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Life-history trait variation in a queen-size dimorphic ant

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Running title: Life-history variation in size-dimorphic ants

ABSTRACT

1. Size polymorphism is often connected to alternative life-history traits, which may eventually lead to distinct size classes. In the ant *Myrmica ruginodis*, larger macrogyne and smaller microgyne queen morphs have been suggested to follow different reproductive strategies, which has presumably resulted in several differences in their key life-history traits.

2. In this study, we examine the association of queen-size morphs with colony queen number (monogyny vs. polygyny), dispersal and queen recruitment patterns as well as habitat associations of the queen morphs. We do this by sampling established queens from a large number of excavated nests from several populations, estimating genetic relatedness among coexisting queens and pitfall trapping free-ranging wingless queens.

3. Our results show that associations of queen morphs with colony queen number and nest-founding strategy holds only partly. The morph frequencies vary widely across populations from practically pure macrogyne to more than 50% microgyne, but the expected association of macrogyne occurrence with monogyny and microgyne with polygyny is not universal. Dispersal and queen recruitment patterns also show that while most macrogyne participate in nuptial flights and most microgyne are recruited back to their natal nests, a fraction of both morphs use the alternative strategy.

4. Polygynous microgyne morph has been suggested to specialize in stable habitats, but our results from Finnish mesic heath forests do not support this. This study shows that other factors than just queen size also influence life-history trait variation and reproductive strategies in ants.

Keywords: Ants, Habitat stability, *Myrmica*, Macrogyne, Microgyne, Size polymorphism, Queen number, Queen recruitment

INTRODUCTION

Alternative reproductive strategies are a widely known phenomenon in the animal kingdom, and they are often associated with intraspecific size variation (Eberhard, 1980; Thornhill & Alcock, 1983; Gross, 1985, 1996; Crespi, 1988; Danforth, 1991). Size often strongly influences fitness, with larger individuals monopolising reproductive opportunities (Roff, 1992; Stearns, 1992). Alternatively, commonly occurring smaller (“sneaker”) males seek reproductive opportunities alongside large, dominant and territorially behaving males (Eberhard, 1980; Thornhill & Alcock, 1983; Gross, 1985; Crespi, 1988; Danforth, 1991). Size-dependent strategies can also be connected to dispersal (Dingle *et al.*, 1980; Derr *et al.*, 1981; Gonzaga & Vasconcellos-Neto, 2001), where smaller individuals are usually assumed to be competitively inferior and thus disperse while larger ones behave philopatrically (Lawrence, 1987; Hanski *et al.*, 1991). Nevertheless, this can also be reversed so that larger individuals disperse further than smaller conspecifics due to larger energy reserves (Anholt, 1990; O’Riain *et al.*, 1996; Léna *et al.*, 1998; Gundersen *et al.*, 2002; Barbraud *et al.*, 2003).

In ants, alternative reproductive strategies have been found especially in females, sometimes causing divergence in queen sizes and life-history strategies (Heinze & Tsuji, 1995; Peeters & Ito, 2001). Typically, queens in nests with only a single reproducing queen (monogyny) are larger than those in nests with many queens (polygyny) (see Peeters & Ito, 2001). Large queens have larger glycogen and fat content than small queens, and they are also typically better equipped for dispersing by flight and independent nest founding (Passera & Keller, 1990; Heinze & Hölldobler, 1993; McInnes & Tschinkel, 1995; Wheeler & Martinez, 1995; Stille, 1996; Lachaud *et al.*, 1999). Positive relatedness among coexisting queens in secondarily polygynous species suggests that new queens are often recruited to their natal nests (e.g. Crozier & Pamilo, 1996). Colony propagation occurs then without dispersal by flying, via splitting of old nests (dependent colony founding), which might stay connected (budding) or not (fission) (Rosengren & Pamilo, 1983; Hölldobler & Wilson, 1990). Life-history traits in ants are often described as suites of linked characteristics centred around queen number (so-called polygyny syndrome, Rosengren & Pamilo, 1983).

The occurrence of polygyny in ants as an indicator of reduced dispersal has been associated with stability, patchiness and longevity of the habitat (Hölldobler & Wilson, 1977; Rosengren & Pamilo, 1983), as well as habitat crowding (Herbers, 1986). In stable habitats, patch size sets limits to the growth and propagation of nests through nest-site limitation, resource limitation and competition in general, selecting for increased polygyny (Hölldobler & Wilson, 1977; Rosengren & Pamilo, 1983; Herbers, 1986). If queen dispersal does not exceed further than the natal habitat patch and the number of nests that can co-occur in a given patch is restricted, such habitat patches are expected to become saturated with time with conspecific nests. In such a situation, the only possibility for newly produced queens to reproduce is to be recruited to an existing nest. Interspecific competition and high queen predation during colony founding can also make nest founding more difficult. Thus, both intra- and interspecific competition may reduce nest-founding opportunities, which increases dispersal risk and promotes queen philopatry (Greenwood, 1980) and recruitment.

Sometimes alternative reproductive strategies in ants lead to the emergence of queen-size dimorphism, with either clearly separated or overlapping size distributions between the larger (macrogyne) and smaller (microgyne) queen morphs (Heinze & Tsuji, 1995; Heinze & Keller, 2000; Peeters & Ito, 2001; Wolf & Seppä, 2016a). Size morphs have been suggested to be associated with differences in life-history traits. Macrogyne queens supposedly disperse and are usually found in monogynous nests, which are founded independently after a nuptial flight (e.g. Elmes 1991; Rüppell & Heinze 1999; Wolf & Seppä 2016a). In contrast, microgyne queens do not usually join nuptial flights, as they are poorly equipped for both dispersal (Passera & Keller, 1990; Heinze & Hölldobler, 1993; Wheeler & Martinez, 1995; Stille, 1996; Wolf & Seppä, 2016b) and independent colony founding (McInnes & Tschinkel, 1995; Peeters & Ito, 2001). Instead, they presumably mate locally and seek adoption in secondarily polygynous nests instead.

In ant genus *Myrmica*, there are two queen-size dimorphic species. The microgyne morph of *M. rubra* is a true inquiline parasite (Seifert 1993) currently speciating from its macrogynous host (e.g. Leppänen et al. 2015), whereas in *M. ruginodis* macrogyne and microgyne are free-living intraspecific queen morphs. The size distributions of the two morphs in *M. ruginodis* are overlapping, however, so that not all queens can be

positively assigned to one of the morphs, and about one fourth of the polygynous nests have a mixture of queen morphs (Elmes, 1991).

Since nest-founding strategies in queen-size dimorphic ants may differ depending on habitat quality and patchiness (Lenoir *et al.*, 2011), size morph frequencies are also expected to differ across habitat types. For instance, in *Temnothorax rugatulus*, microgynes have been suggested to profit from stable nesting substrate, such as rocky outcrops, and to occur more often in high densities in those habitats, whereas macrogynes are better colonisers than microgynes (Rüppell *et al.*, 2001). Previous suggestions about the association of *M. ruginodis* queen morphs with the habitat type are somewhat contradictory. On one hand, Brian and Brian (1949) studied *M. ruginodis* in English and Scottish moor habitats and suggested that macrogynes are predominant in transitory habitats (“seral and mosaic cyclic vegetation types”) and microgynes are found in relatively stable habitats. On the other hand, our study habitat, boreal forest, is a dynamic mosaic of different successional stages due to various disturbances (Kuuluvainen, 2009; Kuuluvainen & Aakala, 2011). In this type of landscape, nests in early-successional habitats following recent disturbance are more polygynous than in more stable late-successional habitats (Seppä *et al.*, 1995). This suggests that the polygynous microgyne morph specialises in rapidly changing early successional stages rather than relatively stable old-growth habitats.

In this study, we investigated to what extent size morphs in the ant *M. ruginodis* are associated with key life-history traits. For this purpose, we assessed queen-morph identity and its association with i) number of established old queens in excavated colonies, ii) queen dispersal patterns based on temporal distribution of free-ranging wingless queens and iii) queen-recruitment patterns by estimating genetic relatedness among co-existing queens separately in macrogyne and microgyne nests. Studying multiple populations and complementing the results with previously published data (Brian & Brian, 1949; Elmes, 1991; Wolf & Seppä 2016b) enabled us to assess the spatial variation in the occurrence of the queen morphs and their association with colony social structure, dispersal and colony founding patterns.

As suggested earlier (Brian & Brian, 1949; Elmes, 1991), we expected to find a bimodal queen-size distribution in *M. ruginodis* and that macrogyne and microgyne queens are

generally found in monogynous and polygynous nests, respectively. Based on the close association of the colony queen number and other key life-history traits (Heinze & Tsuji, 1995; Peeters & Ito, 2001), we also expect to find that macrogyne queens are more prone to dispersing by flight than microgyne queens. This is expected to show in the pitfall data as an excess of free-ranging wingless macrogyne queens after the nuptial flight and more even distribution of wingless microgyne queens across the season. Furthermore, we expected to find that coexisting microgyne queens are related, which would indicate that they disperse locally and are recruited back to their natal nests. The exact prediction of queen relatedness in macrogyne is not clear, because macrogyne queens are rarely expected to reside in polygynous nests. Finally, we analyse habitat association of free-ranging wingless queens and test earlier hypotheses (see above) about the occurrence of the monogynous (macrogyne) and polygynous (microgyne) morphs.

MATERIAL AND METHODS

Sampling

We sampled established old queens by carefully excavating whole nests using previously established procedures (e.g. Seppä 1994; Walin & Seppä 2001) at three sites in southern Finland, i) Savero (Kouvola) in 1992; ii) Antby (Raasepori) in 2004 and iii) TV/Leimann (Hanko). TV/Leimann population was sampled three times, first in 1994 for another study (Walin & Seppä 2001) and again in 2012 and 2014 (combined as TV/Leimann 2010s). Details of excavated populations and sampling are given in Table 1 and Appendix S1.

We also used pitfall-trap data of free-ranging wingless queens collected at two sites. In Musturi old-growth forest (Ruovesi, 1985), the sampling covered a 1.3 ha plot in a spruce-dominated old-growth reserve (Niemelä et al. 1989). In Multiharju (Seitsemäen National Park, Ikaalinen, 1986), the sampling covered a 1.4 km transect consisting of a mixture of mostly old forest and forested mire types (Niemelä et al. 1992), with seven habitat types distinguished for the present study. For both sampling areas, the traps were under operation for most of the snow-free season, which we divided into three periods. Period I started in the early spring after the snowmelt and ended in mid-July before the

M. ruginodis nuptial flight. Period II covered the nuptial flight period, ending in mid-August and period III after that until late autumn. Details of sampled populations and sampling schemes are given in Table 1 and Appendix S1.

Finally, we also used queen data from two earlier excavation studies, one where the data were collected mostly from Scotland (hereafter Scotland, Brian & Brian, 1949) and another where the data were collected from southern England (Elmes, 1991) as a comparison to the Finnish data.

Morphometrics

Until 2004, we measured the head width of queens directly under a binocular to 0.02 mm accuracy. For the more recent excavations, we first photographed the sampled individuals with a Canon EOS 50D camera at a 25× magnification. Then we measured the head width of queens with the software ImageJ (Abràmoff *et al.*, 2004) to 0.02 mm accuracy. High repeatability of this method has been shown previously (Wolf & Seppä, 2016b). By optimising a bimodal fit to the queen-size distribution, head width of 1.065 mm was earlier determined as a cut-off to separate microgynes and macrogynes (Elmes, 1991). Later, this was shown to be in good accordance with a behavioural cut-off value based on the differential participation of queens of different size morphs in the nuptial flight in TV/Leimann population sampled in 2013 (Wolf & Seppä, 2016b). We divided nests to macrogynous, microgynous and mixed nests, or – for some analyses – assigned mixed nests based on their predominant morph type (median queen size in microgynes ≤ 1.06 mm).

Statistical analysis

In each population, we tested queen-size distributions for normality with Kolmogorov-Smirnov tests and for modality with Sarle's bimodality coefficient (BC, SAS Institute Inc, 1990). BC is calculated as a ratio of squared skewness and kurtosis of the size distribution, corrected for the sample size. The BC of a uniform distribution gets a value BC=0.56, while higher values indicate bimodality and lower ones unimodality (SAS Institute Inc, 1990). We tested whether the queen-size distributions in the TV/Leimann population sampled c. 20 years apart were similar with two-sample Kolmogorov-Smirnov-test. We also tested if queens from monogynous nests were larger than queens

from polygynous nests by fitting a linear mixed model to the data with monogyny/polygyny as a fixed factor and populations and nests nested into populations as random factors (Finnish populations only).

We used log-likelihood ratio tests (G test) to test the variation in queen number across populations by testing the difference in frequencies of queenless, monogynous and polygynous nests, and to test the variation in morph occurrence in macrogynous, microgynous and mixed nests across populations. We explored the association of size morphs with the level of polygyny (monogyny/polygyny) in the nests first in general by using log-likelihood ratio tests. Then we used binomial tests to assess if macrogynes and microgynes occurred more often than randomly in monogynous and polygynous nests, respectively, and if monogynous and polygynous nests had more often than randomly macrogynes and microgynes, respectively. Furthermore, we explored the association of the queen number with queen size by calculating Spearman rank correlation between queen number and mean queen size in nests (Finnish and S-English populations) and fitted a linear mixed model to the data to test the relationship between the proportion of microgynes and the number of queens in a nest was tested with the proportion microgynes as a fixed factor and populations as a random factor (Finnish populations only).

For the pitfall-trap data, we tested whether the free-ranging queens were distributed randomly among the sampling periods and habitat types (Multiharju only) using log-likelihood ratio test, calculating the expected occurrence rates based on the distribution of sampling effort among the habitat types and sampling periods. We also used log-likelihood ratio test to test whether the occurrence of macrogynes and microgynes was similar among the habitat types and sampling periods. We used SPSS 25.0 (IBM Corp. Released 2017) and Statistix 9.0 (Analytical Software 1985-2008) for the analyses.

Genetic analyses

The samples from 1990's (Savero, TV/Leimann 1994) were analysed with standard horizontal starch-gel electrophoresis. Genetic variation was resolved in four allozyme loci: α - glycerophosphate dehydrogenase-1 (aGpdh-1), peptidase (Pep, substrate:

glycyl-L-leucine), phosphoglycerate kinase (Pgk), and esterase-2 (Est-2, substrate: 4-methylumbelliferyl acetate) with protocols described in Seppä (1992).

We tested 21 microsatellite primers previously developed for other *Myrmica* species (Evans, 1993; Herbers & Mouser, 1998; Henrich *et al.*, 2003; Azuma *et al.*, 2005; Zeisset *et al.*, 2005). Twelve of these were polymorphic and amplified reliably in PCR in *M. ruginodis*, and we used them for samples from TV/Leimann 2010s. PCR products were analysed with an ABI 3730 sequencer and alleles scored using GeneMapper version 5 (Applied Biosystems). Details of DNA extraction, primer testing and PCR conditions are given in Appendix S2.

We tested queen recruitment pattern in three populations (Savero, TV/Leimann, 1994 and TV/Leimann 2010s) by estimating genetic relatedness among coexisting queens with the software RELATEDNESS 5.0.4. (Queller & Goodnight, 1989). We estimated the overall relatedness for the whole data and separately in macrogyne and microgyne nests and used t-tests to test whether relatedness estimates were significantly different from each other. In the analysis, we added the queens from monogynous nests in the data to calculate the background allele frequencies and jackknifed the estimates over loci and nests for standard errors.

RESULTS

Nest excavations and queen number

Between 32 and 132 entire nests were excavated in the six study populations (including the reference populations in the UK, Table 1). In *Myrmica*, a substantial proportion of nests are queenless (Elmes & Keller, 1993). In our populations, roughly one third of the nests were queenless, one third monogynous and one third polygynous, but the proportions of these categories varied widely and significantly across populations (Table 2; $G = 42.2$, $df = 8$, $P < 0.001$). When regarding queenless nests as monogynous, the average estimated queen number in our populations varied between 1.03 and 3.91. [Previous genetic studies have shown that practically all queenless nests had been monogynous before losing their queen and the observed queen number (one vs. many) is a good proxy for the number of egg-layers in the nests (Seppä, 1994; Walin & Seppä, 2001)]. Disregarding the Antby population with only a single polygynous nest (3%), the

proportion of polygynous nests varied from 35% (Savero) to 60% (TV/Leimann, 1994), but the average queen number in polygynous nests varied only from four (S-England) to six (TV/Leimann, Table 2).

Queen-size distribution

Queen size was normally distributed only in one of our study populations. In the rest of the populations, queen-size distribution was non-normal and negatively skewed in six of eight populations and size distributions were uniform or bimodal ($BC \geq 0.56$) or nearly so in three populations (Fig. 1, 2, Table 3; Appendix S3, S4). In monogynous nests, queen sizes were non-normally distributed in half of the populations, but in all populations in polygynous nests (Table 2). Queen-size distributions in the TV/Leimann population sampled c. 20 years apart were similar (Table 3, Fig. 1, two-sample Kolmogorov-Smirnov-test, $P = 0.22$).

In general, queens in the monogynous nests were significantly larger than in the polygynous nests (linear mixed model, $F = 11.73$, $df = 90.88$, $P = 0.001$). The maximum queen size was similar in all populations (1.18-1.20 mm), but queen-size ranges varied across populations. The size range was largest in TV/Leimann and Multiharju (minimum queen size 0.84 mm) and smallest in predominantly macrogynous populations (Antby, Musturi, Savero; minimum 0.98 mm). In both TV/Leimann data sets, the smallest queens were sampled from two highly polygynous nests and constituted a majority of queens in those nests (Appendix S3).

Queen-number association with the size morphs

The frequency of microgyne queens in excavated populations ranged from c. 25% to c. 65% (Table 2). The frequency of mixed nests (polygynous nests that had queens of both size morphs) ranged from zero to 50% of the polygynous nests (Table 2), and the proportions of nest types (pure macrogyne nests, pure microgyne nests, mixed nests) varied significantly across populations (Table 2, $G = 26.82$, $df = 8$, $P < 0.001$). In the pitfall-trap data sets, 10 and 30% of queens were microgynes.

At the population level, queen morphs were not distributed randomly to monogynous and polygynous nests (Table 2, Finnish populations combined, $G = 14.4$, $df = 1$, $P < 0.001$). Microgynes occurred significantly more often in polygynous than monogynous nests (binomial test, $P < 0.001$), but there was no significant difference in macrogyne occurrence in monogynous and polygynous nests (binomial test, $P = 0.072$). Furthermore, monogynous nests had significantly more often macrogynes than microgynes (binomial test, $P < 0.001$), but there was no significant difference in morph occurrence in polygynous nests (binomial test, $P = 0.082$).

At the nest level, we found a significant positive association when comparing the proportion of microgynes with the number of queens (Finnish populations combined, linear mixed model, $F = 9.54$, $df = 129.00$, $P = 0.002$). Queen number and mean queen size in the nests were also significantly negatively correlated in both Finnish and S-English populations (Spearman rank correlation; all Finnish samples combined: $r_s = -0.39$, $P < 0.001$, $N = 131$; S-England: $r_s = -0.55$, $P < 0.001$, $N = 79$).

Distribution of free-ranging wingless queens within season and across habitats

All queens sampled by pitfall trapping were wingless. In both pitfall data sets, the occurrence of *M. ruginodis* queens across the three sampling periods (before, during and after the nuptial flight) was non-random (Fig. 3, Appendix S4, Musturi: $G = 17.5$, $df = 2$, $p < 0.001$; Multiharju: $G = 8.2$, $df = 2$, $p < 0.05$). Period before the nuptial flight (Period I) in both Musturi and Multiharju had the highest cell contributions (deficiency of gynes in Musturi, excess of gynes in Multiharju) to the test statistics. Distribution of macrogynes and microgynes was similar across the sampling periods in both data sets (Fig. 3, Appendix S4, Musturi: $G = 0.87$, $df = 2$, $P = 0.65$; Multiharju: $G = 0.65$, $df = 2$, $P = 0.69$).

In Multiharju, there was a significant difference in the occurrence rate of queens across habitats (Fig. 4, $G = 34.2$, $df = 6$, $P < 0.001$), with old spruce-pine mire (habitat type 5) showing the largest cell contribution (excess of queens compared to the random expectation) and old spruce-dominated mesic heath forest (habitat type 4) the next largest contribution (deficiency of queens) to the test statistics. However, the queen-

morph distributions across the habitat types did not differ significantly from each other (Fig. 4, Appendix S4, $G = 10.7$, $df = 6$, $P = 0.099$).

Genetic relatedness among queen nest mates

Average relatedness among queen nest mates was similar in all three populations where genetic data were available (Table 4, Saverio vs. TV/Leimann 1994, t-test, $t = 0.85$, $df = 39$, $P = 0.40$; statistical differences to TV/Leimann 2010s population were not tested because the genetic marker used was different). When estimated separately for polygynous macrogyne and microgyne nests, relatedness in macrogyne nests was significantly lower than relatedness in microgyne nests in one population (TV/Leimann 1994: $t = 4.10$, $df = 19$, $P < 0.001$), but not in two others (Saverio: $t = 0.26$, $df = 18$, $P = 0.80$; TV/Leimann 2010s: $t = 0.84$, $df = 32$, $P = 0.41$).

DISCUSSION

Alternative reproductive strategies associated with intraspecific size variation are common (e.g. Eberhard, 1980; Thornhill & Alcock, 1983; Gross, 1985, 1996; Crespi, 1988; Danforth, 1991) and in ants they are usually manifested only in females (Heinze & Tsuji, 1995; Peeters & Ito, 2001). Our work shows that the queen-size morphs in the ant *Myrmica ruginodis* are associated with key life-history traits, such as queen number, dispersal and queen recruitment, but the association is not universal and particularly the number of queens in the nests can be extremely flexible.

Large variation in queen number within and across study populations is in line with previous studies on many facultatively polygynous ants, including other *Myrmica* sp. (e.g. Elmes & Keller, 1993; Crozier & Pamilo, 1996; Pamilo et al 1997; Sundström et al., 2005). Queen number can also vary temporally within single *Myrmica* populations (Elmes, 1987; Elmes & Petal, 1990) due to the irregular gyne production in the nests (Elmes, 1973; Elmes & Petal, 1990). The occurrence of queenless nests (up to 50%) is due to the fast queen turnover in *Myrmica*, which often leaves nests queenless after the demise of the queen(s), particularly when the queen number is low (Seppä, 1994; Evans,

1996). This also emphasises queen number as an unusually flexible trait in *Myrmica* ants (Elmes & Keller, 1993).

Queen morph – queen number association

Queen size was normally distributed only in one of our study populations (Antby), which results from this largely monogynous population being dominated by a single queen morph (macrogyne). In the rest of the populations, the actual size ranges and the non-normal, negatively skewed or bimodal queen-size distributions suggest that they are a mixture of both queen morphs (Figures 1-2, Appendices S3-4), and that the frequency of the morphs varies widely across the populations. Considering that previous work has described *M. ruginodis* as a size-dimorphic species (Brian & Brian, 1949, 1955; Elmes, 1991), finding a strongly macrogyne-dominated population with a normal queen-size distribution is unexpected. Morph frequencies and social structure vary extensively among nests and among (sub)populations also in two other queen-size dimorphic ants, *Temnothorax rugatulus* (Rüppell *et al.*, 1998, 2001) and *Ectatomma ruidum* (Chantal Poteaux, pers. communication).

Queen number was associated with the occurrence of the size morphs in *M. ruginodis*, but not in all populations. The proportion of microgyne and the number of queens in the nest were positively correlated and the average queen size in nest decreased with increasing queen number, which is common in many polygynous ants (e.g. *Formica truncorum*, Sundström, 1995; *F. selysi*, Meunier & Chapuisat, 2009). Furthermore, macrogyne were associated with monogyny and microgyne with polygyny more often than randomly, but all associations were not significant. All these indicate that there is at least an indirect link in *M. ruginodis* between the level of polygyny and the frequency of microgyne. However, in one study population (Savero), the majority of macrogyne and microgyne were found in polygynous and monogynous nests, respectively (Table 2), which is reversed compared to the expected. This suggests that queen number as a plastic trait is also affected by other factors than morph frequencies in *M. ruginodis*. Definite conclusions based on Savero population are difficult to draw, however, because the number of microgyne found is small.

Queen dispersal and recruitment

Seasonal distribution of free-ranging wingless queens caught by pitfall-trapping was not random within the study populations, but the distributions of macrogynes and microgynes caught in the same populations were similar. Thus, our result does not support our prediction of different dispersal patterns in populations with different proportions of microgynes. In the macrogyne dominated Musturi population, we expected to find an excess of free-ranging wingless queens directly after the nuptial flight (period III), but we only found a deficiency of queens during period I (from the start of the season until nuptial flights; Fig. 3, Appendix S4). As microgyne queens are expected to move outside their nests more actively than macrogynes due to commonly occurring dependent colony founding in polygynous ants, we expected to find a more even distribution of free-ranging queens across the season in Multiharju with a relatively large proportion of microgynes. Instead, there was an excess of queens caught from Multiharju only during period I (Fig. 3, Appendix S4).

In accordance with previous results (Seppä, 1994), coexisting queens are related in polygynous nests on average, which indicates that nests must have recruited their own daughters as new queens. Previous dispersal experiments in the TV/Leimann population revealed that most *M. ruginodis* microgynes do not join mating swarms, i.e. they do not disperse by flight (Wolf & Seppä, 2016b). Instead, they probably mate in the vicinity of their natal nest and high relatedness among coexisting microgyne queens corroborates that many microgynes are recruited back to their natal nest. Our relatedness results show that the recruitment process in macrogynes is at least partly different, as coexisting macrogyne queens were not related at all in TV/Leimann 1994 data. However, relatedness among coexisting macrogynes in polygynous nests is difficult to predict since most macrogynes join a mating swarm-(Wolf & Seppä 2016b) and presumably later found new monogynous colonies independently.

If a macrogyne queen first joins a nuptial flight, she has three alternatives that would result in high queen relatedness in a polygynous nest: she can i) return to her natal nest after leaving the mating swarm; ii) found a new nest independently with related queens or iii) seek adoption in a random nest simultaneously with other related queens. All these alternatives are unlikely, however. *M. ruginodis* mating flights are rather extensive

and attract winged sexuals from a large number of nests from a relatively large area (Brian & Brian, 1949; Elmes, 1991; Wolf & Seppä, 2016b, unpublished observations). Orientation of flying *M. ruginodis* queens has not been studied, but returning to the natal nest from a distant nuptial flight site seems unlikely. Furthermore, pleometrosis, independent nest founding by a group of queens, has never been reported in *M. ruginodis* and related queens seeking entrance to the same non-natal nest must be rare. However, queen relatedness patterns in both microgyne and macrogyne nests suggests that not all queens follow the queen-dispersal dichotomy suggested – long-range dispersal of macrogyne by flight and local dispersal of microgyne by foot (Wolf & Seppä, 2016b), and that part can be large enough to change the relatedness regime in the nests. On one hand, positive relatedness in macrogyne nests must be caused by some macrogyne forgoing the nuptial flight and joining their natal nests afterwards. On the other hand, relatedness is rather variable across microgyne nests in TV/Leimann 2010s and Savero populations based on the large standard errors (Table 4), suggesting that nests recruit both their own daughters and unrelated queens.

Habitat association of free-ranging *M. ruginodis* queens

Our results on the distribution of free-ranging wingless queens showed that queens sampled were non-randomly distributed across different habitat types, but that there was no difference in how queen morphs were distributed. Thus, our results do not support the hypothesis that *M. ruginodis* microgyne specialise in stable habitats in successional boreal forests.

Apart from habitat stability, differences in microclimate, such as shadiness, moisture and presence of competitors may have a strong influence on the morph occurrence. Although the moor sites in the UK studied by Elmes (1978, 1991) ranged from dry to very wet, the common feature and major difference to the Finnish sites is presumably the lack of shadiness (absence of trees) in English populations, as most of the English sampling sites were covered by lower plants only (grass, herbs, dwarf shrubs etc). This lower vegetation will generally offer more sun-exposed habitats, especially when grazing animals maintain them relatively open (Elmes, 1978). *Myrmica ruginodis* is the least thermophile of all the *Myrmica* species (Radchenko & Elmes, 2010) and capable

of inhabiting also closed-canopy forests (e.g. Punttila *et al.*, 1991, 1994, 2016, Niemelä *et al.* 1996). Most of our study areas were mesic forests and thus generally rather shady. Thus, a combination of habitat stability and amicable microclimate (lack of closed tree canopy) could in general explain the higher occurrence of the microgyne morph in the UK (c. 70%) compared to the North-European boreal forests (c. 10-50%). In the former habitat type, selection for good dispersal ability is relaxed, which is expected to favour the less-dispersive microgyne morph. This could be tested by studying spatial population structure in the two areas.

Boreal mesic heath forests undergo a continuous successional change and cannot be described as stable, with perhaps the slowly changing old-growth stage as the only exception (Kuuluvainen, 2009; Kuuluvainen & Aakala, 2011). Thus, habitat stability as such cannot be used as a direct predictor of microgyne occurrence in boreal forests, as suggested by Brian & Brian (1949) for the moor habitats in the UK. An earlier genetic study on *M. ruginodis* in successional boreal forests also contrasts the habitat stability hypothesis (Seppä *et al.*, 1995): On one hand, worker nestmate relatedness, a proxy for the level of polygyny and thus for the frequency of microgyne morph, was lowest in the young forest patches following disturbance (clearcutting, sometimes combined with prescribed burning). On the other hand, the population sampled from an old-growth forest that could be considered the most stable habitat compared to the rest of the populations is practically monogynous, suggesting the absence of microgyne (Seppä *et al.*, 1995).

Conclusions

Our study, together with our previous results (Wolf & Seppä, 2016), partly confirms the association of queen-size morphs with key life-history traits in the ant *Myrmica ruginodis*, but the association is not straightforward and universal, with individual populations showing even reversed associations. Particularly the queen number is an unusually plastic trait in *Myrmica* ants (e.g. Elmes & Keller 1993), which evidently applies also separately to the size morphs of *M. ruginodis*. Indeed, the conventional dichotomy to monogynous and polygynous ants with associated distinct life-history traits seems as a simplification. In reality, many ants are facultatively polygynous and

life-history traits are neither simple binary traits nor associated with only a single social colony structure. Especially, a critical distinction in queen number concerning queen dispersal and subsequent nest-founding patterns does not seem to be the transition from monogyny to polygyny, but from facultatively to obligatory polygyny (Sundström *et al.*, 2005; Boomsma *et al.*, 2014).

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CONTRIBUTION OF AUTHORS

All authors contributed to the project design, data collection and analysis, and paper writing.

SUPPORTING INFORMATION

Appendix S1: Sampling Scheme

Appendix S2: Laboratory protocols for DNA extraction and DNA microsatellite analysis

Appendix S3: Queen size data, excavated nests

Appendix S4: Queen size data, pitfall data

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