

Natal Dispersal, Mating Patterns, and Inbreeding in the Ant *Formica exsecta*

Emma I. K. Vitikainen,^{1,2,*} Cathy Haag-Liautard,³ and Liselotte Sundström¹

1. Centre of Excellence in Biological Interactions, Department of Biosciences, University of Helsinki, Helsinki, Finland; and Tvärminne Zoological Station, University of Helsinki, Helsinki, Finland; 2. Centre for Ecology and Conservation, University of Exeter, Penryn Campus, Penryn, United Kingdom; 3. Institut de Sciences de l'Évolution Montpellier, Unité Mixte de Recherche 5554, Station Marine de Sète, 2 rue des Chantiers, 34200 Sète, France

Submitted January 28, 2015; Accepted July 22, 2015; Electronically published October 22, 2015

Online enhancement: appendix. Dryad data: <http://dx.doi.org/10.5061/dryad.b73f3>.

ABSTRACT: Sex-biased dispersal and multiple mating may prevent or alleviate inbreeding and its outcome, inbreeding depression, but studies demonstrating this in the wild are scarce. Perennial ant colonies offer a unique system to investigate the relationships between natal dispersal behavior and inbreeding. Due to the sedentary life of ant colonies and lifetime sperm storage by queens, measures of dispersal distance and mating strategy are easier to obtain than in most taxa. We used a suite of molecular markers to infer the natal colonies of queens and males in a wild population of the ant *Formica exsecta*. Dispersal was male biased, with median male dispersal distances (~140 m) twice those of queens (~60 m). The results also showed that the population was inbred and that inbreeding avoidance behaviors—sex-biased dispersal, queen dispersal distance, and multiple mating—were all ineffective in reducing homozygosity among colony workers. Queen homozygosity did not affect dispersal behavior, but more homozygous queens had lower colony-founding success and were more incestuously mated themselves, with potentially accumulating effects on colony fitness. We also provide independent evidence that dispersal is sex biased and show that our estimate corresponds well with dispersal estimates derived from population-genetic estimates.

Keywords: natal dispersal, inbreeding avoidance, homozygosity, ants, social insects, sex-biased dispersal.

Introduction

Inbreeding reduces fitness through increasing homozygosity and expression of deleterious recessive alleles (e.g., Charlesworth and Charlesworth 1987), and animals have been selected to mitigate its impacts through a suite of behaviors such as sex-biased dispersal, mate choice, and multiple mating (Pusey and Wolf 1996). Yet, inbreeding poses a threat to the welfare and survival of natural pop-

ulations (e.g., Frankham 1995, 2010; Crnokrak and Roff 1999; Keller and Waller 2002; Spielman et al. 2004; Armbruster and Reed 2005), and human-induced habitat loss and fragmentation create boundaries for dispersal, increasing the potential for inbreeding (Frankham 2010; Banks et al. 2013). Therefore, understanding the effectiveness of inbreeding avoidance behaviors in the wild is of crucial importance.

Dispersal dictates the breeding structure in the wild, as it influences the extent to which populations become isolated and therefore susceptible to loss of genetic diversity owing to inbreeding and genetic drift (e.g., Wright 1951; Lande 1988; Clobert et al. 2001). Importantly, more homozygous individuals may also be less able or prone to disperse (e.g., *Erigone* spiders; Bonte 2009), which may predispose populations to inbreeding depression vortices. Direct observations on dispersal distances have been made on mammals and birds, but field data on actual dispersal distances, especially in invertebrates, are scant. In smaller animals, studies often rely on indirect inference of dispersal from experimental release of individuals (e.g., Petit et al. 2008) or estimates of individual mobility (Doak 2000; Wahlberg et al. 2002). Alternatively, genetic markers can be used to infer effective dispersal distances within and between populations (Chapuisat et al. 1997; Sundström et al. 2003, 2005; Mercader et al. 2009; Driscoll et al. 2010; Suni and Gordon 2010). However, these indirect measures of dispersal have not been validated by direct observations of individual dispersal distances in the same populations. Furthermore, population-genetic estimates can describe only the average dispersal behavior of individuals that successfully breed in the new area. Yet, individual attributes and the potential selective disappearance during or after dispersal should be considered in order to fully understand how far and why individuals disperse.

Sex-biased dispersal has been suggested as a key mechanism for inbreeding avoidance, and based on direct ob-

* Corresponding author; e-mail: e.i.k.vitikainen@exeter.ac.uk.

servations, dispersal tends to be male biased in mammals and female biased in birds (Pusey 1987; Perrin and Mazalov 2000; Lawson Handley and Perrin 2007). In invertebrates, the corresponding measures are based on genetic markers, either nuclear or a combination of nuclear and mitochondrial, and have repeatedly demonstrated sex-biased dispersal, especially in social insects (e.g., Sundström et al. 2003, 2005; Seppä et al. 2006). Mating behavior (e.g., polyandry by females) can also reduce inbreeding by increasing the genetic diversity among offspring (Pamilo 1994; Sarhan and Kokko 2007). Polyandrous females may store or use sperm preferentially from an unrelated male as a form of postcopulatory mate choice (e.g., Tregenza and Wedell 2002; Bretman et al. 2009). Surprisingly few studies have combined observation-based and marker-based approaches, yet teasing apart actual individual dispersal patterns, including sex biases, from their population-genetic effects is necessary for detecting changes in dispersal patterns and calibrating genetic estimates with dispersal success, particularly in animals with long-lived individuals or colonies (Lawson Handley and Perrin 2007).

Social insects are among the ecologically most dominant animals, frequently acting as keystone species in terrestrial ecosystems (Folgarait 1998). Owing to reproductive division of labor, only the reproductive females (queens) produce offspring, whereas the sterile females (workers) represent the majority of the individuals but do not contribute to the future gene pool of the population. Thus, despite their important contribution to animal biomass (e.g., Fittkau and Klinge 1973), the effective population sizes of social insects can be very low (Wilson 1963; Pamilo and Crozier 1981; Packer and Owen 2001; Sundström et al. 2005). This creates additional opportunities for mating between close relatives and hence inbreeding. Queens mate for life (Boomsma 2009), typically during a single mating flight, and this determines the genetic diversity among all future offspring of the colony—new reproductives and workers. As a consequence, studies on social insects, especially ants, have demonstrated considerable population structuring, indicating limited dispersal (e.g., Liautard and Keller 2001; Gyllenstrand and Seppä 2003; Sundström et al. 2003; Gyllenstrand et al. 2005; Trontti et al. 2005; Darvill et al. 2006; Mäki-Petäys and Breen 2006; Seppä et al. 2006). In the social hymenoptera, inbreeding creates further potential costs through haplodiploid sex determination. This is because homozygosity at the sex-determining locus leads to a male phenotype also in diploid individuals (Ross and Fletcher 1985; Cook 1993). Diploid males are typically unviable and/or infertile and are produced instead of workers or reproductive females in inbred colonies (Tapy and Page 2002). In addition to diploid male production, inbreeding depression has also been shown to impair colony productivity and life span in ants (Haag-Liautard et al.

2009; Whitehorn et al. 2009; Vitikainen et al. 2011; but see Kureck et al. 2013).

Here we present the first study to examine dispersal distance, mating behavior, inbreeding, and selection during colony founding in a social insect, using a wild population of the ant *Formica exsecta*. First, we assess whether there is sex bias in how far ant queens and males disperse during their nuptial flight and whether this prevents inbreeding. Second, we ask whether queen homozygosity affects her dispersal, survival, or mating behavior. Finally, we ask what the effects of queen mating behavior are on the colonies: whether consanguineous mating affects colony establishment; and whether multiple mating benefits the colonies by decreasing the homozygosity of the workers, by increasing its variance, or by allowing multiply mated queens to selectively use or store sperm in favor of the less related male.

Material and Methods

Study Population

The study population of the narrow-headed ant *Formica exsecta* is located on five islands close to Tvärminne Zoological Station in Hanko on the southwest coast of Finland and has been surveyed for demography, colony kin structure, colony size, productivity, and sex ratio since 1994 (Sundström et al. 2003; Haag-Liautard 2009; Vitikainen et al. 2011). The yearly standing population comprises on average 100 colonies, the majority of which are headed by a single reproductive queen (monogyny), which is either singly or multiply mated. The total number of colonies surveyed since 1994 is 222, including deceased colonies, and a considerable proportion of the colonies reach an age of 20 years or more (Pamilo 1991). Colony sex ratios are split, with on average $56 \pm 12\%$ (SD) of the colonies specializing in production of either >90% males or >90% queens (E. I. K. Vitikainen and L. Sundström, unpublished data). The study population is genetically structured, and there is extensive variation in inbreeding at both the queen and the colony level (Haag-Liautard et al. 2009); previous population-genetic analyses suggest sex-biased dispersal, with males mediating gene flow over longer distances than queens (Sundström et al. 2003).

Incipient Colonies

In order to investigate dispersal behavior and selection during colony founding, we collected queens after the mating flight and extracted the sperm obtained during mating. The newly mated queens and their mates are hereafter referred to as “incipient colonies.” The mating flights of *F. exsecta* occur in July and August at sunrise from 4:00 a.m. onward, only on very calm mornings, so wind di-

rection probably plays a minor role in determining the flight paths or dispersal distance of individuals. Within each colony, the sexual brood matures and leaves the colony within 1 to 2 weeks. However, the colonies differ extensively in the time they release sexuals, with the earliest having released all their sexuals up to 4 weeks earlier than the last ones (E. I. K. Vitikainen and L. Sundström, unpublished data). As a result, the number of colonies contributing to the mating swarm at any one day with suitable weather is probably quite small. We never found mating pairs and do not know whether mating takes place in the air or on the ground. However, newly mated queens can be found walking on the ground after the nuptial flight. We collected a total of 138 young incipient colony queens over several years—2002 ($N = 40$), 2003 ($N = 51$), 2005 ($N = 33$), and 2007 ($N = 14$)—and stored them in 94% alcohol. We recorded the coordinates of the place of capture with a Garmin GPS 12XL receiver with 5-m accuracy. Of the 138 young queens, 133 still had their wings attached at the point of collection, and 130 were mated (127 with wings). Thus, to make sure that queens still having wings would not have dispersed further, 18 queens collected in 2005 were kept in $28 \times 18 \times 20$ -cm plastic boxes covered with mesh for 2–4 days after collection. None of them attempted to fly, and they were all negatively phototactic in contrast to newly emerged pre-flight queens, which are positively phototactic.

Genetic Data, Homozygosity, and Relatedness

The incipient colony queens, 8–16 workers from 222 established colonies, and 5–10 males from 104 colonies where available were genotyped at 10 highly variable microsatellite loci, as described by Haag-Liautard et al. (2009). We used the homozygosity measure of Aparicio et al. (2006), HL, as proxy for inbreeding in queens and workers. The parental genotypes of established colonies were deduced from the workers and offspring males following an established protocol assuming a minimum number of patriline (Sundström et al. 2003). Queen HL was measured from the deduced maternal genotypes, and worker HL was measured as the average of the genotyped workers. For incipient colonies, we directly genotyped the queens and the sperm they had stored during mating to assess the genotypes of their male mates and construct worker genotypes. The average HL of the deduced worker genotypes was then used as a measure of colony/worker homozygosity of the incipient colonies, following Haag-Liautard et al. (2009). In short, the dissected spermatheca was placed in ethanol, and sperm was extracted using sterile needles under a stereomicroscope with $\times 50$ magnification. None of the genotypes indicated contamination of sperm samples by queen DNA.

When multiple patrilines were present in an established colony, we calculated the average HL among offspring

workers, both across patrilines and separately for each patriline. Patrilines were assigned either minority or majority status according to which male fathered most of the offspring. As we could not determine the multilocus genotypes of males from the genotyped sperm from incipient colony queens, we calculated the average HL across the patrilines for multiply mated queens of incipient colonies (see appendix, available online).

For comparison, we also calculated the relatedness between a queen and her male mate(s), which corresponds to the offspring F_{IS} in haplodiploids (Liautard and Sundström 2005). Calculations were done with Relatedness 5.0.2, and standard errors were constructed by averaging across groups (Queller and Goodnight 1989). These calculations were carried out based on the deduced parental genotypes in established colonies and the scored genotypes of queens and their spermathecal contents in incipient colonies.

To assign queens to maternal lineages based on mtDNA, we assessed the restriction fragment length polymorphism haplotype from variable regions within the mitochondrial *cytochrome b* and *NADH1* genes, as described by Liautard and Keller (2001). The fragment ND1a-Fe was cut with NdeI (Finnzymes); ND1b-Fe with HinfI; and Cytb-Fe with HinfI, RSAI, and Sau3AI. The fragments were mixed 1:6 with $\times 6$ DNA loading dye (Fermentas) and run for 1 h 30 min at 110 V on a 1.5% agarose gel with the size standard GeneRuler (Fermentas). For incipient colonies, we analyzed the queens directly from the DNA extracted as described above. For established colonies, two workers per colony were analyzed to confirm the maternal haplotype of the colony. This assignment was not done for colony fathers or sperm from the spermatheca of incipient colony queens, as mtDNA was not accessible from these.

Assignment Analysis

We assigned queens of incipient and established colonies, as well as their male mates, to their putative natal colonies using the guided clustering option available in BAPS 5.3, available at <http://web.abo.fi/fak/mnf//mate/jc/software/baps.html> (Corander and Marttinen 2006; Corander et al. 2008); see the appendix for details of the analysis.

Dispersal Estimates

We calculated the dispersal distance of the queen as the distance between the place of collection (or the colony she had established) and her natal colony. Male dispersal consists of two components: the distance a male has flown and the distance sperm is carried in the spermatheca of their female mate after copulation. We are unable to assess where the mating takes place, and therefore, we calculated two measures of distance for males: the distance from the

natal colony to the place of collection (current location) and the distance between the natal colonies of the queens and their male mates. The former measure therefore describes how far a male's genes dispersed from his place of birth, either by the dispersal of the male himself or in a queen's spermatheca, and the latter is the distance between place of birth of the queen and the male. In cases where the queen was multiply mated, we randomly chose one of the males for analyses of distance to avoid problems of nonindependence. Data underlying the analyses are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.b73f3> (Vitikainen et al. 2015).

Statistical Analyses

We used restricted maximum likelihood mixed models to investigate the relationships between sex, colony stage (incipient/established), and dispersal distance within mating pairs, including colony/queen identity as a random factor. We then looked at factors explaining queen dispersal distance using a general linear model (GLM), with queen mating frequency, mtDNA haplotype, and colony stage (incipient vs. established) as fixed factors and queen homozygosity, male dispersal distance, and distance between male and female natal colonies as covariates. For the subset of queens for which we had measured head widths ($N = 33$), we used partial correlation to separately look at the effect of homozygosity on dispersal distance while accounting for possible effects of homozygosity on size. Dispersal distances were log transformed, and graphs show the transformed data.

We used a GLM to test factors contributing to worker homozygosity, with colony stage (incipient vs. established) and queen mating frequency (single vs. multiple) as fixed factors; island and mtDNA haplotype lineage as random factors; and queen homozygosity, queen and male dispersal distance, and the distance between colonies of origin as covariates. All first-order interactions were included in the full GLM model, and the model was simplified by dropping nonsignificant terms sequentially. The removed terms were then inserted back into the model to confirm that the (non) significance was not contingent on the order of removal. Where model simplification did not improve the model, we present the F values and the significance of factors from the full model.

We compared the average homozygosity in the majority versus minority patriline in multiply mated established colonies with a paired t -test and Levene's test for unequal variances and tested for differences in queen mating frequency between incipient and established queens with a t -test for independent samples. In all other analyses, queens were classified as either singly or multiply mated due to the low number of triply mated queens (six among established and three

among incipient colony queens). All the analyses were done in IBM SPSS Statistics 21.0.000.

Results

Population Structure, Assignment of Individuals, and Offspring Homozygosity

Of the 222 analyzed colonies alive during some time interval between 1994 and 2008, 192 were confirmed to be monogynous (only one reproducing queen). Queen mating frequency ranged from one to three (1.27 ± 0.037 , mean \pm SE, $N = 192$). The queens of incipient colonies (i.e., young queens collected after their mating flight) had also mated with one to three males (1.35 ± 0.048 , $N = 120$), and there was no difference in the mating frequency between the two colony stages ($t_{310} = -1.313$, $P = .19$). We were able to unambiguously assign 65 established queens and 68 of their mating partners to their natal colony. Of the 138 incipient colony queens caught after the mating flight, 132 were assigned to their natal colony within the study area, and of their 162 male mates, 134 could be assigned to their natal colony. Altogether, 45 established colonies were represented among the incipient colony queens, and 55 colonies had contributed to the males the incipient colony queens had mated with.

A significant number of established queens and their male mates could not be assigned to a natal colony, because either they colonized the study area from elsewhere or their natal colony had perished before the focal colony was found. Admixture between immigrants and local individuals may create spurious correlations between parental and offspring homozygosity (e.g., Reid et al. 2006). Hence, we investigated the influence of these unassigned individuals (i.e., potential immigrants) on our estimates of worker homozygosity with GLM by also including the colonies for which parental dispersal distances were not available. Assignment status (i.e., whether the queen or her male mate[s] could be assigned to a colony in the study population) was not associated with offspring homozygosity (queen assignment status: $F_{1,278} = 0.021$, $P = .886$; male assignment status: $F_{1,278} = 0.001$, $P = .969$). Moreover, neither queen homozygosity, mating frequency, nor expected colony worker homozygosity differed between locally assigned and unassigned queens (t -test, equal variances not assumed, mean \pm SE: queen homozygosity: unassigned queens HL = 0.239 ± 0.01 , local queens HL = 0.229 ± 0.01 , $t_{300,3} = -0.625$, $P = .53$; mating frequency: unassigned queens 1.19 ± 0.04 , assigned queens 1.28 ± 0.05 , $t_{299} = 1.53$, $P = .126$; expected worker homozygosity: unassigned queens HL = 0.266 ± 0.01 , assigned queens HL = 0.265 , $t_{290,4} = -0.097$, $P = .923$). This suggests that the subset of colonies, which we were able to assign to their parental colonies, was

a representative sample of the population as a whole in terms of the average level of worker homozygosity.

Queen and Male Dispersal Distances

The median dispersal distance (natal colony to current location) of established queens was 63 m (lower and upper quartiles 34 and 150 m) and of their male mates was 148 m (quartiles 60 and 447 m). The corresponding dispersal distances were 59 m (quartiles 25 and 125 m) and 136 m (quartiles 58 and 344 m) for incipient colony queens and their male mates, respectively (fig. 1). Ninety percent of the queens were found within 600 m of their natal colony, and 90% of their male mates ended up within 1,500 m of their natal colony. Males dispersed significantly farther than queens, and there were no differences in dispersal

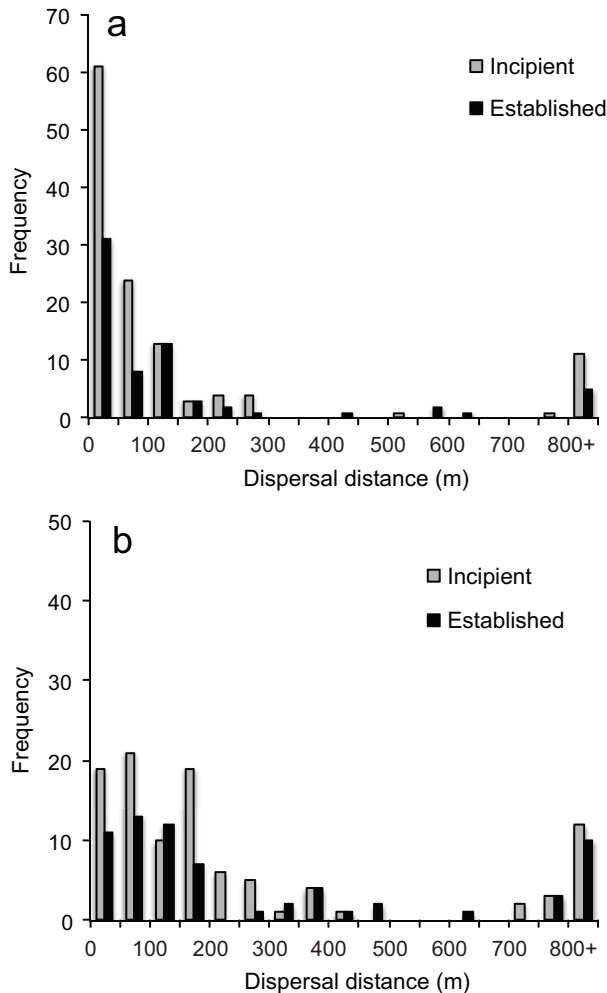


Figure 1: Distribution of dispersal distances of queens (a) and males (b) of incipient (gray bars) and established (black bars) colonies. Tick marks = 50 m.

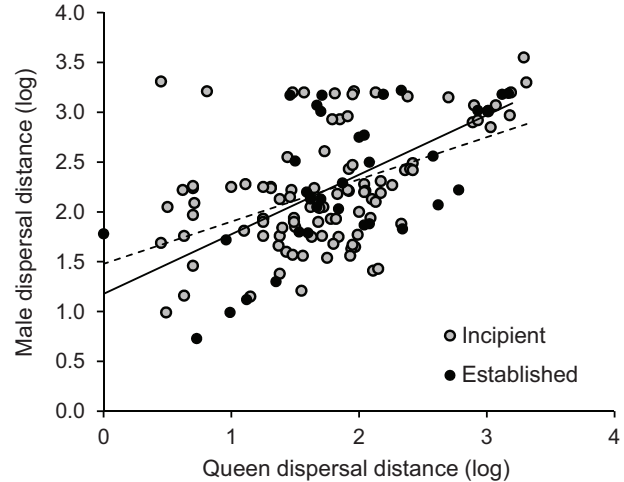


Figure 2: Queen dispersal distance versus dispersal distance of the male she had mated with in incipient (gray dots, dashed line) and established (black dots, solid line) colonies. Lines represent statistically significant relationships.

distance between incipient and established colonies for either sex (sex: $F_{1,357} = 10.891$, $P = .001$; colony stage: $F_{1,357} = 1.563$, $P = .212$; sex \times colony stage: $F_{1,357} = 0.081$, $P = .776$). Within mating pairs, dispersal was conclusively male biased, as 90% of male mates of assigned queens had dispersed a longer distance from their natal colony than the queens they had mated with (fig. 2). The average distance between the natal colonies of mating partners was 108 m (quartiles 57 and 324 m) and did not differ between established and incipient colonies ($F_{1,133} = 1.24$, $P = .26$).

Males were also more likely than queens to move between islands (fig. A1, available online). Only 11% (7 out of 65) of the established colony queens had flown from one island to another, whereas nearly one-third of their male mates originated from another island (28%; 19 out of 68). The corresponding numbers are 8% (11 out of 132) and 20% (27 out of 134) for incipient colony queens and males, respectively. The estimated genetic neighborhood size, as inferred from the average number of colonies (representing the number of reproducing individuals) found within the median dispersal distance, was 17.8 and 42.3 for the median queen and male dispersal distance, respectively.

Neither queen homozygosity ($F_{1,165} = 0.002$, $P = .96$), colony stage (incipient or established: $F_{1,165} = 0.54$, $P = .46$), mating frequency (singly or multiply mated: $F_{1,165} = 0.15$, $P = .70$), nor mtDNA haplotype ($F_{3,165} = 0.48$, $P = .69$) was directly associated with her dispersal distance. However, queen size increased with increasing homozygosity among the subset of incipient colony queens for which we had size measurements ($R = 0.436$, $P = .011$, $N = 33$; fig. 3a). After controlling for the positive effect of homozy-

gosity (HL) on size, dispersal distance decreased with increasing homozygosity; in other words, less homozygous queens had dispersed a longer distance with respect to their size (partial correlation HL vs. distance, controlling for head width: $R = -0.417$, $P = .018$, $df = 30$; fig. 3b). As males themselves cannot be inbred yet may suffer consequences of being raised in a colony that has inbred workers (Vitikainen et al. 2011), we also tested for effects of homo-

zygosity of the natal colony. It had no effect on dispersal distance of either sex ($F_{1,310.7} = 0.06$, $P = .81$).

We found no association between worker homozygosity and the distance between the natal colonies of the parents ($F_{1,130} = 2.16$, $P = .144$; fig. 4a) or the natal dispersal distance of males ($F_{1,130} = 1.35$, $P = .25$; fig. 4b). Surprisingly, and contrary to the expectation that dispersal reduces probability for a consanguineous mating, worker homozy-

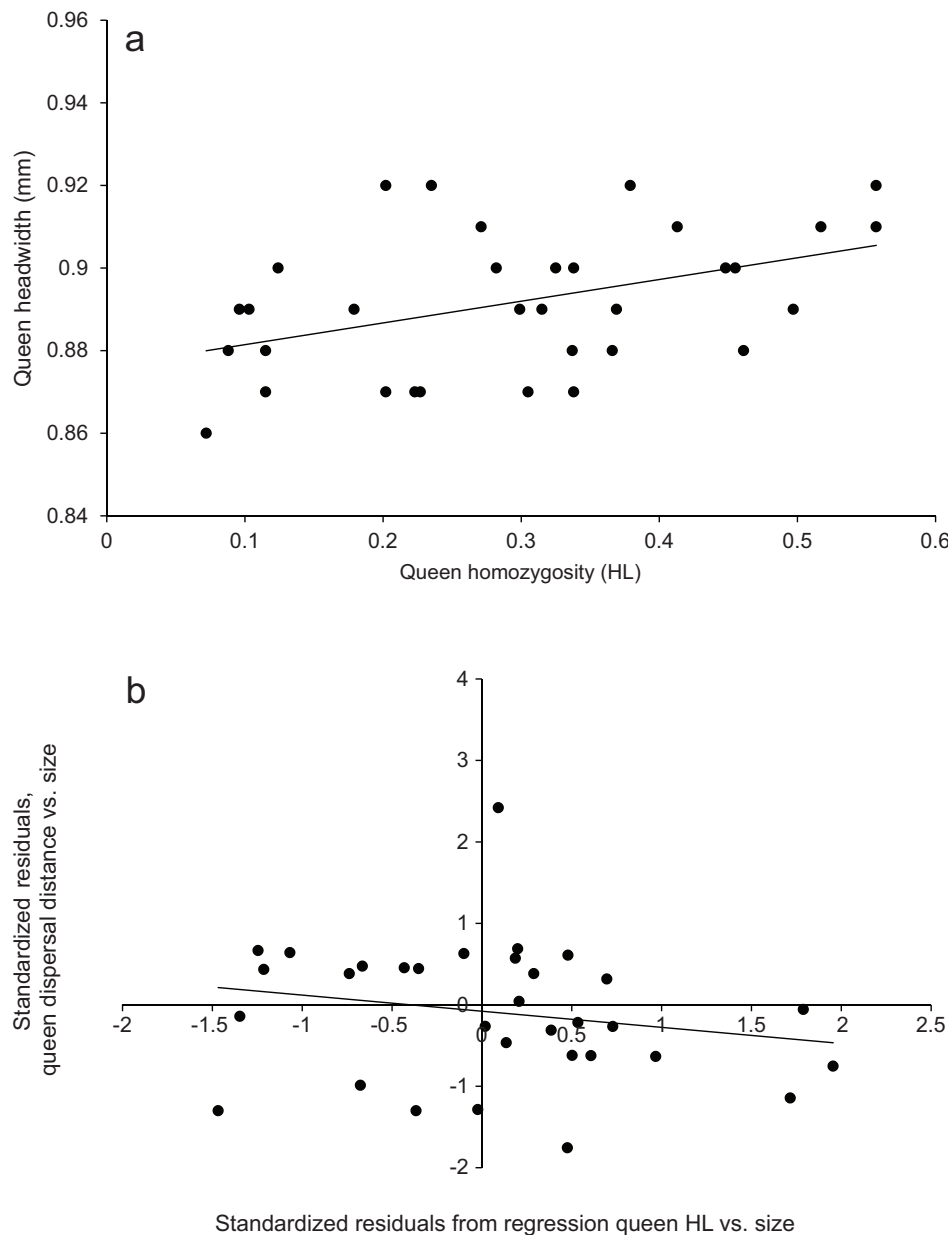


Figure 3: *a*, Size measured as head width (mm) with respect to homozygosity in incipient colony queens collected after the mating flight. *b*, Residuals from partial correlation depicting the association between dispersal distance and homozygosity after correcting for individual size in incipient foundress queens. Lines represent statistically significant relationships between the variables.

gosity increased with increasing queen dispersal distance ($F_{1,178} = 5.24$, $P = .023$; fig. 4c). The effect, however, was negligible and only found among incipient foundress queens (colony stage \times dispersal distance $F_{1,178} = 5.92$, $P = .016$).

Homozygosity, Colony-Founding Success, and Mating Patterns

Incipient colony queens were more homozygous than established ones (incipient: HL = 0.257, SE = 0.013; established queens: HL = 0.219, SE = 0.001; $t_{328} = 2.445$, $P =$

.015; fig. 5). By contrast, we found no significant difference in homozygosity between workers of incipient and established colonies (incipient: HL = 0.277, SE = 0.013; established: HL = 0.258, SE = 0.008; $t_{309} = 1.24$, $P = .231$; fig. 5). This suggests that queen homozygosity but not necessarily worker homozygosity confers a fitness disadvantage at the stage of colony foundation. We also found that worker homozygosity increased with increasing queen homozygosity (queen HL: $F_{1,178} = 6.63$, $P = .011$; fig. 4d; table A3; tables A1–A4 available online). This implies that queen homozygosity influences mating patterns, and indeed, the more homozygous the queen was, the more closely

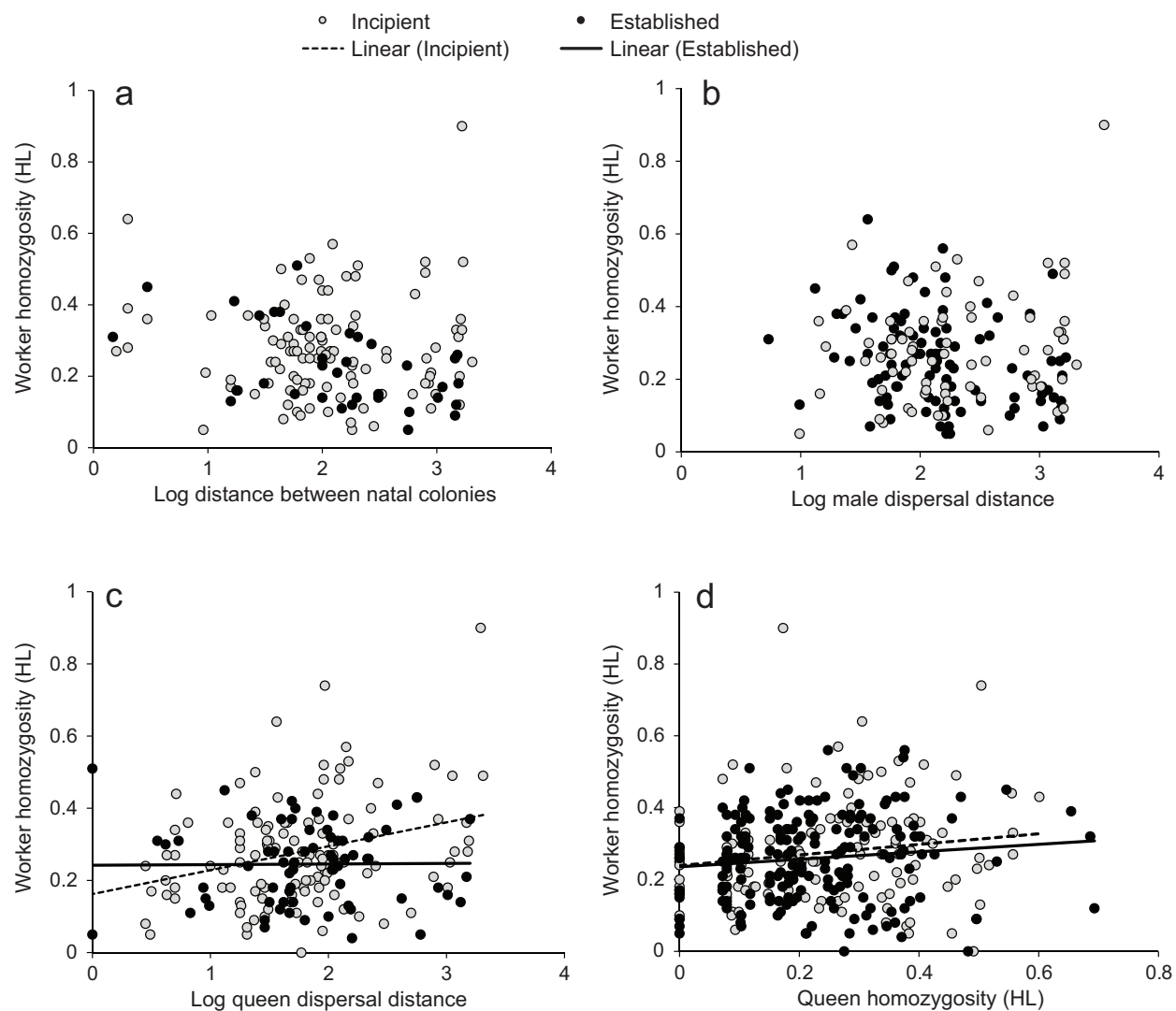


Figure 4: Relationship between worker homozygosity (HL) and distance between colonies of origin (natal distance, log; a), male dispersal distance (log; b), queen dispersal distance (log; c), and queen homozygosity (log; d). Lines depict statistically significant relationships or interactions (colony stage and dispersal distance in c). Gray dots and dashed lines = incipient colonies, black dots and solid lines = established colonies.

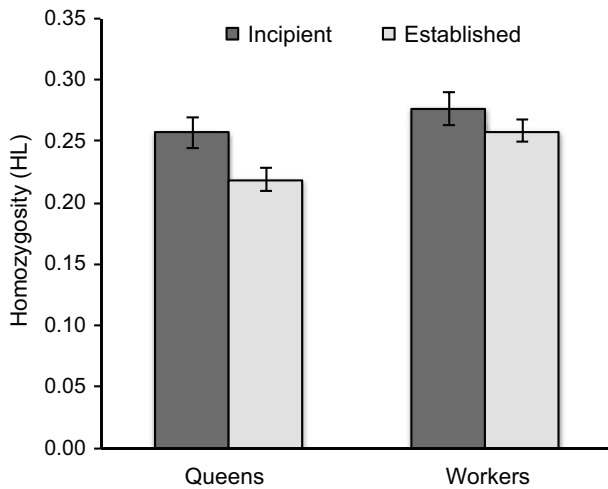


Figure 5: Homozygosity (Aparicio et al. 2006) of queens and their worker offspring, comparing the colony stages. Black bars = incipient colony queens, gray bars = established queens.

related she was to her mating partner(s) (queen HL: $F_{1,179} = 5.01$, $P = .026$; table A4). There was no association between queen mating frequency and queen homozygosity ($F_{2,308} = 0.23$, $P = .797$).

Three of the 65 established queens and four of the 120 incipient colony queens had mated with a brother, which is still within the boundaries expected under panmixis, particularly given the small population size and sex-ratio specialization of the colonies. However, the average relatedness between mating partners was significantly higher than zero in both established and incipient colonies ($r = 0.077 \pm 0.01$ and $r = 0.086 \pm 0.01$, mean \pm SE, respectively; $t_{308} = 0.53$, $P = .59$; overall average $r = 0.08 \pm 0.008$, $N = 310$, mean \pm SE; $t_{309} = 10.29$, $P < .001$, 95% confidence interval 0.07–0.10), which indicates inbreeding in the population. Surprisingly, multiple mating by queens had no effect on the average homozygosity of offspring ($t = -0.882$, $P = .44$, $df = 309$) or the variation in homozygosity (Levene's test for equality of variances: $F_{309} = 1.99$, $P = .153$). The average relatedness between the male mates of queens, however, was very high (average r between males = 0.31 ± 0.03), so the genetic consequences of polyandry in terms of offspring heterogeneity remain negligible. We also found no evidence that multiply mated queens bias their sperm storage or use it to enhance the genetic diversity of their offspring, as there was no difference in the average homozygosity between the minority and the majority patriline (0.24 \pm 0.019, $N = 46$ and 0.27 \pm 0.022, $N = 46$, respectively; $t_{90} = -1.19$, $P = .24$). In agreement with the above results, the relatedness between the queen and her male mate(s) was also highly correlated with the

observed level of homozygosity in offspring (workers; $R = 0.887$, $P = .001$, $N = 309$).

Discussion

Our results show that the majority of individuals in our study population of the ant *Formica exsecta* (90% of queens and 75% of colony fathers) had dispersed 600 m or less from their natal colony, despite the presence of nuptial flights, which are usually considered to lead to long-range dispersal and panmixis in ants (Bourke and Franks 1995; Crozier and Pamilo 1996). Moreover, although males mediate gene flow over distances twice as long as queens, inbreeding is rife in the population; queens are significantly related to their male mates, and males cosiring a colony are highly related. Queens of higher homozygosity also dispersed shorter distances once the impact of queen size on dispersal distance had been accounted for. By contrast, the level of worker homozygosity in the parental colonies had no effect on dispersal distance of males, indicating a lack of "maternal" effects of the rearing colony (cf. Vitikainen et al. 2011). Observed queen dispersal distance does not seem sufficient to avoid inbreeding, as homozygosity of worker offspring was associated with neither dispersal distance of the queen nor the distance between the parental colonies. However, the more homozygous the queens were, the more homozygous offspring they had. This suggests that inbreeding affects mating behavior either directly or via accumulated indirect effects on colonies. Finally, we also show that increased queen homozygosity imposes costs in terms of reduced colony-founding success and that multiple mating does not help reduce the level of homozygosity within colonies. We will examine these conclusions in detail below.

Dispersal Patterns in Males and Queens

Our results based on observed distances to natal colonies suggest that dispersal is limited in our study population of *F. exsecta*. This is unexpected, given that dispersal on the wing during nuptial flights is generally considered to result in long-range dispersal and panmixis. These results are fully congruent with those found in an earlier genetic study on the same population, which suggested population subdivision also within islands (Sundström et al. 2003). The estimated neighborhood size (Rousset 1997), calculated on the basis of nest distances and median dispersal distances of males and queens, was 17 versus 42 (females vs. males) individuals and also fully congruent with that estimated based on genetic data (22 vs. 50; Sundström et al. 2003). Given the population size of, on average, ~100 single-queen colonies alive each year and a maximum of ~40 colonies per island and

the fact that most colonies produce mainly single-sex broods, there is considerable scope for population subdivision and inbreeding. Thus, dispersal is limited, and the colonization of new islands is based on very few longer-range dispersers. Long-distance immigration and emigration necessarily occurs, but the effect on the genetic structure of the local population appears negligible.

Given the geography and the area covered by the study (4 km²), the maximum distance that we could have theoretically detected was 2.1 km (fig. A1). Some long-distance dispersers may have gone undetected, but the proportion is likely to be small, as the median dispersal distance of queens (60 m) is well within the area covered by each individual island (average 9 ha). Ninety percent of the queens remained within a radius of 600 m from their natal colony, and only 9% of queens moved to another island. This suggests that the dispersal barrier posed by water may have especially restricted female dispersal and thus the colonization of new islands. Furthermore, only two new islands within the study area have been colonized since 1993. As most of the incipient colony-founding queens and males were assigned to a natal colony in the area, this suggests that the unassigned established queens and males are likely to be offspring from colonies deceased before the start of the study. Taken together, this suggests that despite dispersal on the wing, *F. exsecta* is restricted in its ability to colonize new suitable habitat patches and establish new populations.

In contrast to the queens, a larger proportion of males (25% vs. 10%) covered distances over 600 m, and accordingly, a larger proportion of males (24% vs. 9%) had crossed water to another island. Thus, dispersal is sex biased, with males dispersing approximately twice as far as queens, and although the ability to colonize new habitat patches may be limited, gene flow between populations and subpopulations should be less restricted in males than in queens. Sex-biased dispersal has indeed been assumed to be widely spread among the social Hymenoptera (Johnstone et al. 2012). In species where individuals cannot be directly tracked, estimates of sex-biased dispersal have mostly relied on comparing maternally inherited mtDNA and biparentally inherited nuclear microsatellites. However, inherent differences in heterozygosity between the two marker types could also explain observed sex bias (Foitzik et al. 2009), leaving male-biased dispersal in Hymenoptera open to debate (Jost 2008). Our results, based on observed dispersal distance, provide independent evidence for sex-biased dispersal and are also fully congruent with an earlier genetic analysis of the same population (Sundström et al. 2003). Arguably, because queens store sperm and may also disperse after mating, gene flow mediated through males also entails passive transport by their female mate. Nonetheless, the observation that many more males than queens were observed to move between islands corroborates

our interpretation that males, not queens, are the long-distance flyers in this species.

Dispersal and Inbreeding Avoidance

Increased dispersal distances per se are expected to counteract inbreeding, as individuals are by chance less likely to encounter relatives farther away from their natal site (Nelson-Flower et al. 2012). We found no support for this in our study population, as neither an increase in distance between the natal colonies of parents nor an increase in the natal dispersal distance of males or females resulted in less homozygous offspring. Surprisingly, offspring homozygosity actually increased with increasing dispersal distance of the queens, although the effect was small and only observed among the incipient colonies. The result could reflect time limitations in colony founding for queens once they leave their natal colony; queens that find their mating partner close by could have more time or resources left for dispersal. Lowered survival of the more inbred queens at the colony-founding stage together with the observed correlation between queen and offspring homozygosity would explain why the pattern was not found among the established colonies.

Under limited dispersal ranges, sex-biased dispersal has been advocated as a strategy to avoid inbreeding in small populations, yet two lines of evidence suggest that inbreeding is rife in our study population. First, neither an increased distance between the natal colonies of parents nor male or female natal dispersal distance resulted in less homozygous offspring. Second, although actual sib mating was rare, queens were significantly related to their male mates. This suggests that the increased gene flow mediated by the longer dispersal distances of males is not sufficient to mitigate inbreeding in the population. Combined with limited dispersal ranges and localized (in time and space) mating swarms, sex-ratio specialization (the production of only male or only female brood) and male protandry (males mature before females), both of which characterize *F. exsecta* (Sundström et al. 1996; Haag-Liautard et al. 2009; Vitikainen et al. 2011), may reduce sib-mating opportunities while allowing mating between individuals from nearby related colonies. Earlier evidence for sex-biased dispersal as an inbreeding avoidance mechanism in the wild is indeed mixed, being apparently effective in some species (e.g., black grouse [Lebigre et al. 2010] and the great tit [Szulkin and Sheldon 2008]) but less so in others (e.g., Seychelles warbler [Eikenaar et al. 2008]).

Homozygosity, Fitness Correlates, and Mating System

Individual homozygosity was associated with a suite of traits related to dispersal, colony-founding success, and

mating behavior in the population. First, homozygosity in conjunction with queen size affected queen dispersal distances such that more homozygous queens, when small, dispersed shorter distances. Where effects have been reported, an increase in homozygosity tends to be associated with a decrease in body size (e.g., Keller and Waller 2002); however, an earlier study on *F. exsecta* involving newly emerged females prior to their nuptial flight found no effect of homozygosity on size, despite a considerably larger sample size (Vitikainen et al. 2011). We therefore suggest that these results may reflect selective disappearance (sensu Nussey et al. 2008), with survival during or after the mating flight of highly homozygous queens being contingent on their size. Thus, if highly homozygous queens survive only if they are large enough (e.g., if size compensates for decreased metabolic efficiency; see Vitikainen and Sundström 2011), a positive relationship between queen size and homozygosity can be seen among the queens that were collected after the flight.

We also found that queens of incipient colonies were more homozygous than those in established colonies, which suggests that homozygosity may compromise colony founding. This supports the results reported in Haag-Liautard et al. (2009), where queen homozygosity was negatively correlated with colony life span. Lower inbreeding coefficients of mature queens compared to those of colony workers have also been reported in other *Formica* species (e.g., Hannonen et al. 2004; Sundström et al. 2005), yet there is no evidence of homozygosity directly affecting the caste fate of females in ants (e.g., Vitikainen et al. 2011). This suggests that selective disappearance of more homozygous queens is likely to account for these differences. Dispersal and mating represent the most energy-demanding phase in the life of ant queens, and as only a fraction survive, any differences in individual quality are likely to manifest at this stage. Indeed, homozygosity is correlated with increased immune response in *F. exsecta* queens (Vitikainen and Sundström 2011), which could reflect compromised energy allocation, ultimately leading to both reduced survival during dispersal and colony founding and a shortened life span of the established colonies with an inbred queen (Haag-Liautard et al. 2009).

Worker homozygosity, in turn, did not significantly differ between incipient and established colonies. Therefore, unlike homozygosity of the queen, the inbreeding level of the resultant colony does not significantly affect colony-founding success. This suggests that, contrary to the bumblebee *Bombus terrestris* (Whitehorn et al. 2009) and the fire ant *Solenopsis invicta* (Ross and Fletcher 1986), diploid male production is unlikely to entail substantial fitness costs during the colony-founding phase in *F. exsecta*. Indeed, *F. exsecta* queens found new colonies by temporary parasitism in colonies of *Serviformica* species (Cze-

chowski et al. 2002), which may have a buffering effect in cases where some of the queen's own offspring develop into diploid males instead of workers.

Queens of higher homozygosity were also more incestuously mated, and consequently, homozygosity of the mother and offspring were positively correlated in both incipient and established colonies. Homozygosity thus appears to be associated with the mating behavior of the queens, either directly or in combination with reduced dispersal as described above; the precise mechanism behind the correlation remains unknown. Mixing between inbred local populations and more heterozygous immigrant individuals can explain this pattern in some species (Reid et al. 2006). However, we found no differences in homozygosity between potential immigrant queens (unassigned queens) and queens of local origin. As both queen and worker homozygosity affect fitness in this population (Haag-Liautard et al. 2009), a tendency for these to be correlated, albeit weakly, means that the effects of inbreeding may be cumulative.

Multiple mating should increase within-colony genetic diversity and help mitigate inbreeding (e.g., Pamilo et al. 1994), yet we found no effects of multiple sires on colony diversity. The apparent reason is that the males cosiring colonies were closely related to each other and were often brothers. This suggests that queens carry out all copulations within a limited time span and that very few colonies contribute to each mating swarm. This conclusion is also corroborated with our observation that only a few colonies at a time tend to launch sexuals at each mating flight, with mating flights spanning up to 4 weeks in time (E. I. K. Vitikainen and L. Sundström, unpublished data). Similarly, we found no evidence for significant sperm bias in favor of unrelated males over related males among the queens that were multiply mated. Hence, queens do not appear to store sperm or adjust sperm use according to relatedness. Indeed, postcopulatory female choice is unlikely to be important in the study population, where localized matings and high relatedness between the mates may undermine any benefits from biased sperm use.

In conclusion, most individuals of our study species *F. exsecta* disperse surprisingly short distances and mate locally, resulting in inbreeding at both the individual and the colony level. The observed sex bias in dispersal distance is not pronounced enough to counteract inbreeding at the scale of the study area. However, inbreeding avoidance may be only a secondary concern, as the failure to find a mate at all may be a more pressing concern (Contarini et al. 2009). Hence, the choice that a queen faces may be between mating and not mating rather than choosing between partners of different quality or relatedness.

To our knowledge, only one study so far has estimated dispersal distance in a natural population of ants: queens

of *Pogonomyrmex barbatus* also show very short dispersal distances despite dispersing on the wing (Suni and Gordon 2010). Indeed, reduced natal dispersal by the queens may be linked with evolution of eusociality and is hence a common feature in social Hymenoptera (Johnstone et al. 2012), even in species with extensive mating flights, and may aggravate the genetic loss caused by habitat fragmentation and low population sizes. These effects may be particularly important in species such as social insects, where mating and dispersal occur during a very limited period of an individual life span and where the size of the breeding population is small.

Acknowledgments

We thank T. Aivelo, H. Luhtasela-El Showk, and U. Vattulainen for help in the field and M. Cant and two anonymous reviewers for helpful comments on the manuscript. This study was supported by the Academy of Finland (grants 54952, 206505, 121216, 251337, and 252411), the LUOVA Doctoral Programme in Wildlife Biology, and the Finnish Society for Sciences and Letters.

Literature Cited

- Aparicio, J. M., J. Ortego, and P. J. Cordero. 2006. What should we weigh to estimate heterozygosity, alleles or loci? *Molecular Ecology* 15:4659–4665.
- Armbruster, P., and D. H. Reed. 2005. Inbreeding depression in benign and stressful environments. *Heredity* 95:235–242.
- Banks, S. C., G. J. Cary, A. L. Smith, I. D. Davies, D. A. Driscoll, A. M. Gill, D. B. Lindenmayer, and R. Peakall. 2013. How does ecological disturbance influence genetic diversity? *Trends in Ecology and Evolution* 28:670–679.
- Bonte, D. 2009. Inbreeding depresses short and long distance dispersal in three congeneric spiders. *Journal of Evolutionary Biology* 22:1429–1434.
- Boomsma, J. J. 2009. Lifetime monogamy and the evolution of eusociality. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364:3191–3207.
- Bourke, A. F. G., and N. R. Franks. 1995. *Social evolution in ants*. Princeton University Press, Princeton, NJ.
- Bretman, A., D. Newcombe, and T. Tregenza. 2009. Promiscuous females avoid inbreeding by controlling sperm storage. *Molecular Ecology* 18:3340–3345.
- Chapuisat, M., J. Goudet, and L. Keller. 1997. Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. *Evolution* 51:475–482.
- Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18:237–268.
- Clobert, J., E. Danchin, A. A. Dhondt, and J. D. Nichols, eds. 2001. *Dispersal*. Oxford University Press, New York.
- Contarini, M., K. S. Onufrieva, K. W. Thorpe, K. F. Raffa, and P. C. Tobin. 2009. Mate-finding failure as an important cause of Allee effects along the leading edge of an invading insect population. *Entomologia Experimentalis et Applicata* 133:307–314.
- Cook, J. M. 1993. Sex determination in the Hymenoptera: a review of models and evidence. *Heredity* 71:421–435.
- Corander, J., and P. Marttinen. 2006. Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology* 15:2833–2843.
- Corander, J., P. Marttinen, J. Sirén, and J. Tang. 2008. Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics* 9:539.
- Crnokrak, P., and D. A. Roff. 1999. Inbreeding depression in the wild. *Heredity* 83:260–270.
- Crozier, R. H., and P. Pamilo. 1996. *Evolution of social insect colonies: sex allocation and kin selection*. Oxford University Press, Oxford.
- Czechowski, W., A. Radchenko, and W. Czechowska. 2002. The ants (Hymenoptera, Formicidae) of Poland. Museum and Institute of Zoology, Warsaw.
- Darvill, B., J. S. Ellis, G. C. Lye, and D. Goulson. 2006. Population structure and inbreeding in a rare and declining bumblebee, *Bombus muscorum* (Hymenoptera: Apidae). *Molecular Ecology* 15:601–611.
- Doak, P. 2000. Population consequences of restricted dispersal for an insect herbivore in a subdivided habitat. *Ecology* 81:1828–1841.
- Driscoll, D. A., J. B. Kirkpatrick, and P. B. McQuillan. 2010. Classic metapopulations are rare among common beetle species from a naturally fragmented landscape. *Journal of Animal Ecology* 79:294–303.
- Eikenaar, C., J. Komdeur, and D. S. Richardson. 2008. Natal dispersal patterns are not associated with inbreeding avoidance in the Seychelles warbler. *Journal of Evolutionary Biology* 21:1106–1116.
- Fittkau, E. J. and H. Klinge. 1973. On biomass and trophic structure of the central Amazonian rain forest ecosystem. *Biotropica* 5:2–14.
- Foitzik, S., S. Bauer, S. Laurent, and P. S. Pennings. 2009. Genetic diversity, population structure and sex-biased dispersal in three co-evolving species. *Journal of Evolutionary Biology* 22:2470–2480.
- Folgarait, P. J. 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. *Biodiversity and Conservation* 7:1221–1244.
- Frankham, R. 1995. Conservation genetics. *Annual Review of Genetics* 29:305–327.
- . 2010. Inbreeding in the wild really does matter. *Heredity* 104:124–124.
- Gyllenstrand, N., and P. Seppä. 2003. Conservation genetics of the wood ant, *Formica lugubris*, in a fragmented landscape. *Molecular Ecology* 12:2931–2940.
- Gyllenstrand, N., P. Seppä, and P. Pamilo. 2005. Restricted gene flow between two social forms in the ant *Formica truncorum*. *Journal of Evolutionary Biology* 18:978–984.
- Haag-Liautard, C., E. Vitikainen, L. Keller, and L. Sundström. 2009. Fitness and the level of homozygosity in a social insect. *Journal of Evolutionary Biology* 22:134–142.
- Hannonen, M., H. Helanterä, and L. Sundström. 2004. Habitat age, breeding system and kinship in the ant *Formica fusca*. *Molecular Ecology* 13:1579–1588.
- Johnstone, R. A., M. A. Cant, and J. Field. 2012. Sex-biased dispersal, haplodiploidy and the evolution of helping in social insects. *Proceedings of the Royal Society B: Biological Sciences* 279:787–793.
- Jost, L. 2008. GST and its relatives do not measure differentiation. *Molecular Ecology* 17:4015–4026.

- Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution* 17:230–241.
- Kureck, I. M., B. Nicolai, and S. Foitzik. 2013. Similar performance of diploid and haploid males in an ant species without inbreeding avoidance. *Ethology* 119:360–367.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science* 241:1455–1460.
- Lawson Handley J., and N. Perrin. 2007. Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology* 16:1559–1578.
- Lebigre, C., R. V. Alatalo, and H. Siitari. 2010. Female-biased dispersal alone can reduce the occurrence of inbreeding in black grouse (*Tetrao tetrix*). *Molecular Ecology* 19:1929–1939.
- Liautard, C., and L. Keller. 2001. Restricted effective queen dispersal at a microgeographic scale in polygynous populations of the ant *Formica exsecta*. *Evolution* 55:2484–2492.
- Liautard, C., and L. Sundström. 2005. Estimation of individual level of inbreeding using relatedness measures in haplodiploids. *Insectes Sociaux* 52:323–326.
- Mäki-Petäys, H., and J. Breen. 2006. Genetic vulnerability of a remnant ant population. *Conservation Genetics* 8:427–435.
- Mercader, R. J., N. W. Siegert, and A. M. Liebhold. 2009. Dispersal of the emerald ash borer, *Agrilus planipennis*, in newly-colonized sites. *Agricultural and Forest Entomology* 11:421–424.
- Nelson-Flower, M. J., P. A. R. Hockey, C. O’Ryan, and A. R. Ridley. 2012. Inbreeding avoidance mechanisms: dispersal dynamics in cooperatively breeding southern pied babblers. *Journal of Animal Ecology* 81:876–883.
- Nussey, D. H., T. Coulson, M. Festa-Bianchet, and J. M. Gaillard. 2008. Measuring senescence in wild animal populations: towards a longitudinal approach. *Functional Ecology* 22:393–406.
- Packer, L., and R. Owen. 2001. Population genetic aspects of pollinator decline. *Conservation Ecology* 5:4.
- Pamilo, P. 1991. Life span of queens in the ant *Formica exsecta*. *Insectes Sociaux* 38:111–119.
- Pamilo, P., and R. H. Crozier. 1981. Genic variation in male haploids under deterministic selection. *Genetics* 98:199–214.
- Pamilo, P., L. Sundström, W. Fortelius, and R. Rosengren. 1994. Diploid males and colony-level selection in *Formica* ants. *Ethology Ecology and Evolution* 6:221–235.
- Perrin, N., and V. Mazalov. 2000. Local competition, inbreeding, and the evolution of sex-biased dispersal. *American Naturalist* 155:116–127.
- Petit, J. N., M. S. Hoddle, J. Grandgirard, G. K. Roderick, and N. Davies. 2008. Short-distance dispersal behavior and establishment of the parasitoid *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae) in Tahiti: implications for its use as a biological control agent against *Hemilodisca vitripennis* (Hemiptera: Cicadellidae). *Biological Control* 45:344–352.
- Pusey, A. E. 1987. Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology and Evolution* 2:295–299.
- Pusey, A., and M. Wolf. 1996. Inbreeding avoidance in animals. *Trends in Ecology and Evolution* 11:201–206.
- Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution* 43:258–275.
- Reid, J. M., P. Arcese, and L. F. Keller. 2006. Intrinsic parent-offspring correlation in inbreeding level in a song sparrow (*Melospiza melodia*) population open to immigration. *American Naturalist* 168:1–13.
- Ross, K. G., and D. Fletcher. 1985. Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), and its evolutionary significance. *Evolution* 39:888–903.
- . 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. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* 145:1219–1228.
- Sarhan, A., and H. Kokko. 2007. Multiple mating in the Glanville fritillary butterfly: a case of within-generation bet hedging? *Evolution* 61:606–616.
- Seppä, P., I. Fernández-Escudero, N. Gyllenstrand, and P. Pamilo. 2006. Obligatory female philopatry affects genetic population structure in the ant *Proformica longiseta*. *Insectes Sociaux* 53:362–368.
- Spielman, D., B. W. Brook, and R. Frankham. 2004. Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the USA* 101:15261–15264.
- Sundström, L., M. Chapuisat, and L. Keller. 1996. Conditional manipulation of sex ratios by ant workers: a test of kin selection theory. *Science* 274:993–995.
- Sundström, L., L. Keller, and M. Chapuisat. 2003. Inbreeding and sex-biased gene flow in the ant *Formica exsecta*. *Evolution* 57:1552–1561.
- Sundström, L., P. Seppä, and P. Pamilo. 2005. Genetic population structure and dispersal patterns in *Formica* ants—a review. *Annales Zoologici Fennici* 42:163–177.
- Suni, S. S., and D. M. Gordon. 2010. Fine-scale genetic structure and dispersal distance in the harvester ant *Pogonomyrmex barbatus*. *Heredity* 104:168–173.
- Szulkin, M., and B. C. Sheldon. 2008. Dispersal as a means of inbreeding avoidance in a wild bird population. *Proceedings of the Royal Society B: Biological Sciences* 275:703–711.
- Tarpy, D. R., and R. E. Page. 2002. Sex determination and the evolution of polyandry in honey bees (*Apis mellifera*). *Behavioral Ecology and Sociobiology* 52:143–150.
- Tregenza, T., and N. Wedell. 2002. Polyandrous females avoid costs of inbreeding. *Nature* 415:71–73.
- Trontti, K., S. Aron, and L. Sundström. 2005. Inbreeding and kinship in the ant *Plagiolepis pygmaea*. *Molecular Ecology* 14:2007–2015.
- Vitikainen, E., C. Haag-Liautard, and L. Sundström. 2011. Inbreeding and reproductive investment in the ant *Formica exsecta*. *Evolution* 65:2026–2037.
- Vitikainen, E. I. K., C. Haag-Liautard, and L. Sundström. 2015. Data from: Natal dispersal, mating patterns, and inbreeding in the ant *Formica exsecta*. *American Naturalist*, Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.b73f3>.
- Vitikainen, E., and L. Sundström. 2011. Inbreeding and caste-specific variation in immune defence in the ant *Formica exsecta*. *Behavioral Ecology and Sociobiology* 65:899–907.
- Wahlberg, N., T. Klemetti, V. Selonen, and I. Hanski. 2002. Metapopulation structure and movements in five species of checkerspot butterflies. *Oecologia* 130:33–43.
- Whitehorn, P. R., M. C. Tinsley, M. J. F. Brown, B. Darvill, and D. Goulson. 2009. Impacts of inbreeding on bumblebee colony fitness under field conditions. *BMC Evolutionary Biology* 9:152.
- Wilson, E. O. 1963. Social modifications related to rareness in ant species. *Evolution* 17:249–253.
- Wright, S. 1951. The genetic structure of populations. *Annals of Eugenics* 15:323–354.