖM & al. (2003)

are predominantly from SW-S-SE (data from Tvärminne zoological station weather station), with relatively rare strong northerly winds especially in late June / early July, at the time of mating flights. Given that the species tends to fly early in the morning and only during calm weather (E. Vitikainen, unpubl.), dispersal would rarely be assisted by strong winds, and if so, the sexuals would be transported towards the mainland. Immigrants from the south are also most likely extremely rare, as the area south of the study site is open sea all the way to Estonia (ca. 70 km). This may have prevented *F. exsecta* from colonising the southernmost large islands. Of the mound-building wood ants, only *Formica truncorum* and *Formica lugubris* have colonised one of the outermost islands (Mellanskär) (L. Sundström, unpubl.).

The inhabited islands encompass an area of about 4 km², with a combined land area of circa 1 km² (Fig. 4). The average distance of the islands from the closest point on the mainland is 0.9 ± 0.42 km (mean ± standard error), and between the islands it is 1.03 ± 0.45 km. Since 1993,

seven islands have been regularly inventoried, GPS-coordinates on colony location obtained, and data on demography, genetics, productivity, and sex ratio consistently collected until 2021 (SUNDRÖM & al. 1996, HAAG-LIAUTARD & al. 2009, VITIKAINEN & al. 2011, 2015). Three additional colonies found on three islands (Lillhamn, Skallotholmen, Halsholmen) were genotyped but not monitored. The colonies are predominantly monogyne, only 33 colonies out of a total number of 252 colonies recorded to date were polygyne, and a few of these were polydomous (E. Vitikainen & L. Sundström, unpubl.). Thus, we have also been able to infer both maternal and paternal genotypes for the great majority of colonies based on worker and offspring male genotypes, and based on this we constructed a pedigree for the population (VITIKAINEN & al. 2015). We have found no evidence for an increased level of polygyny or polydomy during the study period. Three new polygyne colonies were found in 2013 or later, and in total, seven remained alive in 2021 (L. Sundström & E. Vitikainen, unpubl.). Surprisingly, despite the presence of several queens, the

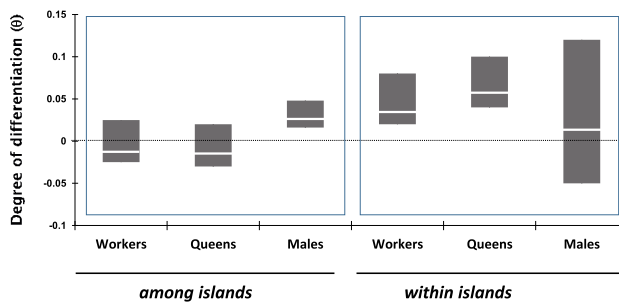


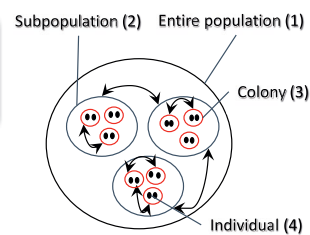
Fig. 6: Genetic differentiation among and within islands for workers and their inferred mothers and fathers. The bars indicate the 5% confidence intervals for the average; those that do not overlap with zero indicate significant differentiation (data from SUNDSTRÖM & al. 2003).

workers of all polygyne colonies were considerably inbred (average homozygosity (APARICIO & al. 2006) $HL = 0.37$, range 0.18 - 0.58, Tab. 1). The corresponding values for monogyne colonies are $HL = 0.28$, range 0.02 - 0.66 (L. Sundström & E. Vitikainen, unpubl.). This suggests that polygyny does not promote increased longevity and lower inbreeding in this population, and that the mortality of polygyne colonies is on par with that of monogyne ones.

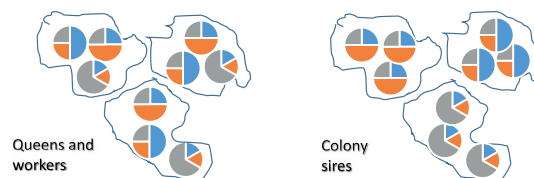
The average life span of a monogyne colony was originally estimated at 6.5 years (HAAG-LIAUTARD & al. 2009) but is now updated to 10 years based on 14 additional years of data on altogether 177 colonies that have died during the study period (Tab. 1). Altogether, 31 colonies have a recorded life span of > 20 years, which tallies with PAMILO'S (1991) estimated maximum life span of 30 years. The average life trajectory of a colony entails a pre-reproductive phase of 3 - 5 years, after which colonies start to produce sexual brood and continue to do so on a yearly basis for the rest of their existence (E. Vitikainen & L. Sundström, unpubl.). Sexual females are monomorphic and anything but adept fliers, but males are dimorphic, with the smaller morph (micraner) classified as a disperser morph (AGOSTI & HAUSCHTECK-JUNGEN 1987, FORTELIUS & al. 1987). This represents a typical example of alternative reproductive and dispersal strategies, in which local habitat patches may be rapidly colonized and monopolized, yet long-range dispersal allows the colonization of new patches further afield (CLOBERT & al. 2001, 2012, and references therein). Nonetheless, although male dispersal will mediate gene flow among populations, a weak aptitude for dispersal in females will introduce considerable constraints on the colonization of new locations as males do not found colonies. The species is essentially specialized on mid-successional and fairly open habitats (SEPPÄ & al. 1995, CZECHOWSKI & al. 2002, MAGGINI & al. 2002). This favours short-range dispersal as this allows rapid monopolization of a habitat patch, yet constrains the colonization of new habitats that become available. Eventually, *Formica* s.str. species will out *Formica exsecta*, as the environment approaches the climax successional stage (SEPPÄ & al. 1995, 2004), at which point selection would favour long-range dispersal

Sociality adds a further dimension as colonies comprise sets of related, yet not genetically identical individuals.

- Three levels of structuring:
1. Structuring among islands
 2. Structuring within islands
 3. Structuring among colonies
 4. Structuring within colonies



The figure below depicts three islands each with three distinct habitable patches indicated by the pie charts. The sectors in the pie charts indicate frequencies of three hypothetical alleles at one gene locus in colony queens, and colony fathers



A pattern with genetic structuring within islands, but not between islands arises if gene flow is limited enough to cause differentiation within islands, such that most of the genetic variation is present within islands, leaving less variation across islands (Fig. 5, queens and workers)

A pattern with significant genetic differences among islands, yet no differentiation within islands arises if dispersal is frequent enough to homogenize allele frequencies within a smaller area, but not across a larger area, as is the case in males and colony fathers (Fig 5, males).

Fig. 7: Gene flow in structured populations. The island model (WRIGHT 1931) describes the accrual of local genetic variation as a result of restricted gene flow. This can create genetically distinct subpopulations.

and colonization of new habitats. Long-term persistence of stable polygynous populations seems possible only in constantly open habitat, such as grazed pastures and alpine meadows (WIEZIK & al. 2017), unsuitable for the wood ants, *Formica* s.str., adapted to climax habitats.

Over time, the landscape matrix within the islands has changed from one with more open expanse towards increased fragmentation into patches of rocky outcrops and open vegetation surrounded by more continuous forest areas (Fig. 5). This may introduce an additional level of structuring, with further limitations on dispersal in its wake. Genetic analysis of the population indeed suggests this is the case, but that the patterns differ between males on the one hand and established queens and workers on the other (SUNDSTRÖM & al. 2003). Males showed significant structuring between islands, but not within islands, whereas the opposite held for queens and workers (SUNDSTRÖM & al. 2003; Figs. 6, 7; Tab. 1). This indicates sex-biased dispersal, by which males disperse relatively randomly within islands but show genetic differentiation among islands. Conversely, female dispersal is reduced to the extent that genetic subdivision within islands accounts for almost all of the total genetic variance, leaving a negligible component for between-island variance (SUNDSTRÖM & al. 2003). In agreement with this result, the established queens also showed significant isolation by distance within islands, whereas males showed none (Fig. 8, Tab. 1). Taken

together, this strongly suggests that queens disperse less than males, a result which gained further support in a later study (VITIKAINEN & al. 2015).

In a later study (VITIKAINEN & al. 2015), we used pedigree data to infer dispersal distances of individual queens and colony fathers, as well as young, yet unestablished queens after their nuptial flights (inasmuch as their pathetic attempts to fly would classify as such, R. Rosengren, pers. comm.). Dispersal was firmly male biased as expected, with an average male dispersal distance (140 m) more than twice that observed in queens (60 m). Based on the pedigree analysis, combined with demographic

data of successfully assigned colonies, only seven out of 65 queens had established a colony on a non-natal island. Correspondingly, only 11 out of 132 young queens collected before colony establishment had dispersed to a different island. Taken together, 90% of all queens, young or old, dispersed less than 550 m. Males crossed islands more often, with 19 / 68 of mates of established queens and 27 / 134 mating partners of young queens originating from a different island (Fig. 9). Nonetheless, only 10% of the males had dispersed further than 1500 m. The number of established queens and their sires that we were able to assign may seem low given the total number of mature

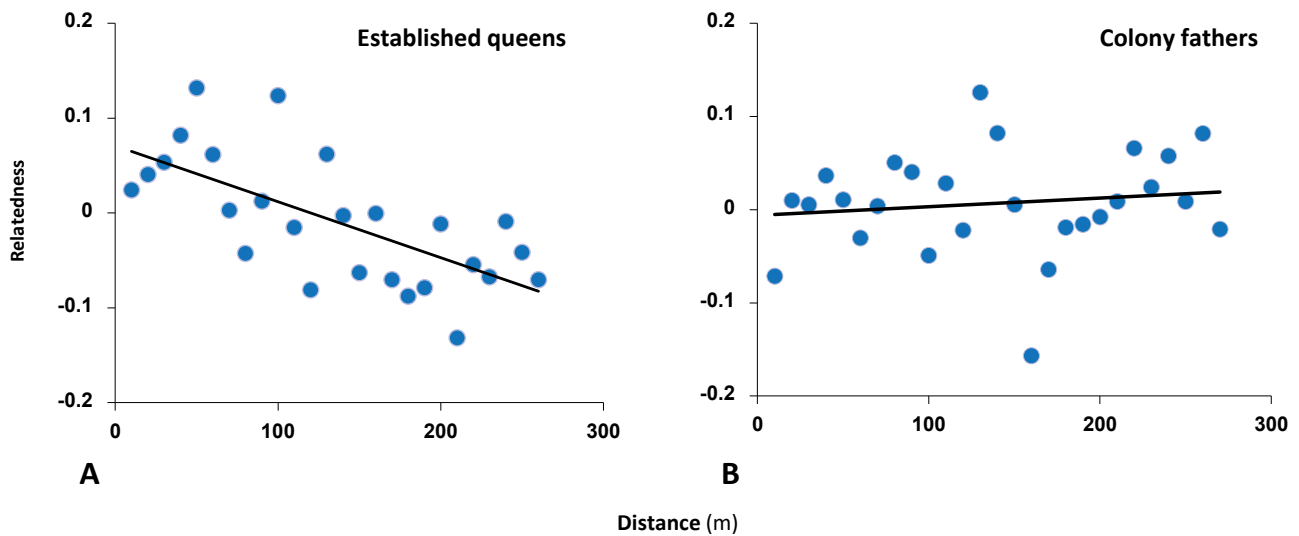


Fig. 8: Pairwise relatedness between queens (A) and colony fathers (B), as a function of the metric distance between nests on the island Joskär (data from SUNDSTRÖM & al. 2003).

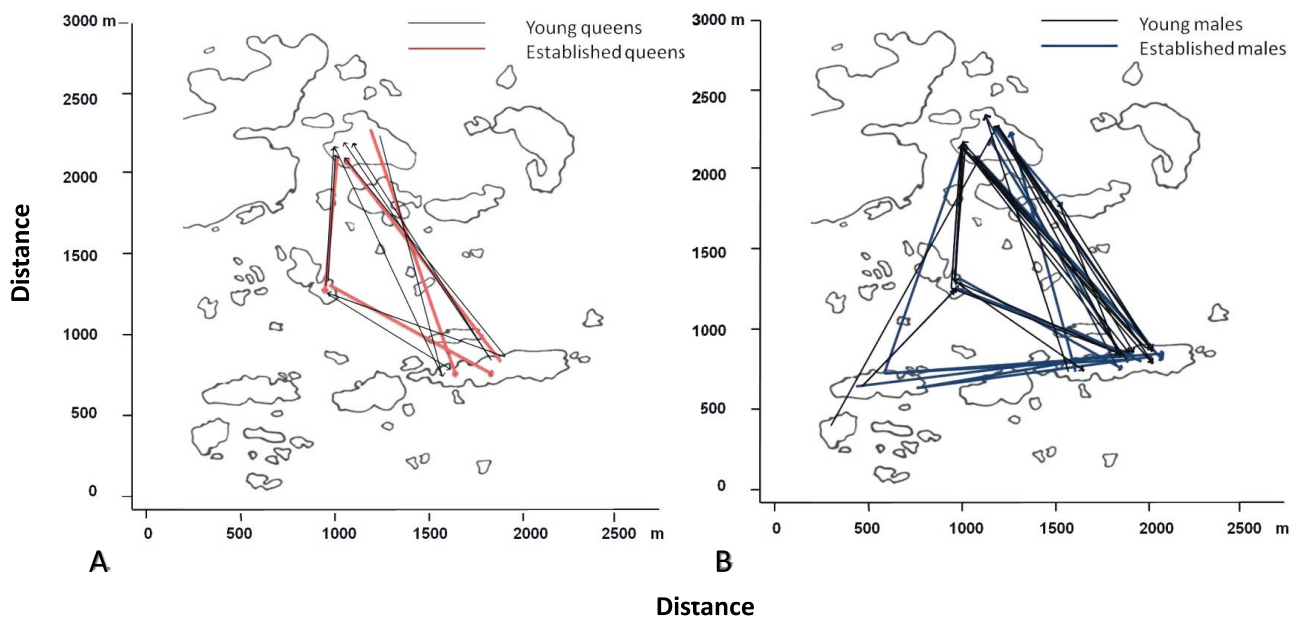


Fig. 9: The number of occasions on which queens (A) or males (B) had crossed from one island to another. Some crossings had occurred before the start of the study, but the maternal or paternal colonies were still alive at the start of the study and were thus genotyped. Four islands have no recorded crossings by queens. These islands were all inhabited at the start of the study, and their parental colonies had probably died earlier (data from VITIKAINEN & SUNDSTRÖM 2015).

colonies (173), but many of the then resident colonies had been established before data collection started, and their maternal colonies had died before the study started and genetic data became available. Furthermore, out of the 173 mature monogyne colonies that existed in the area, the maximum standing population size of mature colonies in any given year was 72, all study islands combined. This highlights the aspect of ant biology that sheer numbers and ubiquity of workers may be misleading as the number of individuals contributing to the genetic variation in the population (effective population size) may be several orders of magnitude lower (SEPPÄ & al. 2009). Indeed, population density thought of this way (in genetic terms) is comparable with medium sized mammals, such as the European hare (e.g., STEPHENS & al. 2019).

The number of colony fathers is higher than that of colony queens because some queens mated with more than one male (SUNDSTRÖM & al. 1996). Similarly, when the information on observed migration events between islands is compared with the genetic estimates, the numbers are in good agreement: The observed migration rate per generation was seven queens + 19 sires, and the estimated rate based on genetic parameters was in total 10 - 15 per generation (data from SUNDSTRÖM & al. 2003, VITIKAINEN & al. 2011, 2015). During the entire study period from 1993 - 2021, only two new islands were colonized (Furuskär, Rovholmar), one of which now is one of only two remaining strongholds of the species in the area (Furuskär, Joskär). Conversely, the species has gone extinct on two islands, Sundholmen (13 colonies) and Brännskär (four colonies), and declined on two more islands (from highest standing crop: Kalvholmen 15 to three, Rovholmar 11 to five) (E. Vitikainen & L. Sundström, unpubl.). On Kalvholmen, the establishment and spread of *Formica lugubris* on the island may have caused the now near demise of both *Formica exsecta* and *Formica truncorum*.

These estimates highlight another aspect of social life. While the monogyne colonies in this population have on average 2300 ± 200 workers (data from VITIKAINEN & al. 2011), each only has one queen mated to one or two (rarely more) males, which contribute to the population gene pool in the following generation. When a population is further divided into subpopulations with reduced gene flow between them, the effective population size is decimated further because of inbreeding (FRANKHAM & al. 2002). A crucial measure that captures population viability is the neighbourhood size, which essentially describes the number of reproductive individuals that form a breeding entity (Tab. 1). Based on data on genetic population division, SUNDSTRÖM & al. (2003) estimated the genetic neighbourhood size to 22 queens and 50 males (following ROUSSET 1997). This agrees well with the observed neighbourhood size, inferred from the mean number of colonies found within the median dispersal distance (on average 17.8 queens and 42.3 males, VITIKAINEN & al. 2015; Tab. 1). These values correspond to an effective population size < 100 , which is below the minimum viable population size (LYNCH & al. 1995a, b, LYNCH & LANDE 1998, FRANKHAM

2005) and tallies well with the observed maximum number of mature colonies (E. Vitikainen & L. Sundström, unpubl.). Similar analyses have been made for long-lived mammals and birds, for example in an analysis of eight common bird species, the genetic neighbourhood sizes ranged from 150 to 7600 (BARROWCLOUGH 1980). In comparison, the neighbourhood size of the endangered European mink (*Mustela lutreola*) was estimated to much less than 100, raising critical concerns regarding population persistence and evolutionary potential in the long term (LODÉ & PELTIER 2005). In general, an effective population size (N_e) of 500 - 1000 is considered to be enough to retain evolutionary potential and to avoid inbreeding depletion (FRANKHAM 1995, FRANKLIN & FRANKHAM 1998, LYNCH & LANDE 1998). However, an N_e as large as 1000 individuals would be necessary to avoid the accumulation of deleterious mutations (LANDE 1995, LYNCH & LANDE 1998).

The low rate of transfer both between and within islands signal population subdivision and genetic structuring. These findings stand in contrast to the generally held view that monogyny usually is associated with extensive nuptial flights and good dispersal abilities (ROSENGREN & al. 1993, ROSS 1993, BOURKE & FRANKS 1995). The results are, however, consistent with the expectations for species that are well adapted to small-scale habitat mosaics, where habitat patches may be short-lived, and dispersal from one suitable patch to another is mediated by short flights, with a premium placed on rapid colonization of such patches (STARRFELT & KOKKO 2012, HAKALA & al. 2019).

Inbreeding, dispersal, and mating patterns

Theory holds that sex-biased dispersal should help mitigate incestuous mating (BENGTSSON 1978, CHARLESWORTH & CHARLESWORTH 1999, GUILLAUME & PERRIN 2009, HARDOUIN & al. 2015; but see LI & KOKKO 2019), yet inbreeding is rife in the study population. This raises the question whether dispersal and / or mating behaviour may contribute to inbreeding. Mate choice is likely absent or rare in ants with large-scale mating swarms as time is short to select a mate (e.g., BOURKE & FRANKS 1995). Nonetheless, incestuous mating may be less likely in genetically structured populations also in the absence of mate choice, especially if mating swarms are large and collect participants from a large area. This may favor increased dispersal distances, which raises the question whether inbreeding influences queen dispersal. *Formica exsecta* performs nuptial flights, which provides the opportunity for outbreeding via dispersal on the wing. Overall, we found no correlation between dispersal distance and queen homozygosity in neither established nor young queens collected after their nuptial flight (VITIKAINEN & al. 2015). However, when we considered only the subset of queens collected after their nuptial flight, for which we also had body size data, we found that when also accounting for size, more inbred queens dispersed a shorter distance (VITIKAINEN & al. 2015). Thus, given that size may critically influence flight ability and stamina (e.g., BROWN & BONHOEFFER 2003, HELMS & KASPARI 2015),

our results suggest that inbreeding in combination with small size places constraints on dispersal. More importantly, however, the flights occur over extended periods (up to a month) as brood maturation is not completely synchronized. This may lead to a limited availability of potential mates, which would further prevent mate choice, even assuming that queens can recognize related males. In the light of these observations, it is not surprising that we found a positive correlation between queen inbreeding and offspring inbreeding, that is, an inbred queen is more likely to have mated with a related male.

Several factors may, nonetheless, reduce incestuous matings. First, sex ratio specialization and male protandry may reduce sib mating, and mating between siblings is indeed rare in the population (only three of the 65 established queens and four of the 120 incipient colony queens, for which we knew the parental colonies of the queens and her male mate, had mated with a brother). However, colonies located close to each other are related, and we found that queens and their male mates were related (incipient and established queens: relatedness, $r = 0.08 \pm 0.008$, mean \pm standard error, $N = 310$; VITIKAINEN & al. 2015). Second, multiple mating or biased sperm use in favour of an unrelated (or less related) sire may alleviate the effects of incestuous mating. In *Formica exsecta*, males share paternity unequally (KELLER & al. 1997), but there was no difference in the expected homozygosity of offspring between the minority and majority patriline of multiply mated colonies ($HL = 0.24 \pm 0.019$ vs. $HL = 0.27 \pm 0.022$, Student's t-test: $t_{90} = -1.19$, $p = 0.24$; VITIKAINEN & al. 2015). However, any such attempts may nonetheless be in vain if all available males come from only a few colonies and are highly related, as is the case in our study population (VITIKAINEN & al. 2015). Indeed, we found that the male mates of doubly mated queens were highly related ($r = 0.31 \pm 0.03$) and often brothers (VITIKAINEN & al. 2015). Beyond actions related to mate choice, longer dispersal distances may lead to more outbred matings and increased offspring heterozygosity. However, we found no association between offspring worker heterozygosity and the distance between mother and daughter colonies. Rather the opposite, longer queen dispersal distance, was associated with higher homozygosity in offspring, that is, the further the queens dispersed from their natal colony, the more related they were to their partners (VITIKAINEN & al. 2015).

We do not know whether queens of *Formica exsecta* mate before or after dispersal, but the observed relatedness pattern suggests that queens may mate close to their maternal colony. If queens could perceive incestuous mating, those that have mated with a closely related male might attempt to disperse further afield in search of a second, less related male. However, given the observed relatedness pattern between queens and their male mates, this does not seem to be the case, perhaps, owing to the spatial and temporal segregation of mating flights, they may not be able to find an unrelated mate. This association between offspring homozygosity and dispersal distance

was only found in young queens and not among established colonies, which implies that there may be selection against queens that are both very incestuously mated and have dispersed a longer distance. These conjectures notwithstanding, the results show that, despite potential means to alleviate inbreeding via multiple mating and increasing dispersal distance, males that co-sire a colony are related, and significant inbreeding prevails to the extent that it may affect both colony performance and potentially population viability. The result of this may thus be an inbreeding vortex, in which inbreeding breeds further inbreeding.

Inbreeding and fitness consequences

An inevitable consequence of small population size, considerable population subdivision, and incestuous matings is an increased risk for inbreeding depression (FRANKHAM 1995). Sociality adds a new dimension to the concept of inbreeding, given that ant queens mate for life. Thus, inbreeding may affect social insects via two routes. First, all the future offspring of a queen that has mated with a related male or males will be inbred and may show signs of inbreeding depression, owing to increased homozygosity or unmasking of deleterious recessive alleles (CHARLESWORTH & WILLIS 2009). Second, the queen herself may be inbred if her mother was mated to a related male, and thus suffer from these effects. Indeed, in eusocial species, both the parental and the offspring generations in a colony may be inbred, with different or even additive negative effects on colony persistence and productivity. Inbreeding may act on key life-history components via several routes, directly on the life span of an individual or colony or indirectly via individual or colony performance. In the latter case, changes may occur directly as a change in reproductive output or be mediated by changes in resource allocation or timing of reproduction. As a result, key life-history components, such as lifetime number of offspring, offspring sex ratio, and age to maturity, may change. Sociality adds an extra dimension to this picture as the responses may occur both at the individual and at the colony level. In addition to visible changes in output or allocation patterns, the responses can often also be measured as physiological or genetic changes, for example, via immune activation or gene expression patterns (Fig. 10).

Our genetic data on the study population in Tvärminne indicate that immigration is negligible as we have found no signs of novel alleles during 25 years of study (L. Sundström & E. Vitikainen, unpubl.). Sex-biased dispersal may reduce the effects of a viscous population structure (BENGTSSON 1978, CHARLESWORTH & CHARLESWORTH 1999, GUILLAUME & PERRIN 2009, HARDOUIN & al. 2015, but see LI & KOKKO 2019), but, as we note above, inbreeding is rife in the population despite a considerable dispersal bias between males and females. This begs the question whether inbreeding in either queens or workers mediates fitness effects in terms of colony longevity or performance. In agreement with the expectations, we found evidence for inbreeding depression in several life-history parameters,

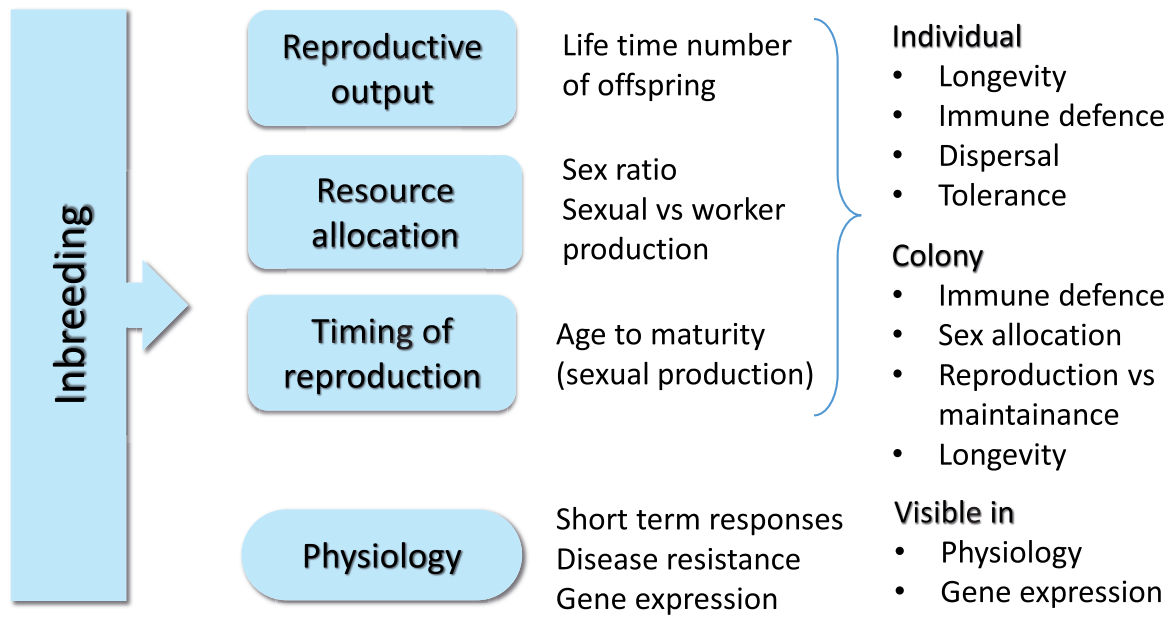


Fig. 10: Potential effects of inbreeding on key life-history-traits. The effects may be visible in either queens or workers – or both. Males cannot be inbred. Trade-offs are present at both the individual and the colony level.

both in queens and workers (SUNDSTRÖM & al. 2003, HAAG-LIAUTARD & al. 2009).

Our observations suggest that inbreeding may hit queens in two ways. First, established colonies headed by inbred queens were younger than those with less inbred ones, explaining 6.8% of the variation in colony age (HAAG-LIAUTARD & al. 2009). This implies that inbred queens either die younger for intrinsic reasons or that such colonies are less viable, perhaps due to a smaller work force, for example as a result of lower fecundity of inbred queens (HAAG-LIAUTARD & al. 2009). However, the latter explanation seems unlikely, given that queen inbreeding affected neither colony size nor productivity (HAAG-LIAUTARD & al. 2009, VITIKAINEN & al. 2011). Second, we found that young queens collected after the mating flight before colony establishment were more inbred than established queens (VITIKAINEN & al. 2015). This suggests that inbred queens have a selective disadvantage during colony founding. The mechanisms remain unknown, but our study, looking at a particular aspect of innate immune defence, using the encapsulation response, found that inbred queens had a heightened immune response, whereas workers showed no such response (VITIKAINEN & SUNDSTRÖM 2011). This could come at a survival cost, particularly at the energetically demanding colony-founding phase, and possibly contribute to a shorter lifespan of inbred queens that have succeeded in establishing a colony.

We found no differences in worker inbreeding between incipient and mature colonies (VITIKAINEN & al. 2015), but workers showed on average higher levels of inbreeding than queens (VITIKAINEN & al. 2011). This suggests that worker inbreeding plays a negligible role in colony founding, initial growth, and survival. Worker inbreeding, nonetheless, comes at a cost to the colony in several

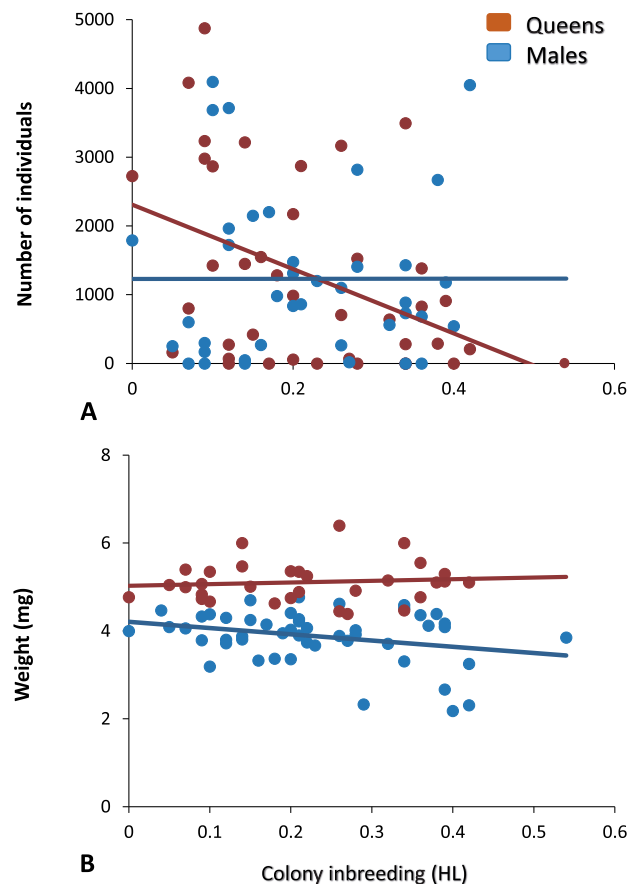


Fig. 11: With increased colony inbreeding, the number of sexual females declines, but the number of males produced remains the same (A). The investment per female sexual, however, remains the same, whereas less resources are invested in each male offspring (B). Originally published in VITIKAINEN & al. (2011).

ways. Perhaps most importantly, the overall production of new reproductives decreased, which confers direct fitness costs in terms of future daughter colonies (VITIKAINEN & al. 2011). This is due to a decrease in the number of new queens produced by colonies with inbred workers (Fig. 11A). No such decline occurred in the number of males produced, and as a result, inbred colonies produce a more male-biased numerical sex ratio. Upon a closer examination of the investment ratios, we found an intriguing pattern: The per capita investment remained the same for new queens, regardless of colony inbreeding, whereas the per capita investment in males decreased (Fig. 11B; VITIKAINEN & al. 2011). This outcome reflects one of the key trade-offs in reproductive allocation, when to produce several small or fewer large offspring (SMITH & FRETWELL 1974). In this case, allocation patterns differ between the sexes, presumably because the colony-founding success of ant queens is contingent on the resources available for the task, whereas male mating success presumably is less dependent on male size or resources, given that they do not accumulate resources post-eclosion (BOOMSMA & al. 2005). Instead, the colony invests more in what could be considered the equivalent to lottery tickets via male offspring. Both effects may stem from overall decreased efficiency of inbred workers in feeding the growing larvae in these colonies.

Apart from direct fitness effects, such as colony survival or productivity, inbreeding also was associated with some physiological responses related to immunity. Thus, the steady state lytic activity, which signals activation of immune functions, was significantly higher in inbred workers than in non-inbred ones (FREITAK & al. 2014). As a result, inbred individuals may be less able than non-inbred ones to handle trade-offs associated with two central life-history traits, immune functions and dietary stress. When exposed to free radicals (ROS) in the diet, lytic activity decreased and mortality increased, as expected, regardless of the level of inbreeding (FREITAK & al. 2014). This stands in contrast to our earlier results, in which we found that workers, regardless of the level of inbreeding, showed a heightened steady-state encapsulation response, compared with young queens, in which the encapsulation response increased with inbreeding (VITIKAINEN & SUNDSTRÖM 2011). This discrepancy is most likely due to differences in the gene regulation pathways that regulate different types of immune responses but nonetheless suggests that inbreeding also may incur costs related to immune defense, perhaps both via workers and queens.

Mating frequency and genetic conflicts

In the long-lived colonies of *Formica exsecta*, queen mating behaviour and dispersal determine both population structure and colony sociogenetic structure for the entire lifespan of the colony. It has well documented theoretical consequences for conflicts both regarding sex allocation and male production (RATNIEKS 1988, BOOMSMA & GRAFEN 1991). Worker reproduction appears absent (HELANTERÄ & SUNDSTRÖM 2007).

According to split sex ratio theory, workers in colonies headed by one singly mated queen gain enhanced inclusive fitness returns under female-biased sex ratios, whereas the queen optimum is always more male biased (BOOMSMA & GRAFEN 1991). The situation differs for colonies with double-mated queens as the genetic value of males converge for workers and the queen. As a result, the population-level sex-investment ratio may be too male-biased for workers in colonies headed by a singly mated queen and too female-biased for workers with a doubly mated queen (BOOMSMA & GRAFEN 1991). An arms race ensues, in that both colony types will drive the colony sex ratio to its extreme – either all female in colonies with a singly mated queen or all male in colonies with a doubly mated queen, driven by the quest to achieve the optimum population sex ratio.

In our study population, queen mating frequency varies between 1 - 3, with 75% of the queens singly mated and 25% multiply mated, and an average mating frequency of 1.27 ± 0.03 (standard error), $N = 225$ (SUNDSTRÖM & al. 1996, VITIKAINEN & al. 2015). We found that colony sex ratios indeed were split in the population such that colonies headed by a singly mated queen specialise in female production and colonies headed by a multiply mated queen specialize on male production (SUNDSTRÖM & al. 1996). The queens, nonetheless, laid equal numbers of unfertilized (male) and fertilized (female) eggs, but male eggs selectively disappeared before maturation in colonies headed by a singly mated queen (SUNDSTRÖM & al. 1996, CHAPUISAT & al. 1997). This demonstrates the presence of an overt conflict regarding resource allocation in the population.

We revisited these results to obtain a more long-term perspective on sex allocation. The new results, which include 28 years of data, confirm the earlier conclusions but also suggest that, in addition to the relatedness asymmetry between sisters and brothers, both inbreeding and colony ageing modulate numerical sex ratios towards an increased male bias (L. Sundström & E. Vitikainen, unpubl.). However, the fact that investment in female brood remains the same but males are smaller partly discounts this effect (VITIKAINEN & al. 2015). The mechanisms whereby workers detect queen mating frequency are unknown, but in *Formica truncorum*, which also shows split sex ratios (SUNDSTRÖM 1994), the cuticular hydrocarbon profile of workers is more diverse in colonies with a doubly mated queen (BOOMSMA & al. 2003). This may provide a cue for the workers whether to bias sex ratios towards new queens or males. In *Formica exsecta*, the cuticular chemistry of adult workers is depauperate (MARTIN & al. 2008, 2013), which may place constraints on the amount of information available to the workers. However, two recent studies suggest that the cuticular chemistry of larvae and pupae is considerably richer, with several short- and long-chained compounds present in pupae and larvae but absent or non-detectable in adults (PEIGNIER & al. 2019, PULLIAINEN & al. 2021). This may convey enough additional information for discrimination (VAN ZWEDEN & al. 2011) if workers assess colony kin structure directly from eggs or larvae.

Standing population and colony performance

Inbreeding may influence population viability via colony performance more generally, but measurable negative effects may not emerge at the level of individual colonies. For example, we found no effect of inbreeding on colony size (HAAG-LIAUTARD & al. 2009, VITIKAINEN & al. 2011), yet average colony size shows a significant declining trend over the years, with a significant decline in the average number of workers produced (Fig. 12; L. Sundström & E. Vitikainen, unpubl.). A weaker trend in the same direction is also visible in sexual production. In agreement with these observations, the yearly standing population has declined from its maximum of a total number of colonies of 126 in the year 2004 to only 45 in 2021. The decline is less drastic for mature colonies, from a maximum of 72 in 2004 to 44 in 2021 (L. Sundström, unpubl.). However, the disparity between the two years of comparison in terms of the proportion of immature colonies is considerable, from about 43% to 2%. For comparison, the average total number of colonies present during 1993 - 2004 was 82.4 colonies, and the net change in population size was positive until 2004 (average + 6.0 new colonies per year). From 2005 onwards, the average number of colonies was 78.6, but the net change was a negative -4.6 colonies per year. For mature colonies, the corresponding numbers were on average 40.6 and + 3.4 during 1994 - 2004, and 57.4 and -1.7 during 2005 - 2021 for the standing population and net change, respectively. Accordingly, in total 136 and on average 9.1 new colonies were founded per year by 2004, whereas in total 52 and on average 3.1 new colonies were founded per year from 2005 to date. Only seven new colonies have been founded since 2012 (L. Sundström & E. Vitikainen, unpubl.).

Colony performance is contingent on longevity, productivity, and output of propagules, and this is in turn contingent on the ability of offspring to handle environmental challenges, such as pathogens or adverse environmental conditions. The capacity to deal with such hazards may depend on the level of inbreeding either at the colony or the individual level. While our research has demonstrated significant effects of inbreeding on several fitness traits, only a few effects of inbreeding emerged in response to environmental challenges via pathogen exposure or climatic conditions either at the colony, individual, cellular, or molecular level (VITIKAINEN & SUNDSTRÖM 2011, FREITAK & al. 2014, BOS & al. 2016). In the case of immune responses, we found that steady state lytic activity was higher in inbred workers, but under oxidative stress the responses of inbred workers were on par with outbred ones (FREITAK & al. 2014). Similarly, the encapsulation response following immune challenge remained unchanged but at an elevated level in workers, yet inbred young queens showed an elevated response (VITIKAINEN & SUNDSTRÖM 2011). A reasonable explanation for this may be that workers are continuously exposed to pathogens when foraging or when in contact with incoming foragers. In a separate study, which did not test for effects of inbreeding, young

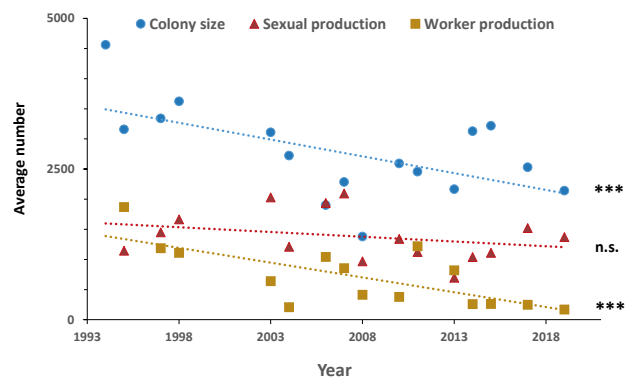


Fig. 12: The change in the yearly average colony size and the number of sexuals and new workers produced across the years. The effect size is small ($R^2 = 0.03 - 0.04$) but significant for colony size and worker production (L. Sundström & E. Vitikainen, unpubl.).

queens, males, nurses, and foragers followed different trade-off patterns upon exposure to the fungus *Beauveria bassiana* (see STUCKI & al. 2017b). In particular, queens and nurses invested in immune functions, at the expense of other functions, whereas males and foragers showed the opposite pattern. This may reflect immune senescence in males and foragers (YOUSEFZADEH & al. 2021).

Starvation may constitute a further challenge, either during hibernation or under resource limitation (MORET & SCHMID-HEMPEL 2000). In laboratory experiments, we found several tissue-specific gene-expression responses to starvation, some of which also suggest differences in gene expression patterns between inbred and non-inbred ants for two genes (Defensin and HSP75) (Bos & al. 2016). Environmental conditions other than starvation, such as drought versus humidity or temperature, may influence survival and induce local adaptation or acclimatization, which may be reflected in gene expression patterns (FEDER & HOFMANN 1999, HOFFMANN & HARSHMAN 1999, TEETS & al. 2012). STUCKI & al. (2017a) furthermore compared the island population with a polygyne mainland population and found repercussions for both survival and gene expression patterns. Temperature drastically influenced survival in the island population, with low survival under hot and dry conditions, whereas no such effects were found for the mainland population. Inbreeding was not included as a factor in this study, but the result begs the question whether overall inbreeding in the island population might have reduced the resilience of the ants. However, in a complementary laboratory experiment conducted in 2019 we found no indications whatsoever of inbreeding on either heat or cold tolerance (N. Bos, E. Vitikainen & L. Sundström, unpubl.). To conclude, there is a paucity of significant effects of inbreeding on either gene expression patterns or physiological functions in response to external stress factors. This may indicate that the capacity for maintaining bodily homeostasis can mitigate stress factors to an extent that renders the responses hard to measure regardless of even a considerable amount of inbreeding.

Furthermore, the gene expression studies only encompass a handful of selected genes, and with a more comprehensive set of genes new patterns may emerge.

Summary and future visions

The long perspective of this study allows us to discern patterns and trends that only become visible after a number of years as well as fluctuations across years that would go undetected in studies that encompass only one or two seasons. Ants also offer a further unique opportunity in that their colonies are largely stationary, allowing reliable tracing of the same individuals or genotypes across years, without the necessity of tagging individuals. This is especially helpful when assessing dispersal rates and distances as well as locating both parents of an individual. Finally, the haplodiploid sex-determination system allows the reconstruction of maternal and paternal genotypes non-destructively by screening offspring workers and males. This is rarely possible in insects (but see EHRlich & HANSKI 2004).

Our genetic studies show a strong signal of limited dispersal and inbreeding in a fragmented landscape matrix (SUNDSTRÖM 2003, HAAG-LIAUTARD 2009, VITIKAINEN & al. 2011, 2015). Consequently, we also find inbreeding depression for several fitness-associated traits, such as sexual production and colony founding success. Inbreeding may crucially affect also individual performance, such as immune responses, efficiency of gene regulation, and ability to combat environmental change (Bos & al. 2016, STUCKI & al. 2017a). Any such downward shifts may not be apparent when judging by the relative performance of colonies if the population as a whole becomes more inbred. To establish any such effect, comparisons with outbred, newly established populations would be required. Some of these consequences are, however, already visible, such as a reduced number of colonies, reduced colony size, reduced production of new sexuals, and indeed signs of increased overall levels of inbreeding in the population (L. Sundström & E. Vitikainen, unpubl.). As a result, the yearly cohorts of new founding queens declines, which may lead to further decline in population size, which may crucially reduce population viability, eventually leading to the demise of the population (LYNCH & al. 1995a, b, FRANKHAM 2005).

In addition to these considerations, the habitat also undergoes changes from an open grass- and shrub-dominated vegetation towards an increased tree cover (Fig. 5). This is a natural process of habitat succession on boreal forests, which leads to an increasingly closed canopy cover, with fewer suitable nest sites for *Formica exsecta*, accompanied by the arrival of competitors, such as the mound building *Formica* s.str. species characteristic for climax boreal forest (SEPPÄ & al. 1995, PUNTTILA & KILPELÄINEN 2009). Indeed, *Formica lugubris* has colonized and started to spread on one of the islands and almost completely ousted *F. exsecta*. This highlights the contrasting short-term and long-term selection pressures on dispersal strategies by which philopatry is selectively favoured when

dispersal is risky, and long-range dispersal to colonise new habitats only pays off when the current environment becomes saturated or uninhabitable (SEPPÄ & al. 2004, STARRFELT & KOKKO 2012, HAKALA & al. 2019). Given the myopic perspective of natural selection, short-range dispersal may prevail despite considerable long-term advantages of long-range dispersal – possibly driving local populations into an inbreeding-mediated extinction vortex (FAGAN & HOLMES 2006).

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