

Causes and consequences of inbreeding
in the ant *Formica exsecta*

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Academic dissertation

To be presented for public examination with the permission of the Faculty of Biosciences
of the University of Helsinki, in the Auditorium 6, B-building in Viikki (Latokartanonkaari 7),
on April 23rd 2010, at 12 o'clock noon.

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Layout © Krista Mäkinen / Potra Girl Design

Cover illustration © Lea Heikkinen

ISBN 978-952-92-7136-8 (paperback)

ISBN 978-952-10-6179-0 (PDF)

<http://ethesis.helsinki.fi>

Yliopistopaino

Helsinki 2010

“These small creatures are within a few inches of our feet,
wherever we go on land—but often, they’re disregarded.
We would do very well to remember them.”

- DAVID ATTENBOROUGH

”..on jokainen muurahainen laulun arvoinen; jokainen Formica on tärkeä.”

- VEIKKO LAVIA MUKAELLEN, LAMMIN KENTTÄKURSSIN MUURAHAISRYHMÄ 1999

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This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

- I Haag-Liautard, C., Vitikainen, E., Keller, L. & Sundström, L. (2009). Fitness and the level of homozygosity in a social insect. – J. Evol. Biol. 22: 134–142.
- II Vitikainen, E., Haag-Liautard, C. & Sundström, L. Inbreeding and reproductive investment in the ant *Formica exsecta* (Submitted manuscript)
- III Vitikainen, E. & Sundström, L.: Inbreeding and caste-specific variation in immune defence in the ant *Formica exsecta*. (Manuscript)
- IV Vitikainen, E., Haag-Liautard, C. & Sundström, L. (Manuscript): Dispersal distance, inbreeding and mating system in the ant *Formica exsecta*
- V Vitikainen, E., O'Hara, B. & Sundström, L. (Manuscript): Sexual production and sex ratio in a natural population of ants: a long term study

Authors contributions to the articles:

- I Sundström designed the study together with Haag-Liautard and Vitikainen. Vitikainen collected the field samples together with Haag-Liautard, and was responsible for compiling and analyzing the microsatellite data. Statistical analyses were conducted jointly by Haag-Liautard and Vitikainen. Haag-Liautard prepared the manuscript and Vitikainen, Sundström and Keller contributed with comments.
- II Vitikainen and Sundström designed the study together. Vitikainen collected and analyzed the field samples, conducted the data analysis and prepared the manuscript. Sundström and Haag-Liautard contributed with comments.
- III Vitikainen designed the study, collected and analyzed the data and prepared the manuscript. Sundström contributed with comments.
- IV Vitikainen designed the study together with Sundström. Vitikainen and Haag-Liautard collected the data. Vitikainen analyzed the data and prepared the manuscript, Sundström contributed with comments.
- V Sundström, Vitikainen and O'Hara designed the study. Vitikainen compiled the data, O'Hara conducted the data analyses. Vitikainen prepared the manuscript, with comments from O'Hara and Sundström.

Abstract

Human actions cause destruction and fragmentation of natural habitats, predisposing populations to loss of genetic diversity and inbreeding, which may further decrease their fitness and survival. Understanding these processes is a main concern in conservation genetics. Yet data from natural populations is scarce, particularly on invertebrates, owing to difficulties in measuring both fitness and inbreeding in the wild. Ants are social insects, and a prime example of an ecologically important group for which the effects of inbreeding remain largely unstudied. Social insects serve key roles in all terrestrial ecosystems, and the division of labor between the females in the colonies – queens reproduce, workers tend to the developing brood – probably is central to their ecological success. Sociality also has important implications for the effects of inbreeding. Despite their relative abundance, the effective population sizes of social insects tend to be small, owing to the low numbers of reproductive individuals relative to the numbers of sterile workers. This may subject social insects to loss of genetic diversity and subsequent inbreeding depression. Moreover, both the workers and queens can be inbred, with different and possibly multiplicative consequences.

The aim of this study was to investigate causes and consequences of inbreeding in a natural population of ants. I used a combination of long-term field and genetic data from colonies of the narrow-headed ant *Formica exsecta* to examine dispersal, mating behavior and the occurrence of inbreeding, and its consequences on individual and colony traits. Mating in this species takes place in nuptial flights that have been assumed to be population-wide and panmictic. My results, however, show that dispersal is local, with queens establishing new colonies as close as 60 meters from their natal colony. Even though actual sib-mating was rare, individuals from different

but related colonies pair, which causes the population to be inbred. Furthermore, multiple mates of queens were related to each other, which also indicates localized mating flights. Hence, known mechanisms of inbreeding avoidance, dispersal and multiple mating, were not effective in this population, as neither reduced inbreeding level of the future colony.

Inbreeding had negative consequences both at the individual and colony level. A queen that has mated with a related male produces inbred workers, which impairs the colony's reproductive success. The inbred colonies were less productive and, specifically, produced fewer new queens, possibly owing to effects of inbreeding on the caste determination of female larvae. A striking finding was that males raised in colonies with inbred workers were smaller, which reflects an effect of the social environment as males, being haploid, cannot be inbred themselves. The queens produced in the inbred colonies, in contrast, were not smaller, but their immune response was up-regulated. Inbreeding had no effect on queen dispersal, but inbred queens had a lower probability of successfully founding a new colony. Ultimately, queens that survived through the colony founding phase had a shorter lifespan. This supports the idea that inbreeding imposes a genetic stress, leading to inbreeding depression on both the queen and the colony level.

My results show that inbreeding can have profound consequences on insects in the wild, and that in social species the effects of inbreeding may be multiplicative and mediated through the diversity of the social environment, as well as the genetic makeup of the individuals themselves. This emphasizes the need to take into account all levels of organization when assessing the effects of genetic diversity in social animals.

Summary

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1. INTRODUCTION

Inbreeding decreases fitness

Human induced environmental change results in fragmentation and destruction of natural habitats, decreasing population sizes and reducing their connectivity. As a result, pairing of related individuals, inbreeding, may become more common, further threatening the well-being and survival of populations (Lande 1988; Frankham 1995; Spielman et al. 2004).

Inbreeding leads to decreased heterozygosity, with individuals inheriting identical copies of the same genes from both their parents. Genetic measures of inbreeding are based on comparing the observed heterozygosity to that expected under random mating (Wright 1951). Inbreeding has deleterious consequences, inbreeding depression, both at the individual and population level (Wright, 1977; Charlesworth & Charlesworth, 1987; Frankham, 1995b; Lynch & Walsh, 1998; Crnokrak & Roff, 1999; Hedrick & Kalinowski, 2000; Keller & Waller, 2002). The two suggested mechanisms underlying inbreeding depression are loss of heterozygote advantage (overdominance), and expression of deleterious recessive alleles (Wright, 1977; Charlesworth & Charlesworth, 1999), with the latter probably contributing more to inbreeding

depression under most circumstances (Charlesworth & Willis, 2009; Ayroles et al., 2009).

Inbreeding depression is typically greatest in fitness-related traits, such as fertility, egg hatching rate and neonatal survival (DeRose and Roff 1999), but inbred individuals may also be more susceptible to environmental stress (Bijlsma et al. 1999; Keller and Waller 2002; Reed et al. 2002; Bijlsma and Loeschcke 2005). Furthermore, interactions with sexual selection or other species may lead to inbreeding depression only being detectable in the wild (Crnokrak & Roff, 1999; Halverson et al., 2006; Joron & Brakefield, 2003; Marr et al., 2006). Stressful environments may hit the inbred individuals harder than outbred ones (Crnokrak & Roff, 1999; Bijlsma et al., 1999; Reed et al., 2002) and a meta-analysis on endangered animals suggests that most suffer from effects of reduced genetic diversity (Spielman et al., 2004).

Challenges in studies in the wild

To date, an important part of our knowledge on the effects of inbreeding in wild populations comes from a few well-studied vertebrate systems (e.g. song sparrow, Keller 1998; Soay sheep, Coltman et al. 1999; red deer, Coulson et al. 1999; reed warbler, Hansson et al. 2002). Large and charismatic vertebrates lend themselves to long-term studies

with extensive field observations, and pedigrees can be built for precise estimation of inbreeding and relatedness coefficients (Pemberton, 2008). In small, more elusive animals these measures are much more difficult to achieve, and only a handful of studies have assessed the direct effects of genetic diversity in natural populations of arthropods (the Glanville fritillary *Melitaea cinxia*, Saccheri et al. 1998; the wolf spiders *Rabidosa punctuata* & *Rabidosa rabida*, Reed et al. 2007; the Japanese bumblebee *Bombus florilegus*, Takahashi et al. 2008).

In practise, indirect estimates of inbreeding, such as marker loci heterozygosity, have to be used in order to investigate the effects of inbreeding in important, yet elusive animal groups, such as insects. The methods are based on observing the loss of heterozygosity on the individual level, or observing bottlenecks at the population-level, and comparing these to individual- or population level estimates of fitness. Yet, concerns have been raised as to what extent these heterozygosity-fitness correlations signify true inbreeding depression. Indeed, heterozygosity in a few marker loci may correlate very poorly with overall genome-wide heterozygosity and true inbreeding (Slate et al., 2004; Balloux et al., 2004), and correlations with fitness may arise due to linkage with loci under selection (Slatkin 1995). However, reliability of these methods depends largely on the population structure. Whereas heterozygosity-based estimates may perform poorly in populations with low average relatedness and low levels of inbreeding, local structuring and variation in relatedness greatly increases the power of detecting inbreeding using marker heterozygosity (Hansson & Westerberg, 2008; Csillery et al., 2006). Furthermore, the correlation between marker and overall genome heterozygosity is expected to be stronger in inbred individuals, so these have to present a reasonable portion of the sample for meaningful conclusions to be drawn (Acevedo-Whitehouse et al., 2003). Hence, in small, bottlenecked populations, the most likely cause for significant heterozygosity-fitness correlations is inbreeding depression, with

heterozygosity estimates reflecting the genetic disequilibrium caused by the pedigree structure of the population (Bierne et al., 2000).

Social insects and inbreeding

Social insects come in great numbers, and have been estimated to comprise over 25% of the animal biomass in tropical forests (Agosti et al., 2001), yet have small effective population sizes (Wilson, 1963; Packer & Owen, 2001), making them vulnerable to loss of genetic diversity and inbreeding depression. A single colony of social Hymenoptera may comprise over a million workers, but only one breeding pair, one of which is haploid and so captures only half of the genetic variation found in diploid organisms (Pamilo & Crozier, 1981; Trivers & Hare, 1976). Indeed, studies on social insects, especially ants, have demonstrated considerable population structuring (Seppä et al., 2006; Liautard & Keller, 2001; Gyllenstrand et al., 2005; Pamilo et al., 2005), but only a few of these have specifically assessed the level of inbreeding (Trontti et al., 2005; Darvill et al., 2006; Gyllenstrand & Seppä, 2003; Mäki-Petäys & Breen, 2007), and even fewer have analyzed fitness consequences of inbreeding in the wild (Whitehorn et al., 2009).

The social organization of the colonies has important implications for studies of genetic diversity and inbreeding. Reproductive division of labor among the females (queens and workers) probably is the key to the success of this group (Oster & Wilson, 1978). While kin selection explains the workers' propensity for giving up their reproductive rights, the theory also predicts conflict among the colony members (Hamilton, 1964). For this reason, inbreeding could, in principle, be even beneficial for the colonies, as it increases the genetic similarity among the colony members, potentially aligning their interests and reducing conflict (Ratnieks et al., 2006). Therefore, social systems might promote inbreeding through increased inclusive fitness, even when inbreeding actually reduces direct fitness (Kokko & Ots, 2006) and systematic inbreeding observed in some ants

could be adaptive, as suggested by Trontti et al. (2005).

One reason why effects of inbreeding have been assumed marginal in the social Hymenoptera is that they are haplodiploid, with males arising from unfertilized eggs. If inbreeding depression is due to the expression of deleterious recessive alleles inherited from both parents, in theory, these should be purged through the haploid males (reviewed in Werren, 1993), alleviating the genetic load. However, purifying selection through haploid males cannot work for traits that have caste-specific expression, such as traits for female fertility or longevity. Consistent with this view, inbreeding depression has been found in a solitary Hymenoptera species (Henter, 2003) and several other haplodiploid organisms (Perrot-Minnot et al., 2004).

On the other hand, social Hymenoptera also have an added cost of inbreeding due to the complementary sex-determination mechanism (Cook, 1993) that leads to production of abnormal diploid males, when queen and male have the same allele in the sex-determining locus. The diploid males are typically unviable or sterile (but see Cowan & Stahlhut, 2004) and may be killed off by the workers (e. g. Petters & Mettus, 1980; Pamilo et al., 1994). As a result, diploid male production reduces colony founding success, productivity and survival (e.g. Tarpy & Page, 2002; Ross & Fletcher, 1986), and is considered a threat to wild populations of social Hymenoptera (Packer & Owen, 2001).

Inbreeding avoidance has been proposed to be the evolutionary reason for many common features of reproductive biology in ants (Wilson, 1963), such as dioecy (colonies produce single sex broods, Wiernasz & Cole, 2009; Freeman et al., 1997), and protandry (early male emergence, Bulmer, 1983), both of which may reduce the probability of sib-mating. Another common reproductive strategy of ant colonies is dispersal and mating flights, where individuals from different colonies mate in what are thought to be panmictic mating swarms. Yet no studies so far have looked at the effectiveness of dispersal in reducing inbreeding in ants.

Studying dispersal in the wild is difficult, due to the difficulties in distinguishing between individuals migrating or dying (e.g. Doligez & Pärt, 2008), and as a result, most of our knowledge on natal dispersal, particularly in insects, is indirectly inferred from the spatial (Mercader et al., 2009; Driscoll et al., 2010), or genetic population structure (e.g. Seppä et al., 2006; Gyllenstrand et al., 2005; Sundström et al., 2003; Holzer et al., 2009). Nevertheless, the relationship between true natal dispersal distances and population genetic estimates remains largely unknown (Rousset, 2004).

Perennial ant colonies provide a tractable system for studies of inbreeding and dispersal in insects. Monogyne colonies, that have only one reproducing queen, are particularly interesting as they can be extremely long-lived and spatially stable (Keller & Genoud, 1997, Pamilo 1991). A monogyne colony, once established, doesn't often change place and as the queens are not replaced, colony life-span will reflect that of the mother queen. Furthermore, queens mate for life before colony establishment (Boomsma, 2009). The stability of these highly related family groups allows for accurate assignment of dispersed individuals to their natal colonies; moreover, the genotypes of the parents of the colonies, queen and the male(s) she has mated with, can be deduced from the workers, allowing for long-term monitoring of reproductive success of the parents of the colony, with minimal disturbance.

2. AIMS OF THE THESIS

The main aim of the thesis was to examine the causes and consequences of inbreeding in a natural ant population. Specifically, the aim was to investigate the effects of inbreeding on both generations present in the colonies: the queens and the workers.

The aim of Chapter I was to see whether inbreeding depression affects colony fitness parameters such as productivity and longevity, and whether the inbreeding level of the queen, and

STUDY QUESTIONS	MAIN RESULTS
I Is there inbreeding depression in <i>Formica exsecta</i> ? Are there different effects if the queen heading the colony is inbred, versus if the workers are inbred?	<i>Queen inbreeding results in reduced longevity of the colony, whereas worker inbreeding leads to reduced proportion of sexual brood in the colonies.</i>
II What are the effects of inbreeding on productivity of the colonies? Does inbreeding level of the colony workers affect the size and numbers of queens and males produced in the colonies?	<i>Sexual production is reduced in colonies with inbred workers, as well as per capita productivity of the workers. Fewer queens are produced in inbred colonies, but their size is not affected. Males produced in inbred colonies are smaller, and the effect is caused by them being raised by inbred sisters.</i>
III Are there differences in the immune defence response between workers and queens of <i>Formica exsecta</i> ? Does inbreeding affect the ant immune defence?	<i>Workers have a higher immune response than queens. Queens from inbred colonies have a heightened immune response, either due to direct effects of inbreeding, or through having been raised in colonies with inbred workers.</i>
IV What is the dispersal system in <i>Formica exsecta</i> ? Are multiple mating or dispersal effective in reducing inbreeding in the population? Does inbreeding level of the queen affect her dispersal or mating behavior, or the success at colony founding?	<i>Dispersal is sex-biased, with males dispersing further (150m) than queens (60m). Neither dispersal nor mating multiply are effective inbreeding avoidance mechanisms in the population. Queen inbreeding did not affect dispersal, but inbred queens were more likely to mate with a relative, and had reduced colony founding success.</i>
V What are the relative effects of environmental variation and genetic variation in determining the sexual production and sex allocation in the population? What is the effect of inbreeding and relatedness on sex ratios of the colonies?	<i>Variation in sexual production is mainly caused by extrinsic factors, while sex ratios mainly depend on colony properties. Inbreeding decreases productivity, and impairs the workers' ability to bias the sex allocation of the colonies according to their kin-selected benefits, as inbred colonies produce more males.</i>

Table 1: Summary of the main study questions and results of the chapters of this thesis

that of the workers, has different effects on colony survival and fitness.

In Chapter II, the aim was to look into the effects of colony inbreeding in more detail to find out whether numbers and size of sexual offspring (males and queens) are traded off in the colonies as a response to inbreeding, and whether the effects of being raised by inbred workers differ for the two sexes.

In Chapter III, the focus was on one possible trait showing inbreeding depression, immune defence. The aim was to find out whether a

commonly used measure, induced encapsulation response, differs between the female castes (queens and workers), and whether the level of inbreeding of the colony has an effect on the immune response.

The aim of Chapter IV was to examine the relationship between mating behavior, dispersal and inbreeding. More specifically, the aim was to see whether inbred queens have an increased tendency for incestuous mating, and whether the queen inbreeding affects dispersal distance. Additionally, the aim was to compare mating behavior and inbreeding among the established

and young queens to examine whether inbred or incestuously mated queens are selected against during the early stages of colony founding.

The aim of Chapter V was to take a different approach to the long-term data and to quantify which proportion of the variance in the biomass production in ant colonies can be attributed to environmental variation, and to what extent it is due to variation in the inbreeding level of the colonies. Additionally, the aim was to examine whether inbreeding affects the sex allocation patterns of the colonies.

3. MATERIAL & METHODS

Study species

Study-species is the narrow-headed ant *Formica exsecta* Nyl. (1846). It is a Palaearctic ant with a wide distribution ranging from the British Isles all the way to Japan. It is an ecotone species that inhabits forest clearings and borders, preferring open and sunny patches in mixed and dry woodland (Czechowski et al., 2002). *F. exsecta* occurs in two social morphs, which may coexist in the same area (Seppä et al. 2004). Polygyne colonies contain tens to hundreds of queens and some hundreds of thousands of workers. Colonies of the monogyne form are much smaller (Ø10-30 cm) with some thousands of workers, and one reproducing queen. The colonies produce new sexuals – males and queens – yearly, and in June-July the sexuals fly out of the colonies to disperse and mate on a mating flight, where queens store the sperm from one or several males in their spermatheca; males die after the mating flight. Young queens found colonies independently (without the help of workers from the natal colony) or by temporary social parasitism in colonies of *Serviformica* ants. The colonies can be extremely long-lived, with the same queen reproducing for several decades (Pamilo, 1991), whereas workers typically only survive for one year.

Study population & data collection

The study population is located on 8 islands close to Tvärminne Zoological station in Hanko, on the SW coast of Finland (59°50'N, 23°15'E), and it has been surveyed since 1994 for yearly estimates of colony size, productivity, sex ratio and longevity (Chapuisat et al., 1997; Sundström et al., 1996; Sundström et al., 2003). During the study, genetic samples have been collected of 235 colonies in total, out of which 222 unique colonies were detected. In between 10-16 workers and 5-10 males per colony, altogether 4400 individuals, were genotyped at 10 highly polymorphic microsatellite loci to determine the level of inbreeding and the sociogenetic structure of the colony – the number of reproducing queens and the males they had mated with. Monogyne colonies form a majority of the population (87%) with a single queen mated with one to three males (average mating frequency 1.27, range 1-3). The population is inbred (worker F_{IS} 0.09, 95% CI 0.041-0.16) with high variation in the inbreeding level between the colonies.

In 2007, I measured immune responses of newly emerged workers and queens, using the encapsulation assay, from individuals collected from the study colonies and reared in the lab (III). This allowed me to compare the immune responses of the two castes and the effect of colony inbreeding on them. In addition to the yearly numerical estimates of productivity obtained by mark-recapture of the brood, I collected males and queens from on top of the colonies before the mating flights in summer 2008 to determine their mature weight, and to examine colony-level effects of inbreeding on the number and size of males and queens (II).

To obtain estimates of effects of inbreeding on the colony founding success of the queens, young queens were collected after the mating flights, in July-August 2003-2007. Altogether 138 young queens were caught walking on the ground after dispersal from their natal colony. They were genotyped at the 10 microsatellite loci to determine their level of inbreeding, and their

mating frequency and relatedness to the males they had mated with was determined from the sperm they had stored during the mating flight.

The detailed data on population demography and colony genetic structure allowed me to assign a majority of young queens and their male mates back to a colony of origin, by using the microsatellite markers and haplotype data available from the population. Similarly, I deduced the genotypes of queens heading established colonies, and that of their mates, from their offspring, and assigned them to a colony of origin within the study area (IV). Dispersal was then calculated as the distance between the present colony (established queens) or the place of capture (young queens) and the colony of origin. I could then look at effects of inbreeding depression during the early stages of colony foundation (I,IV) and the effects of natal dispersal on the inbreeding level of the future colony, by comparing the dispersal distance, mating frequency and inbreeding between the young queens and those that had already successfully established a colony.

Estimation of inbreeding

I used two methods to estimate colony inbreeding which have slightly different theoretical founding. The first is based on relatedness between parents (Queller & Goodnight 1989), where allele frequency in the background population is taken into account, so that individuals that share a rare allele are considered to be more closely related (and their offspring more inbred) than individuals that share a common allele. When calculated as the one-way relatedness of male to the queen, the relatedness value is equal to the inbreeding coefficient of the offspring, the F_{IS} (Liautard & Sundström, 2005), in haplodiploids.

The second method used to estimate inbreeding was the homozygosity by loci (HL) estimate of Aparicio et al. (2006). I estimated the average homozygosity across loci, weighted by the information content of the locus, for each individual. The colony inbreeding is then the

average value of workers in the colony, which also equals that of new queens produced in the colonies if caste-determination is independent of the heterozygosity of the individual (II). The advantage of this method is that in established colonies, no deduction of parental genotypes is needed, as the inbreeding coefficient can be calculated directly from the workers of the colony. However, to compare this estimate to young queens that have not yet established a colony, the genotypes of their offspring need to be constructed from their spermathecal contents (I, IV). These two measures of colony inbreeding were, however, highly correlated, and choice of method did not affect interpretation of the results (II).

Concerns have been raised over the use of heterozygosity-based measures of inbreeding, so I tested the consistency of the inbreeding measure from the different marker loci to estimate whether the signal between markers is consistent and likely to reflect heterozygosity at the genome level (I). To achieve this, the microsatellite loci were randomly divided into two groups of five and the correlation between the estimate from the two groups estimated by resampling 10 000 times. A significant correlation between the two groups of markers confirms that the signal from different loci is consistent and likely to reflect inbreeding in this population (Balloux et al. 2004).

4. MAIN RESULTS AND THEIR INTERPRETATION

Inbreeding impairs fitness of ants

My results show that inbreeding decreases fitness with respect to several traits in the ant *Formica exsecta*, both at the level of the queens and the workers. The inbreeding level of the two castes had different effects, with worker inbreeding reducing the productivity of the colonies, and queen inbreeding reducing her survival and longevity. Below, I will discuss these findings in more detail.

Colony level effects of inbreeding

An ant colony is inbred if the mother queen has mated with a related male. This results in the reduced heterozygosity of workers of the colonies. As a result of this colony-level inbreeding, the sexual productivity of the colonies decreased (Fig. 1; I, II), owing to reduction in the number of queens produced in inbred colonies (Fig. 2. II, V), increased numbers of worker brood being produced, instead of queens (I, II) and reduced size of males (Fig. 3, II).

Colony inbreeding decreased the number of new queens produced in the colonies (II), and sex ratios in colonies with inbred workers were consistently male-biased (V). The reduced numbers of queens could, in principle, result from caste determination being conditioned on the individual's level of heterozygosity; however, this was not observed (II), and it would be an unlikely mechanism taking into account the high number of chromosomes and typically high rates of recombination in the social Hymenoptera (Wilfert et al. 2007). A more likely explanation is that inbreeding impairs the overall efficacy of the workers tending to the brood. If growing diploid larvae get insufficient nutrition, this could translate into fewer of them entering the queen developmental pathway (Evans & Wheeler, 2001).

In a previous study of the same population (Sundström et al., 1996), sex ratios of the colonies varied consistent with the workers controlling the sex ratios in order to increase their own inclusive fitness (Trivers & Hare, 1976; Boomsma & Grafen, 1990; 1991), with colonies of high relatedness producing new queens, and colonies with low relatedness more males. Inbreeding appears to interfere with this process, as the effect of relatedness on the sex ratios was weak, with inbreeding accounting for most explained variation at the colony level (V).

A shift to more female biased sex ratios with inbreeding would be expected if workers were controlling the ratio according to their kin-selected benefits, because inbreeding further

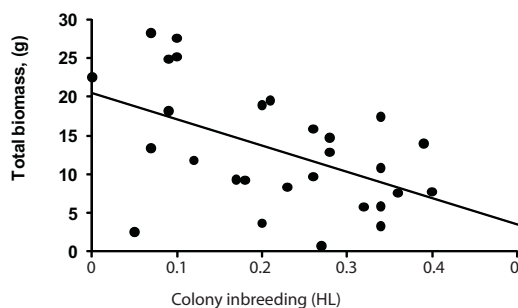


Fig. 1. The biomass production of ant colonies decreases if the workers of the colonies are inbred.

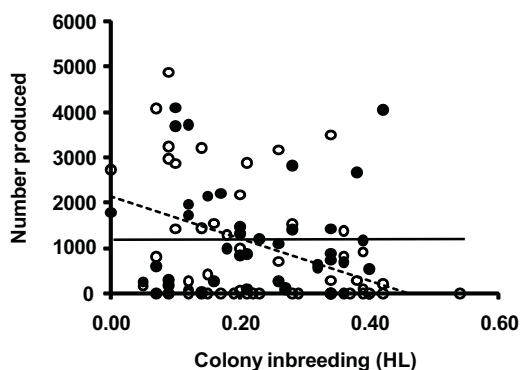


Fig 2. Fewer new queens (dashed line) were produced in inbred colonies, whereas number of males (solid line) was not affected by colony inbreeding.

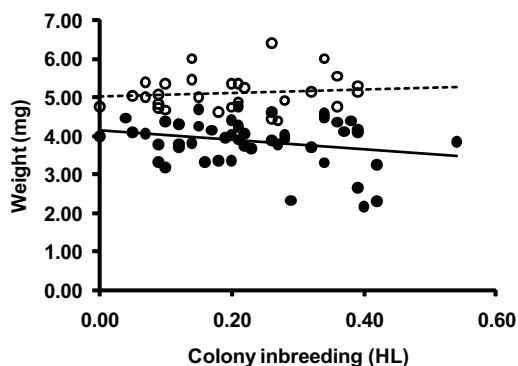


Fig 3. Males (solid line) produced in inbred colonies were smaller, while queen size (dashed line) did not change with colony inbreeding.

increases the genetic similarity within the colonies (Trivers & Hare, 1976; Boomsma & Grafen, 1990; 1991). However, the opposite was observed, a shift towards male bias in inbred colonies. Hence, inbred workers may not be able to produce the sex ratio most beneficial to them, possibly due to limited resources of the inbred colonies. The results show that depending on the mechanism, negative effects of inbreeding can also constrain the effect of kin selection in social insects.

Effects of inbreeding on males

Male size decreased with the increasing inbreeding level of the colony. As males are haploid, individual heterozygosity cannot account for their reduced size, and the effects have to be mediated through the inbred workers tending to the brood. In theory, the result could also be due to the smaller males being diploid, abnormal males that colonies of Hymenoptera produce as result of a matched mating in the sex-determining locus (Cook 1993). However, Hymenopteran diploid males tend to be bigger, not smaller, than haploid males (e.g. Naito & Suzuki, 1991; Yamauchi et al., 2001) and the prevalence (5%) of diploid males is too low to account for the observed reduction in the size of males. Therefore, reduced male size represents socially mediated inbreeding depression, whereby the diversity of the rearing environment, rather

than the genetic make-up of the individual itself, results in inbreeding depression.

Diploid male production may, however, play a role in reducing the overall productivity of the colonies, if the diploid males are destroyed by the workers. However, the number of diploid offspring doesn't change with colony inbreeding (II), which suggests that the effect, if present, is negligible. Also, the cost of diploid male production is typically largest at the colony-founding stage, when queens mated with related males produce diploid male offspring instead of workers, leading to lower growth rate and survival of the colonies (e.g. Ross & Fletcher, 1986). As colony inbreeding had no effect on establishment of the colonies, conclusion is that diploid male production is not a major cost at this stage. The result may reflect the biology of *F. exsecta*, as queens parasitizing existing *Serviformica* colonies would be buffered against effects of diploid male production at the colony-founding stage.

Effects of inbreeding on queens

Inbred colonies produced fewer queens, but some inbred young queens occur in the population, as on the individual level, heterozygosity did not affect caste determination (II). In contrast to males, the size of queens remained unaffected by inbreeding. However, immune defence of the queens was affected, with young queens produced in inbred colonies having a heightened encapsulation response. This can be a direct effect of inbreeding of the queens themselves, as their inbreeding coefficient will be equal to that of their sister workers from which the colony inbreeding was measured. Alternatively, the heightened response may reflect physiological consequences of having been raised by inbred workers, or even effects of colony comprising inbred workers, if for instance such colonies have increased parasitic loads.

Perhaps surprisingly, the immune response was higher in inbred than in non-inbred queens. This may reflect modified stress response of the inbred queens, or the workers tending them,

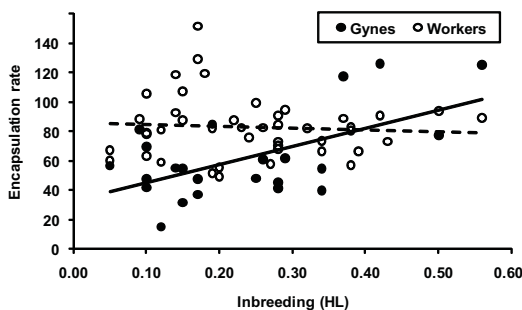


Fig. 4. Queen immune response (solid line) was upregulated in inbred colonies, worker response (dashed line) was not.

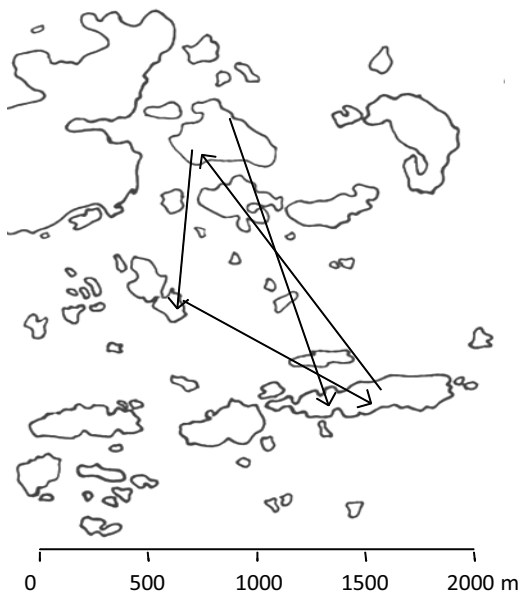


Fig. 5. Between-island dispersal paths of established queens. Only 4 out of 69 queens that originated from the study area were observed to have dispersed from one island to another.

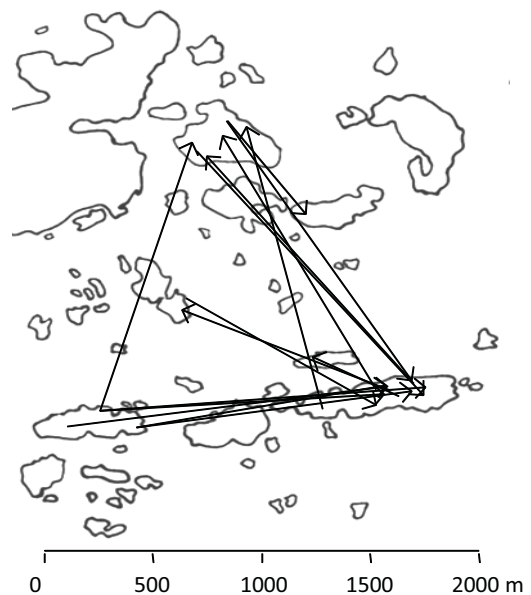


Fig. 6. Between-island dispersal paths of males of established colonies. Out of 74 males that originated from the study area, 14 were observed to have dispersed from one island to another.

or impaired capability of inbred queens to regulate the response at an appropriate level (III). Alternatively, inbreeding level of the colony could reflect in the response of queens through increased parasite pressure of the inbred colonies, eliciting up-regulated immune response, in which case the response would be adaptive. Whichever the mechanism, the inbred queens are later selected against, as their proportion decreases during the colony foundation stage (IV) and the ones that survive have reduced longevity (I). Altogether, effects of inbreeding depression in queens, together with male biased sex-ratios of inbred colonies are a probable cause for the lower inbreeding level of mature queens as compared to workers in several *Formica* species (Hannonen et al., 2004; Sundström et al., 2005). This warrants further research on the effects of inbreeding on the queens of social insects.

Mating behavior

The effects of inbreeding in the population are multiplicative, as the inbred queens themselves have a higher probability of incestuous mating (IV), although the effect was small. Inbred individuals could be more prone to incestuous mating for a reason or another, either due to heritability in mating behavior, or their condition affecting the mating behavior or dispersal. However, the result was not mediated through reduced dispersal, as queen heterozygosity had no effect on the queen dispersal distance (IV). Sister-brother matings were rare (around 5%; see IV) and do not account for the observed inbreeding level of the population. Indeed, colonies often specialize in producing one sex only (Sundström et al., 1996) which will prevent sib mating. Instead, population level inbreeding seems to arise from a substantial part

of the pairings taking place between individuals from related, yet different colonies. Also, when queens were multiply mated, the males were highly related. This further supports the idea that mating flights are localized, perhaps with only a few colonies contributing to a swarm at any one time. Indeed, based on field observations, colonies release sexuals at very different times, sometimes weeks apart, which leads to mating flights being well separated both in time and space, and far from panmictic. The results show, that mechanisms curbing sib-mating are not necessarily effective in preventing inbreeding.

Dispersal behavior

The results show that dispersal is sex-biased in *Formica exsecta*, with majority of the queens staying as close as 60m to their natal colony. Males fly further, but not for more than 150 meters on average. Also, many more males were observed having flown from island to another, as compared to queens (Fig. 5. & 6.) which further confirms the result. The dispersal distances were strikingly similar among the established and the young queens, which indicates that the distances measured are what most individuals in the population would experience. The number of colonies that a dispersing queen or male would have within its typical range of dispersal (17.4 for queens, and 44 for males), corresponded very well to the neighborhood sizes previously estimated from the population genetic data (22 for queens, 50 for males, Sundström et al., 2003). This suggests that while some dispersal occurs to and from outside the study area, the effect of emigrants on the population genetic structure is negligible.

The study shows that despite dispersal on the wing, ant queens do not end up establishing new colonies far away from their natal colony; also, despite mating flights and a low fraction of actual sister-brother matings, the population is inbred, owing to local dispersal. To our knowledge, only one study so far has estimated dispersal distance in a natural population of ants (Suní & Gordon, 2010),

where based on population genetic estimates, queens of *Pogonomyrmex barbatus* also show very short dispersal distances despite dispersing on the wing. Indeed, reduced natal dispersal by the queens may be a common feature in social Hymenoptera, even in species with extensive mating flights, which may aggravate the genetic loss caused by habitat fragmentation and low population sizes (Packer & Owen, 2001, Goulson et al., 2008). Reduced dispersal and subsequent inbreeding contribute to the decline and extinction of local populations of endangered animals (Daniels & Walters, 2000), and these effects may be particularly important in species like social insects, where mating and dispersal occur during a very limited period of an individual's lifespan, and where the size of the breeding population is small.

Possible mechanisms of inbreeding depression in ants

The results presented here suggest that inbreeding, measured as heterozygosity, compromises the efficiency of ant workers, and reduces longevity and survival of ant queens. Recent studies on the fruit fly show that, at the genome level, inbreeding depression may present a form of genetic stress that changes the regulation of genes involved in metabolism, defence and stress responses, thus decreasing the energetic efficiency of inbred individuals (Kristensen, 2006; Ayroles et al., 2009). Consistent with this view, inbreeding reduces growth rate (Roff 1998), and increases resting metabolic rate in crickets (Ketola & Kotiaho, 2009), and reduces mass gain in white-footed mouse (Jimenez et al., 1994). If this change in gene expression reflects a common mechanism of inbreeding depression in insects, it would explain the observed effects on ant colonies. Compromised energy allocation of the workers would lead to decreased efficiency or increased energy expenditure of the workers, reducing the growth of larvae with possible cascading effects on caste determination, as discussed in II and V. Inbred queens would have heightened stress response

leading to up-regulation of their immune response (III), lower survival during the colony foundation phase (IV) and reduced lifespan (I). This calls for studies on the metabolic effects of inbreeding in Hymenopteran species.

5. FINAL REMARKS AND FUTURE CONSIDERATIONS

Effects of inbreeding on the social Hymenoptera have so far been considered marginal, or only attributable to the production of diploid males. The results in this thesis show that inbreeding has deleterious effects on fitness of ants, and that these effects are mediated by the both generations present at the colonies. The social mode of life can make the situation complex, as the levels also interact: the lower survival of inbred queens turns into a shorter lifespan of the colony, and the lower efficiency of inbred workers translates into smaller size of their brothers. In social animals, not just the genetic diversity of the individual itself, but also that of the rearing environment may have profound effects on the fitness of the individuals. All levels of inbreeding should be considered when evaluating sustainability of social insect populations.

The results raise many interesting questions that could not be examined within the scope of this work. An obvious line for future research would be to follow up on the mechanism of inbreeding depression at the individual level. If costs of inbreeding are mediated through the increased energy expenditure of workers, this could be shown using metabolic arrays such as respirometry, or by directly looking at gene expression to confirm whether the patterns presented in Ayroles et al. (2009) reflect general effects of inbreeding among insects.

The increased immune response of the queens coming from inbred colonies raises the possibility that inbred colonies might be harboring higher levels of pathogens; at present, we have no data on occurrence of pathogens or natural parasites in the colonies, but this would be interesting to look at. The study also focused on one aspect of the immune

defense, and as there may be trade-offs between different components of the insect immune defense, it would be interesting to see whether there are caste-specific or inbreeding-related differences in other immune traits as well.

Finally, some of the effects stayed the same throughout the study years, while others, such as the overall reduction in productivity, could only be seen during some study years (II), or when taking the yearly variation into account (V), but not when using yearly averages (I). This calls for careful evaluation of the analysis methods when looking at long-term data. With the current data, we could not answer the question whether this is due to inbred colonies having more variation, or being more vulnerable to environmental fluctuations. To what extent environment and inbreeding interact is a big question in conservation genetics, and warrants further studies.

6. ACKNOWLEDGEMENTS

Lotta, you have been the most inspiring and supportive supervisor. I am very grateful I had the opportunity to work with you and to learn so much from you. You encouraged me to pursue my ideas, and introduced me to the scientific community from very early on. You were always there to help and advice, at times when it was least deserved and most needed, such as the hectic write-up of this thesis. I could not have wished for a better supervisor.

Cathy, my second supervisor at the start of this project – thank you for all your help, guidance and friendship through these years. The other co-authors of the papers, Bob - - you dragged me into the slightly scary, yet beautiful world of Bayesian statistics; thanks for the shared cat-enthusiasm as well. Thanks to Laurent Keller, for collaboration and inspiring discussions, and for your support in applying for the PhD funding.

Pekka, thank you for being my Custos, and also for all the ant advice along the way - but most

of all, thank you for the Tvärminne badger story. Michel Chapuisat and Jouni Aspi are gratefully acknowledged for very efficient and helpful reviewing of this thesis.

During my studies I got to travel and meet so many wonderful people, within the social insect research (yes, it is both the scientists and insects that are social!), and beyond. Thanks to the organizers and participants of the network meetings, and the nights at the pub around these occasions.

Naomi Pierce, thank you for so kindly welcoming me as a hang-around member in your lab, and for your encouragement and ideas on my work. Thanks to Sophie Armitage for teaching me the details of the immune assays, the whole Copenhagen lab and Koos Boomsma for hosting the visit, and the scientific advice along the way.

During these years, I've had the pleasure of working with many people in the various side-projects not included in this thesis, and it has been a great joy to broaden my view to areas further than inbreeding in ants. Thanks to Jelle Van Zweden and Patricia D'Ettore, and Stephen Martin for fruitful collaborations involving ant chemistry; Jouni Laakso, for introducing me to the world of bacteria and helping with the immune assays; Markus Öst, Mika Kilpi and Kai Lindström for the fascinating eider duck project.

Thanks to Tvärminne Zoological Station personnel for making it all go smoothly, and for providing the most beautiful surroundings for the field work. Thanks to all the fellow inmates of Tvärminne during the summers ever since 2002 when my Master's project first came about. Katja H, Marja & Maria, Topi and the other Tokko's; Riggert, Magnus, Markus, Cathy & Christoph, and all the other station dwellers through these years – thanks for the Bear-flag parties and the smoked fish on the rock. Thomas, you're such a wonderful person to talk with. Olli, thanks for your friendship, for the mushroom picking, skiing and the seals.

You dragged me out of the office, and made me remember again why I am a biologist.

Team::Antzz, all members present and past! Perttu, Cathy, Vienna, Minttu, Kalle, Heikki, Katja, Kriko, Mikko, Martin, Tuomo, Riitta, Sedeer, Cédric, Terhi, Hannele, Ulla, Lea, Unni, Anton and Martina. Working with such wonderful people has been a joy and a privilege. Thanks for all those summer days in Tvärminne, counting the ants, sharing the work, and having so much fun at it too. Thanks for the conference trips and courses, all the help in the lab and the field, and the massive midsummer feasts!

Special thanks to Heikki, for your friendship and advice, and for the very contagious happiness and calm you spread around you - and for the murkiest pots of coffee ever. To Perttu, also keeping the caffeine-flow constant, and for all the advice and support to go with it. To Kalle, for secret rebellion and a surprisingly dark sense of humor. And to Katja, for being my biggest idol. From those scary first meetings abroad, to even scarier talks at the department, "What would Katja do" seems to have worked very well. Thanks for setting a wonderful example, and for being a good friend. Hannele and Tuomas, my wonderful MSc-students, I have learned at least as much from you as you have from me! Thanks also for your help in the field and in the lab, and for the company for those early morning ant watches. Ulla, my dearest field assistant, thanks for everything, and particularly for those sweet cheer-ups during stressful times – you know exactly which cord to pull! ☺ Lea, a particularly big thank you for the cover illustration that manages to capture so beautifully what this work was all about. Thanks to Sedeer for all the philosophical conversations, and a special thanks for pancakes.

As I even have trouble counting the numerous field assistants, clearly counting all the ants would not have been possible without all of you who have been involved in the *Formica exsecta* project from

its very start. Special thanks to my personal field helpers. Eva E & Riitta L, you kept me on the right track with your cheerful reminding me of the date and the island we were on. Markus H, you counted not only the ants, but their mites as well, with an elephant's patience! Eva and John, thank you also for the help with the genotypings. Many other people have also provided help and advice in the MES-lab over the years: Soile Kupiainen, Heikki Ryyänen, Leena Laaksonen, Suvi Saarnio, Minttu Koskinen, and Heini Ali-Kovero. A great big thank you to Krista, for the layout of this thesis!

Our department, with its ever-changing names, has been a wonderful place to work in. Thanks to Veijo Kaitala and Ilkka Teräs, for all help in jumping through the necessary administrative hoops, and to the LUOVA-coordinators Anna-Liisa Laine, Jonna Katajisto and Anni Tonteri for help and guidance. Kari Vepsäläinen, Hannu Pietiäinen and Kristian Donner, your insightful courses during my Master's studies were an inspiration to continue on the science path. Thanks for all the fellow PhD-students and other department hang-arounds, past and present, for creating a good atmosphere. Daniel and Andres, Chris, Jenni L – thanks for interesting conversations of science and non-science. Jussi A and Wouter, for statistics and beyond, and Johan, Kimi, Outi and Tuomas for all the practical advice and encouragement at the last stretch. Thanks to Jostein, for the musical contributions and to Vilppu, for cat caretake and general enthusiasm.

Thank you, all my friends in and outside biology, for reminding me what is important in life. Lasse, I fondly remember all the rambling discussions we've had, and I'm grateful for you also taking care of the cats. Pirita, your cheerful company at the dancing classes helped me take my mind of work. Ville, you taught me most of what I know about pseudobiology, and have made a valuable contribution to my collection of puujalkavitsit. Kevin, you showed me a whole new world – what it is to truly be inspired by science. Minna, thanks for all the shared laughs, lunches, coffees,

conversations of all things possible, over the years – I can't imagine having gone through this without you. Leon, thank you for your friendship, that has recently become so much more.

Tiina, my science writer friend, ever since the early days of Elukat & Ötökät. Thank you for all the shared things and ideas, I feel that no matter what it is (perhaps, the stranger it is!), you always understand. Thank you for my godson Emil who I see way, way too seldom. Veera, you are a dear friend and I only wish I could see more of you and the family. Thanks for always providing the sensible view on things, and for the honor of inviting me as godmother for Tuomas. Anni, nothing's ever boring around you. Thank you for everything, but particularly for all the giggles and silliness.

Thanks to my family, for your unconditional love and support. You never questioned my choices, and always encouraged me to do things that would make me happy. You endured my endless ramblings about ants, and everything else. Janne, Kaisa ja Lauri – kiitos kaikesta tuesta. Anna, my (little, yes, still!) sister. What a joy it is that you are my best friend too. Äiti – you always trusted and believed in me. Isä – all those walks in the woods, with your kind example, you planted in me the love for nature, the curiosity and appreciation for all the creatures great and small. That is why I wrote this thesis.

This work has been financially supported by the LUOVA graduate school, Academy of Finland, the INSECTS network, the Nordforsk network for Social evolution, Suomen Biologian Seura Vanamo, Societas pro Fauna et Flora Fennica, and travel grants by the Chancellor of University of Helsinki.

7. REFERENCES

- Acevedo-Whitehouse, K., Gulland, F., Greig, D., & Amos, W. 2003. Disease susceptibility in California sea lions. *Nature* 422: 35-35.
- Agosti, D., Majer, J. D., Alonso, L. E., & Schultz, T. R. (Eds.). 2001. *Ants: Standard Methods for Measuring and Monitoring Biodiversity*. Washington DC: Smithsonian Institution Press.
- Aparicio, J. M., Ortego, J., & Cordero, P. J. 2006. What should we weigh to estimate heterozygosity, alleles or loci? *Mol. Ecol.* 15: 4659-4665.
- Ayroles, J. F., Hughes, K. A., Rowe, K. C., Reedy, M. M., Rodriguez-Zas, S. L., Drnevich, J. M., CáCeres, C. E., & Paige, K. N. 2009. A Genomewide Assessment of Inbreeding Depression: Gene Number, Function, and Mode of Action. *Conservation Biology* 23: 920-930.
- Balloux, F., Amos, W., & Coulson, T. 2004. Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology* 13: 3021-3031.
- Bierne, N., Tsitrone, A., & David, P. 2000. An Inbreeding Model of Associative Overdominance During a Population Bottleneck. *Genetics* 155: 1981-1990.
- Bijlsma, R., Bundgaard, J., & Van Putten, W. F. 1999. Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. *J. Evol. Biol.* 12: 1125-1137.
- Bijlsma, R., & Loeschcke, V. 2005. Environmental stress, adaptation and evolution: an overview. *Journal of Evolutionary Biology* 18: 744-749.
- Boomsma, J. J. 2009. Lifetime monogamy and the evolution of eusociality. *Philos. Trans. R. Soc. B-Biol. Sci.* 364: 3191-3207.
- Boomsma, J. J., & Grafen, A. 1991. Colony-Level Sex-Ratio Selection in the Eusocial Hymenoptera. *J. Evol. Biol.* 4: 383-407.
- Boomsma, J. J., & Grafen, A. 1990. Intraspecific Variation in Ant Sex-Ratios and the Trivers-Hare Hypothesis. *Evolution* 44: 1026-1034.
- Bulmer, M. G. 1983. The Significance of Protandry in Social Hymenoptera. *The American Naturalist* 121: 540.
- Chapuisat, M., Sundström, L., & Keller, L. 1997. Sex-ratio regulation: the economics of fratricide in ants. *Proc R Soc B* 264: 1255-1260.
- Charlesworth, B., & Charlesworth, D. 1999. The genetic basis of inbreeding depression. *Genet. Res.* 74: 329-340.
- Charlesworth, D., & Charlesworth, B. 1987. Inbreeding Depression and Its Evolutionary Consequences. *Annu. Rev. Ecol. Syst.* 18: 237-268.
- Charlesworth, D., & Willis, J. H. 2009. The genetics of inbreeding depression. *Nat Rev Genet* 10: 783-796.
- Coltman, D. W., Pilkington, J. G., Smith, J. A., & Pemberton, J. M. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* 53: 1259-1267.
- Cook, J. M. 1993. Sex Determination in the Hymenoptera - a Review of Models and Evidence. *Heredity* 71: 421-435.
- Coulson, T., Albon, S., Slate, J., & Pemberton, J. 1999. Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. *Evolution* 53: 1951-1960.
- Cowan, D. P., & Stahlhut, J. K. 2004. Functionally reproductive diploid and haploid males in an inbreeding hymenopteran with complementary sex determination. *Proceedings of the National Academy of Sciences of the United States of America* 101: 10374-10379.
- Crnokrak, P., & Roff, D. A. 1999. Inbreeding depression in the wild. *Heredity* 83: 260-270.
- Csillery, K., Johnson, T., Beraldi, D., Clutton-Brock, T., Coltman, D., Hansson, B., Spong, G., & Pemberton, J. M. 2006. Performance of Marker-Based Relatedness Estimators in Natural Populations of Outbred Vertebrates. *Genetics* 173: 2091-2101.
- Czechowski, W., Radchenko, A., & Czechowska, W. 2002. *The ants (Hymenoptera, Formicidae) of Poland*. Warszawa.
- Daniels, S. J., & Walters, J. R. 2000. Inbreeding Depression and Its Effects on Natal Dispersal in Red-Cockaded Woodpeckers. *The Condor* 102: 482-491.
- Darvill, B., Ellis, J. S., Lye, G. C., & Goulson, D. 2006. Population structure and inbreeding in a rare and declining bumblebee, *Bombus muscorum* (Hymenoptera : Apidae). *Mol. Ecol.* 15: 601-611.
- DeRose, M. A., & Roff, D. A. 1999. A Comparison of Inbreeding Depression in Life-History and Morphological Traits in Animals. *Evolution* 53: 1288-1292.
- Doligez, B., & Pärt, T. 2008. Estimating fitness consequences of dispersal: a road to 'know-where'? Non-random dispersal and the underestimation of dispersers' fitness. *Journal of Animal Ecology* 77: 1199-1211.
- Driscoll, D. A., Kirkpatrick, J. B., McQuillan, P. B., & Bonham, K. J. 2010. Classic metapopulations are rare among common beetle species from a naturally fragmented landscape. *Journal of Animal Ecology* 79: 294-303.
- Evans, J. D., & Wheeler, D. E. 2001. Gene expression and the evolution of insect polyphenisms. *Bioessays* 23: 62-68.
- Frankham, R. 1995a. Conservation genetics. *Annu. Rev. Genet.* 29: 305-327.
- Frankham, R. 1995b. Inbreeding and Extinction - a Threshold Effect. *Conservation Biology* 9: 792-799.
- Freeman, D. C., Doust, J. L., ElKeblawy, A., Miglia, K. J., & McArthur, E. D. 1997. Sexual specialization and inbreeding avoidance in the evolution of dioecy. *Bot. Rev.* 63: 65-92.
- Goulson, D., Lye, G., & Darvill, B. 2008. Decline and Conservation of Bumble Bees. *Annual Review of Entomology* 53: 191-208.
- Gyllenstrand, N., & Seppä, P. 2003. Conservation genetics of the wood ant, *Formica lugubris*, in a fragmented

- landscape. *Mol. Ecol.* 12: 2931-2940.
- Gyllenstrand, N., Seppä, P., & Pamilo, P. 2005. Restricted gene flow between two social forms in the ant *Formica truncorum*. *J. Evol. Biol.* 18: 978-984.
- Halverson, M. A., Skelly, D. K., & Caccone, A. 2006. Inbreeding linked to amphibian survival in the wild but not in the laboratory. *J. Hered.* 97: 499-507.
- Hamilton, W. D. 1964. The genetical evolution of social behaviour. II. *J. Theor Biol* 7: 17 - 52.
- Hannonen, M., Helanterä, H., & Sundstrom, L. 2004. Habitat age, breeding system and kinship in the ant *Formica fusca*. *Molecular Ecology* 13: 1579-1588.
- Hansson, B., Bensch, S., Hasselquist, D., & Nielsen, B. 2002. Restricted dispersal in a long-distance migrant bird with patchy distribution, the great reed warbler. *Oecologia* 130: 536-542.
- Hansson, B., & Westerberg, L. 2008. Heterozygosity-fitness correlations within inbreeding classes: local or genome-wide effects? *Conserv. Genet.* 9: 73-83.
- Hedrick, P. W., & Kalinowski, S. T. 2000. Inbreeding depression in conservation biology. *Annu. Rev. Ecol. Syst.* 31: 139-162.
- Henter, H. J. 2003. Inbreeding depression and haplodiploidy: Experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. *Evolution* 57: 1793-1803.
- Holzer, B., Keller, L., & Chapuisat, M. 2009. Genetic clusters and sex-biased gene flow in a unicolonial *Formica* ant. *BMC Evolutionary Biology* 9: 69.
- Jimenez, J., Hughes, K., Alaks, G., Graham, L., & Lacy, R. 1994. An experimental study of inbreeding depression in a natural habitat. *Science* 266: 271-273.
- Joron, M., & Brakefield, P. M. 2003. Captivity masks inbreeding effects on male mating success in butterflies. *Nature* 424: 191-194.
- Keller, L., & Genoud, M. 1997. Extraordinary lifespans in ants: a test of evolutionary theories of ageing. *Nature* 389: 958-960.
- Keller, L. F. 1998. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution* 52: 240-250.
- Keller, L. F., & Waller, D. M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.* 17: 230-241.
- Ketola, T., & Kotiaho, J. S. 2009. Inbreeding, energy use and condition. *Journal of Evolutionary Biology* 22: 770-781.
- Kokko, H., & Ots, I. 2006. When not to avoid inbreeding. *Evolution* 60: 467-475.
- Kristensen, T. N. 2006. Inbreeding by Environmental Interactions Affect Gene Expression in *Drosophila melanogaster*. *Genetics* 173: 1329-1336.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science* 241: 1455-1460.
- Liautard, C., & Keller, L. 2001. Restricted effective queen dispersal at a microgeographic scale in polygynous populations of the ant *Formica* exsecta. *Evolution* 55: 2484-2492.
- Liautard, C., & Sundström, L. 2005. Estimation of individual level of inbreeding using relatedness measures in haplodiploids. *Insect. Soc.* 52: 323-326.
- Lynch, M., & Walsh, B. 1998. Genetics and analysis of quantitative traits. Sunderland, Massachusetts, USA.: Sinauer.
- Marr, A. B., Arcese, P., Hochachka, W. M., Reid, J. M., & Keller, L. F. 2006. Interactive effects of environmental stress and inbreeding on reproductive traits in a wild bird population. *J. Anim. Ecol.* 75: 1406-1415.
- Mercader, R. J., Siegert, N. W., Liebhold, A. M., & McCullough, D. G. 2009. Dispersal of the emerald ash borer, *Agrilus planipennis*, in newly-colonized sites. *Agricultural and Forest Entomology* 11: 421-424.
- Mäki-Petäys, H., & Breen, J. 2007. Genetic vulnerability of a remnant ant population. *Conserv. Genet.* 8: 427-435.
- Naito, T., & Suzuki, H. 1991. Sex determination in the sawfly, *Athalia rosae ruficornis* (Hymenoptera) - Occurrence of triploid males. *J. Hered.* 82: 101-104.
- Oster, G. F., & Wilson, E. O. 1978. Caste and Ecology in the Social Insects. Princeton, New Jersey, U.S.A.: Princeton University Press.
- Packer, L., & Owen, R. 2001. Population genetic aspects of pollinator decline. *Conserv. Ecol.* 5: 36.
- Pamilo, P. 1991. Life-Span of Queens in the Ant *Formica*-Exsecta. *Insect. Soc.* 38: 111-119.
- Pamilo, P., & Crozier, R. H. 1981. Genic Variation in Male Haplods Under Deterministic Selection. *Genetics* 98: 199-214.
- Pamilo, P., Sundstrom, L., Fortelius, W., & Rosengren, R. 1994. Diploid Males and Colony-Level Selection in *Formica* Ants. *Ethol. Ecol. Evol.* 6: 221-235.
- Pamilo, P., Zhu, D. Q., Fortelius, W., Rosengren, R., Seppä, P., & Sundstrom, L. 2005. Genetic patchwork of network-building wood ant populations. *Annales Zoologici Fennici* 42: 179-187.
- Pemberton, J. M. 2008. Wild pedigrees: the way forward. *Proc. R. Soc. B-Biol. Sci.* 275: 613-621.
- Perrot-Minnot, M., Migeon, A., & Navajas, M. 2004. Intergenomic interactions affect female reproduction: evidence from introgression and inbreeding depression in a haplodiploid mite. *Heredity* 93: 551-558.
- Petters, R. M., & Mettus, R. V. 1980. Decreased Diploid Male Viability in the Parasitic Wasp, *Bracon*-Hebetor. *J. Hered.* 71: 353-356.
- Queller, D. C., & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution* 43: 258 - 275.
- Ratnieks, F. L. W., Foster, K. R., & Wenseleers, T. 2006. Conflict resolution in insect societies. *Annu. Rev. Entomol.* 51: 581-608.
- Reed, D. H., Briscoe, D. A., & Frankham, R. 2002. Inbreeding and extinction: The effect of environmental stress and lineage. *Conserv. Genet.* 3: 301-307.
- Reed, D. H., Nicholas, A. C., & Stratton, G. E. 2007. Inbreeding levels and prey abundance interact to determine fecundity in natural populations of two species of wolf spider. *Conserv. Genet.* 8: 1061-1071.

- Roff, D. A. 1998. Effects of inbreeding on morphological and life history traits of the sand cricket, *Gryllus firmus*. *Heredity* 81: 28–37.
- Ross, K. G., & Fletcher, D. J. 1986. Diploid male production—a significant colony mortality factor in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology* 19: 283–291.
- Rousset, F. 2004. *Genetic Structure and Selection in Subdivided Populations*. Princeton: Princeton University Press.
- Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W., & Hanski, I. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392: 491–494.
- Seppä P., Gyllenstrand N., Corander, J. & Pamilo, P. 2004. Coexistence of the Social Types: Genetic Population Structure in the Ant *Formica exsecta*. *Evolution* 58: 2462–2471.
- Seppä, P., Fernandez-Escudero, I., Gyllenstrand, N., & Pamilo, P. 2006. Obligatory female philopatry affects genetic population structure in the ant *Proformica longisetata*. *Insect. Soc.* 53: 362–368.
- Slate, J., David, P., Dodds, K. G., Veenvliet, B. A., Glass, B. C., Broad, T. E., & McEwan, J. C. 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* 93: 255–265.
- Slatkin, M. 1995: Hitchhiking and associative overdominance at a microsatellite locus. *Mol Biol Evol* 12: 473–480.
- Spielman, D., Brook, B. W., & Frankham, R. 2004. Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the United States of America* 101: 15261–15264.
- Sundström, L., Chapuisat, M., & Keller, L. 1996. Conditional manipulation of sex ratios by ant workers: A test of kin selection theory. *Science* 274: 993–995.
- Sundström, L., Keller, L., & Chapuisat, M. 2003. Inbreeding and sex-biased gene flow in the ant *Formica exsecta*. *Evolution* 57: 1552–1561.
- Sundström, L., Seppä, P., & Pamilo, P. 2005. Genetic population structure and dispersal patterns in *Formica* ants - a review. *Ann. Zool. Fenn.* 42: 163–177.
- Suni, S. S., & Gordon, D. M. 2010. Fine-scale genetic structure and dispersal distance in the harvester ant *Pogonomyrmex barbatus*. *Heredity* 104: 168–173.
- Takahashi, J., Ayabe, T., Mitsuhata, M., Shimizu, I., & Ono, M. 2008. Diploid male production in a rare and locally distributed bumblebee, *Bombus florilegus* (Hymenoptera, Apidae). *Insect. Soc.* 55: 43–50.
- Tarpy, D. R., & Page, R. E. 2002. Sex determination and the evolution of polyandry in honey bees (*Apis mellifera*). *Behav. Ecol. Sociobiol.* 52: 143–150.
- Trivers, R., & Hare, H. 1976. Haplodiploidy and the evolution of the social insect. *Science* 191: 249–263.
- Trontti, K., Aron, S., & Sundstrom, L. 2005. Inbreeding and kinship in the ant *Plagiolepis pygmaea*. *Mol. Ecol.* 14: 2007–2015.
- Whitehorn, P., Tinsley, M., Brown, M., Darvill, B., & Goulson, D. 2009. Impacts of inbreeding on bumblebee colony fitness under field conditions. *BMC Evolutionary Biology* 9: 152.
- Wiernasz, D. C., & Cole, B. J. 2009. Dioecy and the evolution of sex ratios in ants. *Proceedings of the Royal Society B-Biological Sciences* 276: 2125–2132.
- Wilfert L, Gadau J. and Schmid-Hempel P. 2007: Variation in genomic recombination rates among animal taxa and the case of social insects. *Heredity* 98, 189–197.
- Wilson, E. O. 1963. Social Modifications Related to Rareness in Ant Species. *Evolution* 17: 249–253.
- Wright, S. 1951. The genetical structure of populations. *Ann. Eugenics* 15: 323–354.
- Wright, S. 1977. *Evolution and the Genetics of Populations. Vol. 3 . Experimental Results and Evolutionary Deductions*. Chicago: University of Chicago Press.
- Yamauchi, K., Yoshida, T., Ogawa, T., Itoh, S., Ogawa, Y., Jimbo, S., & Imai, H. T. 2001. Spermatogenesis of diploid males in the formicine ant, *Lasius sakagamii*. *Insect. Soc.* 48: 28–32.
- Zayed, A., & Packer, L. 2005. Complementary sex determination substantially increases extinction proneness of haplodiploid populations. *Proceedings of the National Academy of Sciences of the United States of America* 102: 10742–10746.