

## Colony kin structure and queen recruitment in the ant *Formica fusca* (Hymenoptera: Formicidae)

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### Abstract

The acceptance of extra queens in social insects changes colony kin structure and may reduce the inclusive fitness of workers. Nonetheless, colonies do not always reject intruding queens, although more so in colonies with high rather than low relatedness among colony members (e.g., mono- and polygyne colonies, respectively). This begs the question to what extent initially accepted queens come to reproduce in the colony and whether the outcome depends on colony kin structure regardless of the number of queens actually present in the colony. Here we test whether related daughter queens are more amenable for adoption than unrelated non-nestmate queens, whether acceptance is contingent on within-colony relatedness, and whether resident queens are favoured over new ones. We compared adoption and over-wintering survival of young queens of the facultatively polygyne black ant *Formica fusca* and found that nearly 100% of the introduced queens were killed or died within four months, whereas nearly all resident queens were retained regardless of colony kin structure. Contrary to our expectations based on inclusive fitness arguments, related daughter queens were not more eligible for adoption than unrelated prospective adoptees. Although colony kin structure had no effect on the tendency to adopt additional queens, dead queens were more often dismembered and thus presumably killed in high-relatedness than low-relatedness colonies.

**Key words:** Facultative, monogyne, polygyne, relatedness, adoption, rejection, *Formica*, social.

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### Introduction

The presence of multiple queens within a colony involves costs for all parties involved, yet multiple queening is common in ants (HÖLLDOBLER & WILSON 1990, KELLER 1995, GADAU & FEWELL 2009). Queens face loss of personal reproduction as colony resources are divided between several reproductives (ELMES 1973, HERBERS 1984, KELLER 1988, SUNDSTRÖM 1995; but see WALIN & al. 2001, DALECKY & al. 2005), whereas workers face reduced inclusive fitness returns, as polygyny dilutes worker relatedness to the brood they rear, all else being equal (DALECKY & al. 2005). However, both workers and resident queens may favour adoption of related queens if multiple breeders enhance colony productivity or colony longevity sufficiently (NONACS 1988, DALECKY & al. 2005), or if increased genetic diversity brings better colony resistance against parasites (HUGHES & BOOMSMA 2004). Conversely, young queens may seek adoption, even at the expense of personal reproduction, if dispersal risks are high and their independent reproductive prospects therefore are low (ROSENGREN & PAMILO 1983, NONACS 1988, KELLER 1995, HELMS CAHAN & al. 2002). Adopted daughter queens may also replace deceased queens and so provide inclusive fitness returns for resident queen(s) and workers if the average queen life span is shorter than that of the colony (NONACS 1988).

The propensity to adopt additional queens may be influenced by the kin structure of recipient colonies. Thus, based on inclusive fitness arguments monogyne colonies, or colonies with high relatedness among colony members, should be more reluctant to accept additional queens than polygyne colonies, or colonies with low relatedness, given that the decrement in inclusive fitness returns is greater when the first additional queen is adopted compared to the adoption of further queens (PAMILO 1991). Consequently, polygyne, i.e., low-relatedness colonies may show a higher rate of queen turnover, as old queens become replaced with new ones (NONACS 1988). This indeed is the case in several species (SEPPÄ 1994, SEPPÄ & WALIN 1996, EVANS 1996, PEDERSEN & BOOMSMA 1997, BARGUM & al. 2007).

Although polygyne colonies may be expected to be more lenient than monogyne ones towards daughter queens seeking adoption, both colony types are predicted to discriminate against non-nestmate queens (PAMILO 1991). Indeed, empirical studies show that nestmate queens are often accepted but non-nestmate queens are mostly rejected in polygyne colonies (BENNETT 1988, STUART & al. 1993, KIKUCHI & al. 2007, VÁSQUEZ & SILVERMAN 2008), whereas both queen types are usually rejected in monogyne colonies (BENNETT 1988, SUNDSTRÖM 1997, VÁSQUEZ & SILVERMAN 2008; but see MEUNIER & al. 2011). Nonetheless,

also unrelated queens may successfully enter colonies (ELMES 1973, KELLER 1995, RÜPPELL & HEINZE 1999, EVANS 1996, SUNDSTRÖM 1997, SOUZA & al. 2005, HOLZER & al. 2008a, ZINCK & al. 2009), conferring full benefits to the intruding queen, but only costs in terms of loss of inclusive fitness returns for resident colony members. Especially polygyne colonies have been considered vulnerable to such intrusions (KELLER 1995). The argument goes that low relatedness is associated with high genetic diversity (GIRAUD & al. 2001; but see TRONTTI & al. 2007), which is hypothesized to compromise recognition owing to increased cue diversity (HÖLDOBLER & WILSON 1977, BREED & BENNETT 1987, VANDER MEER & MOREL 1998; but see ROSSET & al. 2007, MARTIN & al. 2009, HELANTERÄ & al. 2011). Alternatively, common ancestry of nests within polydomous networks, and / or a low incidence of non-network members as intruders may have selected for a more permissive recognition threshold to avoid the mistaken rejection of nest mates (CHAPUISAT & al. 2005; but see MARTIN & al. 2009). Thus, we expect a difference in the tendency to make recognition errors mediated by differences in kin structure, as has been shown for several species (FORTELIUS & al. 1993, SUNDSTRÖM 1997, HOLZER & al. 2008a, b; but see SOUZA & al. 2005, ZINCK & al. 2009). The question is whether the physical presence of one versus multiple queens, or the change in within-colony relatedness through the presence of multiple breeders conveys potential changes in worker attitudes.

To date most tests of queen adoption have been conducted over a short time span following intrusion (BENNETT 1988, STUART & al. 1993, FORTELIUS & al. 1993, SUNDSTRÖM 1997, KIKUCHI & al. 2007, HOLZER & al. 2008b, MEUNIER & al. 2011) and on either monogyne or highly polygyne species or populations (BENNETT 1988, STUART & al. 1993, FORTELIUS & al. 1993, KÜMMERLI & al. 2005, KIKUCHI & al. 2007, HOLZER & al. 2008a, b, VÁSQUEZ & SILVERMAN 2008, VÁSQUEZ & al. 2008). In many of these studies a considerable proportion of intruding queens remained alive throughout the duration of the experiments, yet the ultimate fitness returns from adoption are determined by the reproductive success of adoptee queens, a process which may span an entire seasonal cycle. Indeed, in many highly polygyne species, such as *Linepithema humile* a considerable number of additional queens is recruited each reproductive season, but most of them are executed the next year (KELLER & al. 1989, REUTER & al. 2001). The high rates of queen turnover found in several facultatively polygyne species (EVANS 1996, PEDERSEN & BOOMSMA 1999, BARGUM & al. 2007) may also entail execution.

Here we test whether high versus low within-colony relatedness per se, rather than the actual number of queens present in the colony affect the adoption and over-wintering survival of young queens in the black ant *Formica fusca*. In this species variation in queen number creates variation in kin structure, with an average effective number of queens of circa three (HANNONEN & al. 2004). Monogyne colonies retain their resident queen across several seasons, whereas one third of the queens are replaced each breeding season in polygyne colonies (BARGUM & al. 2007). Also the colony type tends to remain unchanged across seasons, with monogyne colonies remaining monogyne and polygyne colonies remaining polygyne (BARGUM & SUND-

STRÖM 2007). Polygyny is nonetheless thought to arise from monogyne colonies via the adoption of extra queens (SEIFERT 2007). Resident queens are usually related to each other and to the workers (HANNONEN & SUNDSTRÖM 2002, HANNONEN & al. 2004, BARGUM & al. 2007), suggesting that polygyne colonies predominantly adopt daughter queens. However, sometimes queen relatedness in polygyne colonies is low ( $< 0.25$ , HANNONEN & SUNDSTRÖM 2003) suggesting the occasional adoption of unrelated queens. We hypothesize that related daughter queens are more amenable for adoption than unrelated non-nestmate queens, that colony kin structure per se, rather than the physical presence of one or many queens, affects acceptance such that high-relatedness (monogyne) colonies are more reluctant to accept additional queens than low-relatedness (polygyne) ones. In addition, given the difference in queen turnover between mono- and polygyne colonies (BARGUM & al. 2007) as well as the greater kin value of siblings compared to nieces and nephews under monogyny (BOURKE & FRANKS 1995, CROZIER & PAMILO 1996), we hypothesize that resident queens are favoured over new adoptees in high-relatedness, but not necessarily in low-relatedness colonies. In the latter type of colony the residual reproductive value of young queens may exceed that of old ones hence young queens may be favoured over old ones.

## Material and methods

**Study colonies:** Thirty-nine entire colonies of *Formica fusca* were excavated in 2008 and 58 in 2009 in the vicinity of Tvärminne Zoological Station in southern Finland. At the time of collection queens had not resumed oviposition after hibernation. The excavated colonies were brought to the laboratory and queens and workers were separated. In 2008 we found no queens in 20 colonies, and one queen in 19 colonies, whereas in 2009 we found no queens in 20 colonies, one queen in nine colonies and multiple (2 - 60) queens in 29 colonies. Each colony was used only once, in the year it was collected. Once sorted, all colonies were established in laboratory nests made of plastic trays (30 × 30 × 40 cm), the walls of which were coated with flulon™ to prevent the ants from escaping, the bottom lined with peat, and a ceramic tile added to provide shelter.

**Establishment of laboratory colonies and rearing of gynes:** To obtain female sexuals (gynes) we first divided each queen-right field colony into a queen-right fragment with about 300 workers and an orphaned fragment with 100-150 workers in the conditions described above (Fig. 1). As the number of queens found in the field colony does not necessarily reflect the true kin structure of the colony because polygyne colonies may be functionally monogyne, and monogyne colonies may have had more queens than the one we collected, we chose to standardize queen number to one in all queen-right nests. Thus, regardless of the number of queens in the field colonies each queen-right laboratory nest had only one queen, but the kin structure among workers differed. From the polygyne colonies this queen was chosen randomly from those available.

We allowed the queen in each queen-right fragment to start laying, and once over 100 eggs were present in the nests we transferred these to their corresponding orphaned fragment. The egg sex ratio in queen-right colonies is highly female-biased so in the absence of a queen most eggs would develop into new queens (HELANTERÄ & SUNDSTRÖM

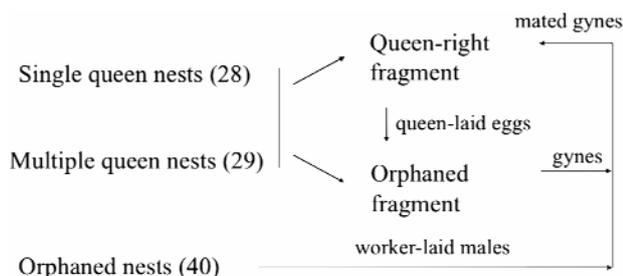


Fig. 1: Experimental design and number of colonies used, the numbers in brackets indicate the number of field colonies used in the experiment.

2005). The eggs the queens laid after this transfer were left in the queen-right fragment and were allowed to develop into new workers. The orphaned nests were used to produce males (Fig. 1); workers of *Formica fusca* readily start to lay eggs when orphaned (HELANTERÄ & SUNDSTRÖM 2005, 2007). These nests contained over 400 workers. All nests were maintained under controlled temperature conditions (25 - 27°C), fed with Bhatkar-Whitcomb diet (BHATKAR & WHITCOMB 1970), and moistened daily during brood development. Once the brood had pupated we counted the number of worker pupae in the queen-right fragments. After emergence, gynes and males were allowed to mature in their natal fragments for ca two weeks.

**Production of mated gynes and establishment of experimental colonies:** Once the sexuals became positively phototactic and were found on the top of the nest, they were entered in mating chambers made of glass (50 × 50 × 50 cm). In each chamber we placed all gynes from one nest fragment and twice the number of males from several of the orphaned colonies. The behaviour of each gyne was observed to ensure that all those that were used in the experiment copulated. Dissections made in earlier experiments have shown that all gynes that copulate are also inseminated (K. Bargum, pers. comm.). Similarly dissections of mated and unmated worker-produced males have shown that they have viable sperm and transfer sperm during mating (A. Chernenko, unpubl.). Each gyne was immediately after mating placed in a separate plastic box 15 × 15 × 15 cm with peat and *Sphagnum* moss for moisture. The mated gynes were kept with ca 20 workers from their natal nest, fed Bhatkar-Whitcomb diet (BHATKAR & WHITCOMB 1970) and watered daily until introduced into their host colonies. By the time matings were staged, the queen-right fragments had on average  $42.01 \pm 32.89$  (N = 57) newly emerged workers in addition to the over 300 old workers collected in spring.

When all gynes had mated we discarded the orphaned nest fragments and prepared the queen-right fragments and the newly mated gynes for adoption. As our focus was to test the long-term survival and possible reproduction of prospective adoptee queens, we used a design, which allowed the mated gynes to bypass the risks of entering colonies. This procedure also allowed assessing the egg-laying rates of any surviving queens after hibernation. To accomplish this we used a modification of the ROSS & KELLER (2002) procedure, where we first gradually decreased the temperatures from 25°C to + 5°C, with a decrement of one degree per day over 20 days. Once the temperature had reached ca + 5°C we introduced one gyne into each queen-

right nest fragment. As we obtained gynes from only four colonies in 2008 (all monogyne) and five in 2009 (two monogyne and three polygyne), we used these queens for introduction into both their natal queen-right fragment, and the remaining unrelated queen-right fragments. All but two polygyne colonies received gynes from a polygyne colony, and all monogyne colonies received gynes from a monogyne colony. No gynes were introduced into orphaned fragments. Each gyne-producing fragment donated mated gynes to at most four recipient fragments. Therefore individual gynes do not represent independent samples. However, the focus of our analyses was not the influence of gyne origin on their acceptance, but the colonies responding to the gynes, and these represent independent observations. Altogether we introduced related gynes into nine host colonies and unrelated gynes into 48 host colonies, all of which already contained one resident queen. All introduced gynes as well as resident queens were individually marked with a colour dot on the thorax (*Edding 750<sup>TM</sup>* paint markers).

We then maintained the fragments at + 5°C to simulate hibernation. During this period the fragments were moistened once a week, and the colonies and queens were checked after two, and four months to avoid disturbing the colonies too often. On these occasions dead queens were removed and their status (intact or dismembered) noted. At the end of the experiment we collected all remaining queens and identified their origin based on the colour dot. Intact dead queens were likely to have died by themselves, i.e., having either left the colony on their own or been expelled by the workers (defined here as neglect), whereas dismembered queens were considered killed by the workers. At this point the condition of the colonies was also evaluated on the scale "weak" (less than 50 workers), "adequate" (50 - 100 workers), and "good" (over 100 workers). Three colonies were excluded from subsequent analyses, as both queens were dead at the final check. It is likely that the queens in these colonies had died because the colony was unhealthy, rather than neglect or execution by the workers.

**Relatedness estimates:** To assess colony kin structure and the extent to which this agrees with the number of queens found in the colonies collected from the field, we estimated the average relatedness among nestmate workers from the genotype data. We genotyped the resident queens and eight to 24 workers per colony at eight polymorphic DNA-microsatellite loci (FL12, FL20: CHAPUISAT 1996; FE13, FE17, FE19, FE21, FE51: GYLLENSTRAND & al. 2002; FY7: HASEGAWA & IMAI 2004). DNA-extraction and PCR amplification followed the protocols described in HANNONEN & al. (2004). The PCR-products were then diluted 1:26 with ddH<sub>2</sub>O, separated using automated capillary sequencer MegaBACE 1000 and sized against ET400-R standard GE Healthcare. The genotypes were scored using the program Fragment Profiler v1.2 GE Healthcare and allele calling was confirmed manually.

**Statistical procedures:** As a measure of colony kin structure we estimated the average relatedness among the workers collected from the field colonies using Relatedness 5.0.8 (QUELLER & GOODNIGHT 1989). We weighed the nests equally, and obtained standard errors by jack-knifing over colonies. We then tested the effect of worker relatedness on queen survival and mode of death using a binomially distributed generalized linear model. The response variables were queen fate (alive or dead; GLZ 1) or mode

Tab. 1: Numbers of dead and alive introduced and resident queens at first check and at the second check at the end of the experiment.

		First check			Second check		
		Introduced		Resident	Introduced		Resident
		Related	Unrelated	Related	Related	Unrelated	Related
High relatedness	alive	3	13	31	0	3	26
	dead	4	11	0	7	21	5
Low relatedness	alive	1	8	23	0	0	21
	dead	1	13	0	2	21	2
Total		9	45	54	9	45	54

of death (neglect or killed; GLZ 2), and the explanatory variables were in both cases worker relatedness as a covariate, and queen type (introduced or resident) as a discrete factor, and the interaction between the two. The significance of cell differences in the interaction term was assessed from the Wald confidence intervals (95%) of pairwise contrasts. Similar pairwise comparisons were carried out for the interaction, when significant. We did not include the factor year, as each colony was used only in one year, and all polygyne colonies were collected in 2009.

## Results

The average worker relatedness was  $0.63 \pm 0.32$  across all colonies. In colonies where one queen was found upon collection relatedness was  $0.87 \pm 0.11$ , and in colonies with multiple queens at collection it was  $0.39 \pm 0.28$ . In four colonies worker relatedness was relatively high (0.50 - 0.63) although they contained several queens at excavation, but their genotype distribution was not consistent with monogyny. In a further four polygyne colonies worker relatedness was very high (range 0.82 - 1.0), indicating functional monogyny or the adoption of extra queens after the present workers were born. The genotype distributions within these and all remaining colonies with high worker relatedness ( $r > 0.62$ ) were fully congruent with monogyny and single or double mating (in four colonies out of 31 the queen was doubly mated). When the four functionally monogynous colonies were omitted the average relatedness in the remaining polygyne colonies was  $0.30 \pm 0.18$ . Thus we classified colony kin structure as high- or low-relatedness colonies as appropriate for some of the analyses below. At the end of the experiment, not counting the three colonies that were excluded from the analyses, 27 of the high-relatedness colonies were classified as being in good condition, two in adequate condition, and two in weak condition. The corresponding values for the low-relatedness colonies were 20, two and one. We found no significant differences in these classifications between the two types of colonies ( $\chi^2(2) = 0.195$ ,  $p = 0.91$ ).

The origin of the introduced queens (daughter or foreign) had no effect on their fate: all related queens and almost all unrelated queens died or were killed (Tab. 1, Fisher's exact test  $p = 1.0$ ). The fraction of dead introduced queens did not differ between high- and low-relatedness colonies neither at the first check after two months, nor at

the end of experiment (Tab. 1, Fisher's exact test  $p = 0.26$ ). Related introduced queens were no more likely to die later than unrelated queens (Tab. 1, Fisher's exact test  $p = 1.0$ ). Thus, only queen type, introduced vs. resident, dictated queen fate: introduced queens were more likely to die while resident queens were more likely to survive (GLZ 1, factor: queen type, Wald  $\chi^2(1) = 6.46$ ,  $p = 0.01$ ). Neither relatedness (GLZ 1: relatedness Wald,  $\chi^2(1) = 0.89$ ,  $p = 0.344$ ) nor the interaction queen type \* relatedness (GLZ 1, Wald  $\chi^2(1) = 1.39$ ,  $p = 0.239$ ) had a significant effect on queen fate (Fig. 2).

The dead queens or their pieces were always found outside the nests at the periphery of the nest boxes, never inside the nest. In 24 of the 31 high-relatedness colonies the introduced queen was classified as killed (dismembered) and in four colonies it had died intact, the corresponding values for the low-relatedness colonies were 13 and ten, respectively out of 23. In high-relatedness colonies the resident queen was killed in four cases, and died intact in one case, whereas none of the resident queens were killed and two died intact in the low-relatedness colonies. In agreement with this, relatedness was the main determinant for mode of death (GLZ 2, covariate: relatedness, Wald  $\chi^2(1) = 3.95$ ,  $p = 0.047$ ), with both queen types being more likely to be killed in high-relatedness colonies (Fig. 3). Neither the effect of queen type (GLZ 2, factor: queen type, Wald  $\chi^2(1) = 0.74$ ,  $p = 0.39$ ) nor the interaction between relatedness and queen type were significant (GLZ 2, Wald  $\chi^2(1) = 0.34$ ,  $p = 0.56$ ). The number of killed and intact introduced queens did not differ between the first and the last check: 22 queens were killed and seven were intact at first check and 16 were killed and seven were intact at the last check ( $\chi^2(1) = 0.26$ ,  $p = 0.61$ ).

## Discussion

We show that regardless of colony kin structure workers of *Formica fusca* retain resident queens rather than accepting introduced queens. Indeed, in 78% of the cases the introduced queen died and the resident queen was retained. Contrary to our expectations, however, related daughter queens were not more eligible for adoption than unrelated queens, and none of the retained introduced queens were related to the workers of the recipient colony. Furthermore, we found no effect of colony kin structure on the tendency to adopt additional queens. However, queens were more often found

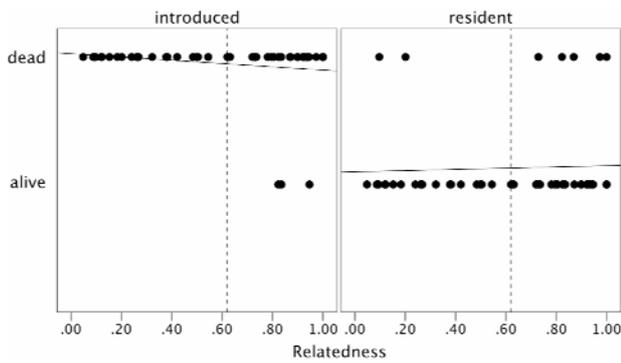


Fig. 2: Survival among introduced and resident queens as a function of worker relatedness in the recipient colonies. Dashed lines separate high- and low-relatedness colonies (single-queen and multi-queen, respectively).

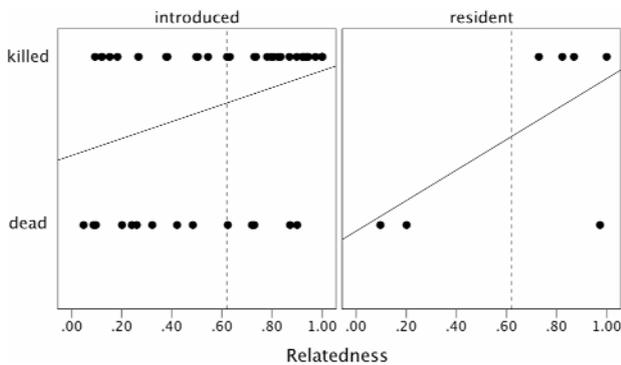


Fig. 3: Distribution of dead and killed queens among introduced and resident queens as a function of worker relatedness in the recipient colonies. Dashed lines separate high- and low-relatedness colonies (single-queen and multi-queen, respectively).

dismembered in high-relatedness colonies, in which workers were the descendants of a single queen, whereas most dead queens were found intact outside the nest without evident intervention by workers in low-relatedness colonies comprising several matriline.

Although we found that related introduced queens were no more likely than unrelated ones to be adopted the question whether polygyne colonies are more lenient towards related queens remains unresolved, as we had rather few replicates with related queens. Also earlier studies are divided in this respect, as some have found no difference in acceptance rates of related versus unrelated queens in polygyne colonies (FORTELIUS & al. 1993, SUNDSTRÖM 1997, HOLZER & al. 2008a, b), whereas others have found a preference for related queens (BENNETT 1988, STUART & al. 1993, KIKUCHI & al. 2007, VÁSQUEZ & SILVERMAN 2008, MEUNIER & al. 2011).

Resident queens were retained rather than introduced ones with few exceptions, but in roughly half the cases the introduced queen was retained for more than two months. Therefore, although intruding queens may be initially left alive, very few may ever come to reproduce in the colony. The fact that introduced, rather than resident, queens were rejected suggests that workers opt for a closely related or familiar queen, rather than a queen with greater residual reproductive value (BOURKE & FRANKS 1995, CROZIER &

PAMILO 1996). A related, but not an unrelated, adoptee queen may be preferable if the fecundity of the resident queen is lower (FORSYTH 1980, REEVE & KELLER 2001, HANNONEN & al. 2002), yet in our study none of the related introduced queens were alive at the end of the experiment. Similarly, neither HOLZER & al. (2008a) nor VÁSQUEZ & SILVERMAN (2008) found an effect of queen fecundity on her survival in *Formica exsecta* and *Linepithema humile*, respectively. We also found that contrary to the pattern observed in *Linepithema humile* (see KELLER & al. 1989), execution in *F. fusca* occurred already during hibernation, not at the onset of reproduction in spring.

The introduced queens may, however, have been rejected because the workers in the queen right experimental nest fragments had not experienced the presence of sexual brood before the young queens were introduced. The queens were reared by orphaned fragments and these workers were not present in the experimental nests. A corresponding pattern of conditional killing of adoptee queens has previously been documented in *Formica exsecta* (see BROWN & al. 2003) and *F. paralugubris* (see FORTELIUS & al. 1993). However, these studies specifically examined initial acceptance of young queens into colonies, not the continued acceptance during hibernation. To our knowledge only two studies have addressed long-term acceptance of supernumerary queens and in both cases these were accepted despite the absence of prior exposure to sexual brood (HOLZER & al. 2008a, ROSS & KELLER 2002). This suggests that the rejection of young queens is not an artefact of lack of prior exposure to sexual brood, but a selective response towards young queens per se, perhaps mediated by low fertility or lack of appropriate fertility signals (sensu HANNONEN & al. 2002).

All introduced gynes in the experiment were produced under laboratory conditions and also mated with laboratory-produced males. Such gynes may be less viable than those produced in field colonies. Alternatively, the viability of mated gynes may be low irrespective of their origin, but no studies have tested this. However, earlier observations on isolated mated *Formica fusca* gynes did not indicate exceptionally high mortality (K. Bargum, pers. comm.), and none of the numerous studies on laboratory-reared queens have reported elevated mortality rates in these (e.g., YAMAUCHI & al. 2006, 2007, SCHREMPF & HEINZE 2008). Also, worker-produced males may be of lower quality and affect gyne survival, perhaps through sperm number and quality. Our earlier observations nonetheless have shown that worker-produced males are fertile (A. Chernenko, unpubl.). Moreover, given that orphaned colonies are presumably quite common and worker reproduction not rare (HELANTERÄ & SUNDSTRÖM 2005, 2007), there is no reason to assume that worker-produced males would be of poor quality due to weak selection. If workers produce males, selection should favour the production of good-quality males.

Regardless of colony kin structure, adoptee queens were rejected in all colonies. This stands in contrast to theoretical predictions (HÖLLDOBLER & WILSON 1977) and earlier empirical results (FORTELIUS & al. 1993, SUNDSTRÖM 1997, HOLZER & al. 2008a, b), which show a greater degree of colony closure in monogyne than in polygyne species or populations. Such a pattern could arise if high genetic and chemical diversity compromise recognition in polygyne colonies (BREED & BENNETT 1987, VANDER MEER & MOREL

1998). However, high genetic diversity produces neither a high diversity of recognition cues in workers nor a more permissive attitude towards non-nestmate workers in *Formica fusca* (see HELANTERÄ & al. 2011) or *F. exsecta* (see MARTIN & al. 2009). Hence nestmate recognition is not a simple function of genetic make-up and recognition cue phenotype (HOLZER & al. 2006, MARTIN & al. 2009, VAN ZWEDEN & D'ETTORRE 2010, HELANTERÄ & al. 2011). Our results show that low relatedness per se does not render colonies more lenient towards intruders, but maintain a degree of genetic integrity, apparently mediated by the behavior of workers. The physical presence of multiple queens and the odor cue diversity they mediate may, however, change also worker attitudes in this respect.

Although introduced queens met the same fate regardless of the kin structure of their adoption colony, workers in high and low-relatedness colonies responded differently to the introduced queens, irrespective of the actual number of queens present. Introduced queens were more often found dismembered in high-relatedness colonies than in low-relatedness colonies. Observations on the fate of non-nestmate workers show that dismembered individuals usually have been killed, whereas individuals that have died for other reasons are dumped intact on a refuse pile (A. Chernenko, unpubl.). Thus, if our interpretation of mode of death is correct, workers from monogyne colonies actively execute intruding queens, whereas workers from polygyne colonies expel or allow them to escape from the nest. Without the care of workers, and failing to escape from the nest box to establish a nest elsewhere, these queens would eventually succumb outside the nest, where they indeed were always found. Thus the fate of the queens introduced into the polygyne nests may be dictated more by queen behaviour than worker intervention. Nevertheless, dismembering does not necessarily mean that a queen was killed, as workers may have been chewing on dead queens. If so, the mode of death rather reflects the time of death with dismembered queens dying earlier than intact ones during the time span between checks. Hence, queens in high-relatedness colonies nonetheless died earlier than those in low-relatedness colonies.

Our result, that low-relatedness colonies show as stringent a colony closure towards unrelated intruding queens as high-relatedness colonies, stands in contrast to most other studies on queen recruitment in polygyne colonies (ROSENGREN & al. 1986, FORTELIUS & al. 1993, SUNDSTRÖM 1997, ROSS & KELLER 1998, HOLZER & al. 2008a, b, VÁSQUEZ & SILVERMAN 2008). Indeed, in some cases unrelated queens from either the same or a different population were not only accepted, but also laid eggs the next breeding season (HOLZER & al. 2008a, VÁSQUEZ & SILVERMAN 2008). However, these studies used either uniclonal or highly polygyne species or populations, in which colonies within a network usually have shared ancestry due to budding (HELANTERÄ & al. 2009), or workers rarely encounter individuals from other networks. As a result they may have evolved a more permissive recognition threshold to avoid the mistaken rejection of nest mates (CHAPUISAT & al. 2005, MARTIN & al. 2009). In contrast, our study system entails both monogyne and polygyne colonies in sympatry, with a gradual transition in queen number, and a moderate number of queens (HANNONEN & al. 2004). Furthermore, colony networks with shared ancestry of nests are absent

(BARGUM & al. 2007), and unrelated intruders are therefore frequent. In addition, continuous gene flow between the two types is likely to prevent behavioural divergence (CHAPUISAT & al. 2004, DEHEER & HERBERS 2004, BARGUM & al. 2007). This may crucially alter the premises for discrimination. Indeed, *Formica selysi* (see CHAPUISAT & al. 2004), which is similar to *F. fusca* in this respect, also shows discrimination against non-kin in polygyne colonies (MEUNIER & al. 2011). Thus the composition of populations in terms of the kin structure of colonies and the opportunity for gene flow between colonies of different kin structure may crucially shape the degree to which colonies form closed units.

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