

Genetic and behavioural divergence of queen size morphs in the red ant *Myrmica ruginodis*

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When you eliminate the impossible, whatever remains, however improbable, must be the truth.

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III. Wolf JI and Seppä P: Reproductive specialization in the size-dimorphic ant *Myrmica ruginodis*: Do microgynes act as inquilines? – manuscript.

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Abstract

Size is a feature that is often connected to different life history-traits and individuals can vary in many fitness aspects related to size, such as reproductive strategies, dispersal and mating behaviour, dominance and many more. If size variation is not continuous in a species, individuals can be divided into two or more distinct size morphs. In social insects, queen size dimorphism (QSD) is an interesting phenomenon as it separates queens into two different morphs, with macrogynes defined as the larger and microgynes as the smaller morph.

The aim of this thesis is to improve our understanding of this phenomenon in social insects with a special focus on a palaeartic ant species, *Myrmica ruginodis*. This thesis demonstrates how several taxa differ in their cause, prevalence and evolutionary processes involved in QSD. Four different hypotheses have been identified as reasons to QSD. First, intraspecific polymorphism in connection with alternative reproductive strategies. Second, inquilinism, where microgynes mainly produce sexual offspring and forego worker production. Third, speciation, where the two morphs represent separate species with intraspecific polymorphism as an ancestral state and speciation processes often combined with inquilinism. Fourth, selfish larval development, which has only been observed in stingless bees so far.

This thesis shows how the first three scenarios represent transitional stages and are valid in ants, which provide the necessary evolutionary framework via high levels of obligate secondary polygyny. This thesis also examines the cause for QSD in *Myrmica ruginodis* by analyzing several traits. These are morphology, offspring production, dispersal and mating behaviour, as well as gene flow.

Overall my results suggest that macrogynes and microgynes in the ant *M. ruginodis* represent an intraspecific polymorphism with alternative reproductive strategies without inquilinism involved in it. Incomplete mating isolation among morphs indicate that this species is at a very early transitional stage from intraspecific polymorphism along the divergence continuum.

Introduction

Queen size dimorphism in social insects

Size-dependent alternative strategies are a well-known phenomenon in the animal kingdom and can be connected to many behavioural and especially reproductive strategies (Eberhard 1980; Dingle et al. 1980b; Derr et al. 1981; Thornhill and Alcock 1983; Gross 1985; Crespi 1988; Danforth 1991; Gonzaga and Vasconcellos-Neto 2001). Nevertheless, size variation is not always continuous and in some species, individuals can be divided into two or more distinct size morphs. Size and size morphs are often connected to adult fitness with larger individuals being able to monopolise reproductive opportunities (Roff 1992; Stearns 1992) and to different reproductive strategies (Eberhard 1980; Thornhill and Alcock 1983; Gross 1985; Crespi 1988; Danforth 1991).

In social insects, the best-known example of body size variation is the difference between queens and workers, which is often a result of differential developmental pathways (Wheeler 1986; Wheeler 1991). Another, less obvious, phenomenon in social insects is the occurrence of queen size dimorphism (QSD), which leads to discrete queen phenotypes (Heinze and Tsuji 1995; Heinze and Keller 2000; Chapter I). In species with QSD, the smaller morph is defined as microgyne and the larger morph as macrogyne. Depending on the species, size distributions of the morphs can be either clearly separated or overlapping (Chapter I). Each of the two morphs are usually associated with differences in reproductive strategies and life-history traits such as dispersal, mating behaviour and social colony structure (Heinze and Tsuji 1995; Ruppell and Heinze 1999; Peeters and Ito 2001; Chapter I, II, IV).

Causes of QSD and occurrence in social insects

Four different hypotheses for the emergence of QSD have been proposed (Chapter I).

Hypothesis 1: Size morphs might be a polymorphism representing two different reproductive strategies (Ruppell and Heinze 1999), including different mating behaviour, mode of colony foundation, dispersal and other behavioural traits. In ants, for example, different reproductive strategies are widespread and could be due to phenotypic plasticity (i.e. differences are induced by the environment), be genetically maintained, or be an interaction of environmental and genetic factors. Queen morphs in connection with alternative reproductive strategies might be a route to social parasitism (H2) or a speciation process (H3) (often via H2).

Hypothesis 2: QSD might reveal a parasitic relationship, where microgynes take advantage of macrogynes by specializing in producing (mainly) sexual offspring instead of workers. Thus, coexistence of the size morphs may indicate that a form of social parasitism – here inquilinism – is present or evolving. Consequently, this

initially intraspecific parasitic relationship might provide enough barriers to gene flow between morphs to evolve into interspecific parasitism (Buschinger 1990; Bourke and Franks 1991; Leppänen et al. 2015).

Hypothesis 3: A third possible cause for the occurrence of two different queen morphs is the beginning of a speciation process. Here the morphs represent two ecotypes, where populations may have reduced gene flow due to divergent selection in different environments. Not much research has been conducted on speciation via ecotypes so far, and the best-described example comes from cichlids (Seehausen and Magalhaes 2010; Nosil 2012). In ants, two ecologically distinct forms of *Camponotus nawai* (Satoh 1989) seem to qualify best for this scenario and the species has been later split in two separate species, namely *C. nawai* and *C. yamaokai* (Terayama and Satoh 1990).

Hypothesis 4: A fourth possible cause for QSD is that larvae which were originally destined to become workers, selfishly develop into queens (Bourke and Ratnieks 1999; Beekman et al. 2003) but due to genetic, nutritional or social impairment, they develop into smaller queens. This phenomenon is only known in stingless bees so far (Imperatriz-Fonseca and Zucchi 1995; Wenseleers et al. 2005; Ribeiro et al. 2006).

QSD has been found in three groups of social insect: ants, stingless bees and one termite species (Chapter I). In contrast, no QSD seems to occur in honeybees, bumble bees or eusocial wasps. In ants, QSD can be caused by either alternative reproductive strategies, as a route to social parasitism or as a route to ecological speciation (H1–3), while the suggested cause of QSD in stingless bees is primarily selfish behaviour of female larvae (H4, see Chapter I). Alternative reproductive strategies have also been suggested as an explanation in the only termite with QSD, *Nasutitermes princeps* (Roisin and Pasteels 1985).

How to distinguish the different hypotheses connected to QSD?

So far, researchers have tried to identify the cause for queen dimorphism in many species, with varying results (Chapter I). Depending on the species, a single trait is often not sufficient to distinguish between the four hypotheses. Thus, ideally, several traits should be examined in combination to reach a conclusive result about the causes of QSD. Traits that will help to identify the cause of queen size dimorphism are:

- Morphology
- Offspring production by the microgynes
- Gene flow and reproductive isolation between the morphs
- Heritability of size, i.e. if the morphs breed true
- Cuticular profile of the queen morphs

QSD in *Myrmica ruginodis*

Myrmica ruginodis Nylander is a common palaeartic ant (Collingwood 1979; Radchenko and Elmes 2010; Czechowski et al. 2012), with two queen morphs that can occur in sympatry. The smaller morph is defined as microgyna and the larger morph as macrogyna (Brian and Brian 1949). The size distributions of the queens are overlapping, but a cut-off value based on head width has been established (Elmes 1991) that enables the assignment of queens to either morph with a high certainty. This division was later confirmed by a behavioural cut-off value based on dispersal behaviour (Chapter IV). The two queen morphs are connected to different life history traits, such as social colony structure, dispersal, and mating behaviour (Elmes 1978b, 1991; Seppä 1994; Chapter II; Chapter IV). While macrogynes are assumed to participate in the nuptial flight and disperse further away to found their own colony independently, microgynes supposedly forego the nuptial flight, mate close to their nest of origin and found colonies dependently (Brian and Brian 1949, 1955; Elmes 1991). This means they will be readopted to an existing colony (secondary polygyny) and may later found a colony by budding, i.e. splitting of the nest. Different hypotheses (see above) for the evolution of the two size forms have been proposed (Brian and Brian 1949, 1955; Pearson 1981; Elmes 1991; Chapter I). In my PhD project, I have studied the different causes and consequences of queen size dimorphism in the ant *Myrmica ruginodis*. For this, I have analysed traits that could separate the queen morphs, such as size, social colony structure, reproductive specialization of queens, gene flow, mating behaviour and dispersal. The approach is to compare the traits in the two morphs when in sympatry, so that the role of environmental, genetic and behavioural traits, influencing the different prevalence of the queen morphs, can be assessed.

Aims of this thesis

The overall aim of my thesis is to provide new insights into the occurrence, mechanisms and evolution of queen size dimorphism with special focus on a case study in the ant – *Myrmica ruginodis*.

I begin by reviewing the phenomenon of queen size dimorphism in eusocial insects in general and discuss four different hypotheses that explain the occurrence of the two queen morphs. I also identify precursors and occurrences in the different taxa and explain the evolutionary processes involved (Chapter I).

I aim to examine the phenomenon by analysing queen size dimorphism in the red ant *Myrmica ruginodis*. For this, I combine data from several populations and compare the suggested differences in life-history traits between the morphs. These

factors are size distribution, social colony structure, queen relatedness and recruitment, as well as association to habitat and timing of dispersal of the morphs (Chapter II).

I continue my study by investigating the tentative behavioural variation of the two queen morphs (Chapter III and IV), which can additionally help identifying the cause for the existence of the two queen morphs. First, I explore if the smaller morph acts as an inquiline by specialising in sexual offspring production. For this, I analyse reproductive skew in polygynous colonies to determine if microgynes mainly produce sexual offspring and only few (or no) workers (Chapter III). This kind of social parasitism has been found especially often in the genus *Myrmica* but has so far never been tested in *Myrmica ruginodis*. In the following chapter (IV), I examine differences in dispersal and mating behaviour of the two morphs. With a newly developed technique, I am able to distinguish individuals dispersing by flight or on the ground, and compare dispersal propensity as well as morph ratios at the sexual emitting nests and at the mating place. This allows me to investigate the potential for spatial or temporal isolation between the two morphs. Furthermore, I analyse if the morphs mate size-assortatively, which can initiate genetic differentiation between macrogynes and microgynes.

I conclude by testing if and how much genetic differentiation occurs between the two size morphs (Chapter V) in order to identify if the two morphs represent intraspecific polymorphism connected to different reproductive strategies or if they represent an incipient stage of divergence.

Material and Methods

In this thesis, I used a multimodal approach and combined morphological, behavioural and genetic data to investigate my research questions from as many different perspectives as possible. The following chapter provides an overview of the techniques used and more detailed information can be found in the corresponding chapters.

Data collection

The different types of analyses used in the four different data-based chapters (II-V) are summarised in table I. Note that Chapter I is a review and is thus not included in the table.

Table 1: Summary of the data, samples and analyses used for the four data-based chapters

Chapter	Data			Data analysis		
	Source	Samples	Population and year of collection	Morphology	Behaviour	Genetics
II.	Excavated nests	Old queens	Savero 1992*; TV/Leimann 1994*; Antby 2004; TV/Leimann 2010s; S-England ¹ ; Scotland ²	Size, Dissections	Queen recruitment	Relatedness
	Pitfall traps	Wingless free-ranging queens	Musturi 1985; Multiharju 1986	Size	Dispersal, Habitat	
III.	Excavated nests	Old queens and adult offspring	TV/Leimann 2012	Size, Dissections		Offspring production
	Mating place	Alate gynes and males	TV/Leimann 2007-2013 (6 years)	Size	Dispersal, Mating	
IV.	Colonies	Alate gynes and males	TV/Leimann 2013	Size, Dissections	Dispersal	
	Excavated nests	Old queens	Savero 1992*; TV/Leimann 1994*; Antby 2004; Harpaskog 2012; TV/Leimann 2010s; Tåktom 2012;	Size		Differentiation

¹data derived from Elmes 1991

²data derived from Brian and Brian 1949

*analyses performed with allozyme marker. All other genetic analyses were performed with DNA microsatellite marker

Morphology

To assess size, I first photographed the sampled individuals with a Canon EOS 50D camera at 25x magnification under a dissection microscope. Then I measured the head width of old queens, gynes and males as well as the thorax width, length and height of the old queens with Image J to 0.02 mm accuracy (Chapter II, III, V). High repeatability of this method was shown by Wolf and Seppä (Chapter IV). For queens, I also calculated the thorax volume as thorax width x length x height, which was shown to be directly correlated with head width. Thus, it was sufficient to use only head width as a measure of size. Head width and wing area of dispersing females were measured with the same procedure (Chapter IV). From the earlier collections (until 2004), head width of the queens was measured directly under a light microscope to 0.02 mm accuracy, a commonly used measure to describe size in ants.

Sampled individuals were assigned to either morph depending on the cut-off value established by Elmes (1991), which was in good accordance with our behavioural cut-off value (Chapter IV, see also subchapter “Behaviour”). Size distributions were tested for several populations in SW-Finland (Chapter II). Furthermore, I analysed social colony structure and its association with queen morphs and compared the Finnish data with data from the UK to investigate flexibility of those traits (Chapter II). Queens from the 2010’s TV/Leimann collection were dissected to investigate if they had mated and laid eggs, i.e. if they had a filled spermatheca (Chapter II, III). Dispersing gynes were dissected to exclude the possibility they had mated inside their nest of origin (Chapter IV).

Behaviour

To assess the ability and propensity of individuals to participate in the nuptial flight, I trapped dispersing alates directly at their natal colonies (Fig. 1). This enabled me to collect and distinguish individuals that disperse by flight or on the ground and relate their dispersal behaviour to their size by scoring the alates according to their mode of dispersal (Chapter IV).

The size, where the majority of gynes chose to disperse by flight rather than on the ground was taken as a behavioural cut-off value for the morphs. Afterwards, I compared the expected and observed propensities of the morphs at the emitting nests and the nuptial flight. I collected mating couples and unmated males during the nuptial flight and recorded time of collection and mating duration. This enabled me to test if size assortative mating occurs and if larger males have an advantage in securing a mating partner in comparison to smaller males. Finally, I investigated if temporal isolation occurs between the two morphs, i.e. if morphs participate at the nuptial flight during different days (Chapter IV). Dispersal behaviour was also analysed by collecting free-ranging wingless queens from pitfall traps and relating their size to season of dispersal and type of habitat (Chapter II).



Fig. 1 Construction (tent) for the dispersal experiment, with tulle supported by sticks, deployed above the nest and sealed at the bottom with sand. Female and male ants were collected from the ground and top of the construction, which could be opened and sealed conveniently, and were subsequently measured for head width. Height of each tent was approximately 115 cm and width about 70 cm.

Genetic analyses

I tested 21 microsatellite primers, which had previously been developed for other *Myrmica* species (Evans 1993; Herbers and Mouser 1998; Henrich et al. 2003; Azuma et al. 2005; Zeisset et al. 2005). Of those, twelve were polymorphic in *Myrmica ruginodis* and were used for genotyping queens, alates and workers. I performed a maternity assignment with the programme COLONY 2.0.3.1 (Wang 2004). With this information I was able to determine if size of mother and male or female offspring are connected to each other (thesis summary, Results II) and to test the reproductive specialisation of queens in relation to their size (Chapter III).

I also calculated mean nestmate relatedness for the queens in the TV/Leimann 2010s population with the same 12 polymorphic markers, as well as for the queens from previous collections with allozyme data (Tvärminne 1994 and Savero 1992). The analysis was performed with the software RELATEDNESS v. 5.0.4. (Queller and Goodnight 1989). This was done to investigate if predominantly macrogyne and microgyne colonies differ in their queen recruitment pattern and thus colony founding strategies (Chapter II).

Moreover, I used the genetic data to test queens of several populations in SW-Finland (see table I) for genetic differentiation of the two queen morphs with the programme ARLEQUIN 3.5.2.2 (Excoffier and Lischer 2010). For this, I used two alternative hierarchical approaches, with either queen morph or population as the higher level. To rule out tentative nest effects, I performed the same analysis with nests grouped within either populations or morphs.

Additionally, I calculated pairwise F_{ST} estimates among all populations and morphs with ARLEQUIN. The above-described analyses were performed with DNA microsatellite marker as well as allozyme data. In addition, the following tests were performed with microsatellite data alone: isolation by distance with a Mantel test (IBD) in GENEPOP ON THE WEB (Raymond and Rousset 1995; Rousset 2008) and

examination of the number of genetic clusters in the data by using model-based Bayesian clustering analyses with the software BAPS 6.0 (Corander and Marttinen 2006; Corander et al. 2008).

Results and Discussion

I. The dividing phenomenon: Queen size dimorphism in social insects

In Chapter I, I aimed to provide new insights into the phenomenon of queen size dimorphism in social insects in general. In particular, I wanted to examine different causes for queen size dimorphism and fill existing gaps in our knowledge of its occurrence. My extensive search of literature revealed that taxa do not only differ in their prevalence, but also in their cause for occurrence of QSD. Alternative reproductive strategies and social parasitism, in the form of inquilinism, were mostly described for ants. By identifying that the occurrence of microgynes is not a stable state in ants but rather a transitional process (Fig. 2), with intraspecific polymorphism as a starting point, I was also able to recognize ecological speciation as part of this process.

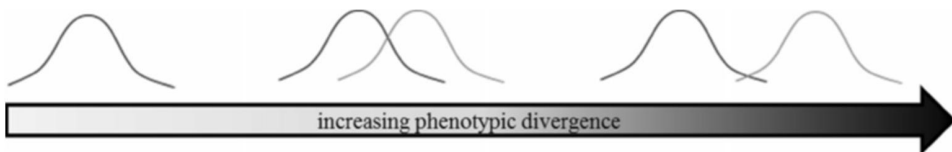


Fig. 2 Phenotypic distributions at different stages of the divergence continuum.

Accordingly, divergence of size distributions can vary, depending on the species and stage in this continuum, so that an ancestral species with a single queen morph can (but does not necessarily) lead to two separated species with two size distributions, i.e. microgynes and the macrogynes.

At the same time, I revealed in my review article (Chapter I) that QSD occurs just rarely in termites and never in honeybees, bumble bees or highly social wasps. Also the cause for queen size dimorphism in stingless bees is very different than that in ants, namely by selfish larvae development (Imperatriz-Fonseca and Zucchi 1995; Wenseleers et al. 2005; Ribeiro et al. 2006), which is not connected to the continuum model outlined above. By highlighting these differences in prevalence and cause of QSD, I was able to identify that secondary polygyny – at a high or obligate level – plays the key role in providing the necessary evolutionary time-frame for the three

speciation related hypotheses. To be more detailed, the long co-existence of queens permits enough time for the subsequent changes in morphology, manifestation of reproductive strategies in connection to the morphs and restrictions of gene flow to evolve.

Since secondary polygyny is mostly absent or rare at best in social bees, vespine wasps and higher termites (Hölldobler and Wilson 1977; Boomsma and Nash 2014; Boomsma et al. 2014), it explains why QSD is neither expected nor found in these groups. It also illustrated why the cause of QSD in stingless bees is consistently different than in ants.

Furthermore, my review article (Chapter I) provided new ideas and tools how to distinguish between the different causes of QSD. Especially in ants, where morphs might be in a transitional phase, it is essential to investigate more than one trait and take a multimodal approach. Especially offspring production, gene flow and cuticular hydrocarbons in addition to morphology will prove useful in identifying the cause for QSD in any of the social insect taxa. By compiling information from many studies - not only of ants but of all social insects - I was able to provide a general resource for researchers, interested in this complex phenomenon in ant, bees, termites as well as researches working with size dimorphism in other species. Studying this interesting subject will stimulate our understanding of evolutionary steps in social parasitism and speciation processes in general.

II. Queen morphs and their differences in life-history traits in *Myrmica ruginodis*

In contrast to previous studies (Brian and Brian 1949; Elmes 1991), size distributions of *M. ruginodis* queens proved to be much more flexible than previously assumed. Size distributions in the study populations ranged from normal to bimodal distributions, including anything in between (Chapter II). Furthermore all analysed queens had mated, i.e. a filled spermathecal (Chapter II). Life-history traits like social colony structure and queen recruitment processes were also more flexible than expected. I showed that the classic association of macrogynes with large size, low queen number in nests and independent founding strategies holds as an overall concept, but there was large variation among individual nests or even individual populations. The same results were found for microgynes in association with their suggested life-history traits (small size, high queen number in nests and dependent colony founding).

Previously suggested habitat characteristics in relation to polygyny and microgynes, i.e. habitat stability (Brian and Brian 1949; Hölldobler and Wilson 1977; Rosengren and Pamilo 1983) could not be verified, however. Also, no seasonal pattern of dispersal could be clearly attributed to a specific morph. Nevertheless, the peaks of

movement were either in late summer, which coincides with the end of the nuptial flight and thus independent colony founding processes, or in late spring, which is in accordance with dependent colony founding such as budding or the search adoptive nests.

Often, body size can be either genetically maintained (Smith 1993; Ruppell et al. 2001a; Fjerdingstad 2005; Meunier and Chapuisat 2009) or determined by environmental factors (Owen and McCorquodale 1994; Speight et al. 1999) or both (Bargum et al. 2004; Howard and Jeanne 2013). If size is heritable, it can offer valuable insight into the reason for QSD in a species (Chapter I). Accordingly, I analysed how maternal size affects offspring size in *M. ruginodis* and performed a regression analysis for males and gynes from several nests.

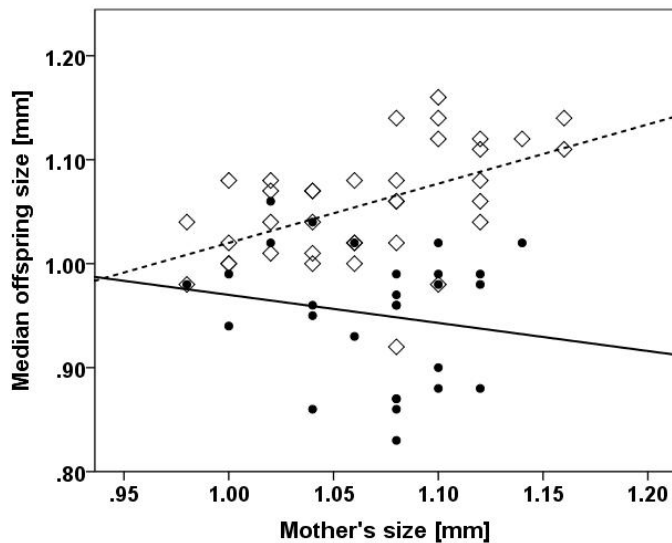


Fig. 3 Linear regression between mother's size and the median size of her offspring. Gynes shown as open diamonds with dashed line and males as closed circles with solid line.

I showed that offspring size was indeed strongly dependent on their mother's size in gynes (Fig. 3), but not in males (linear regression: gynes: $P=0.001$; males: $P=0.37$). This analysis does not reveal if this connection is due to genetic or environmental (i.e. nest) effects or a combination of both. However, since male and female offspring should be both affected by environmental factors in their colony, I hypothesise that a genetic component is responsible for the strong connection between mother's size and size of their female offspring.

III. Inquilinism in *Myrmica ruginodis*?

In several ant species, microgynes have been verified to act as inquilines (Savolainen and Vepsäläinen 2003; Hora et al. 2005; Feitosa et al. 2008; Buschinger 2009) and as I have illustrated in the review article (Chapter I), social parasitism can enhance the transition process from an intraspecific polymorphism towards two separate species. Here, I investigated if microgynes in the species *M. ruginodis* behave as inquilines (Chapter III) and demonstrated that microgynes do neither specialise in sexual offspring production, nor refrain from worker production.

Instead, I showed that larger queen size is positively connected to a higher gyne production, but has no influence on male or worker production (Fig. 4). The higher gyne production is probably due to a higher fecundity of larger individuals and indeed it has been shown in other species, e.g. *Ectatomma ruidum* and *Manica rubida*, that macrogynes are more fecund than microgynes (Lachaud et al. 1999; Lenoir et al. 2010). All analysed queens had a filled spermatheca.

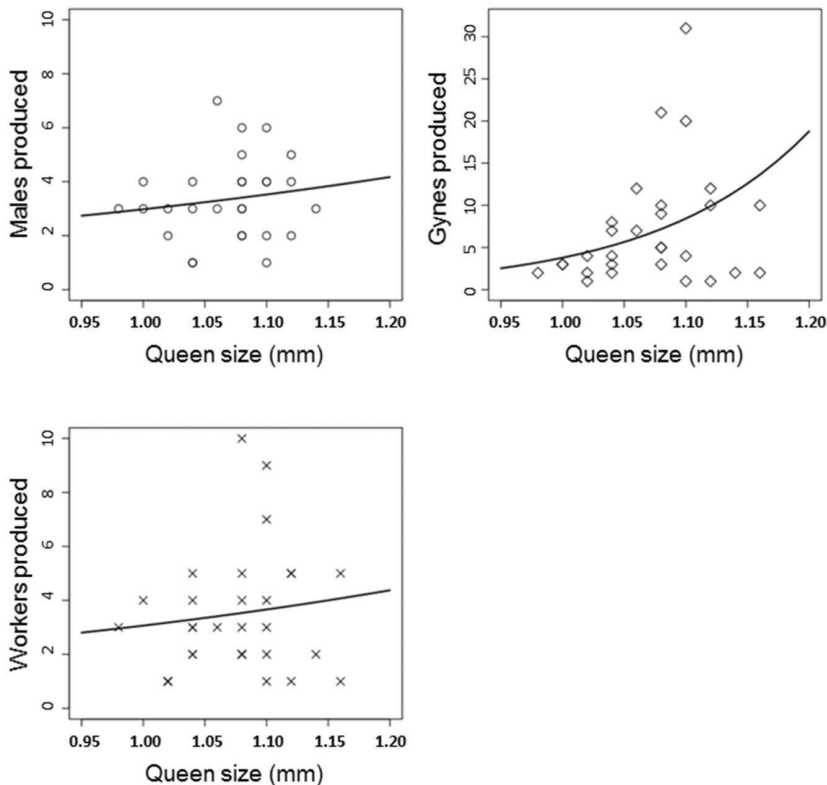


Fig 4 Number of offspring produced as a function of queen size in twelve colonies. Males shown as circles (upper left panel), gynes as diamonds (upper right panel) and workers as crosses (lower panel).

IV. Size in connection to dispersal and mating strategies

Size is a trait that is in several species connected to alternative reproductive strategies (Eberhard 1980; Dingle et al. 1980b; Thornhill and Alcock 1983; Gross 1985; Crespi 1988; Danforth 1991; Gonzaga and Vasconcellos-Neto 2001). In ants, queens can display intraspecific size polymorphism, i.e. size morphs, with alternative reproductive strategies (Heinze and Hölldobler 1993; McInnes and Tschinkel 1995; Hamaguchi and Kinomura 1996; Rüppell et al. 2001a; Schlick-Steiner et al. 2005; Lenoir et al. 2010; Lenoir et al. 2011). Consequently, I analysed reproductive strategies in the ant *M. ruginodis* by investigating dispersal and mating behaviour of sexuals during the nuptial flight (Chapter IV). For this purpose, I developed a new technique to trap dispersing individuals and demonstrated that size was indeed connected to the mode of dispersal in gynes, but not in males.

I showed that mostly larger gynes (macrogyenes) disperse by flight while smaller gynes (microgyenes) disperse on the ground. The difference in dispersal behaviour indicates a reduced flight ability in microgyenes, which I confirmed by the significantly reduced ratio of wing area to head width that is in accordance with other results in ants (Rüppell et al. 1998; Kikuchi 2002). Furthermore, the difference found in dispersal behaviour in females was in good accordance with a previously established size cut-off value (Elmes 1991) and could be taken as a behavioural cut-off value between size morphs. Dissections confirmed that none of the dispersing gynes had mated inside their nest of origin.

Depending on the morph proportions emitted from the colonies, I was also able to compare observed and expected ratios of the morphs at the nuptial flight. I showed that predominantly macrogyenes participated in the nuptial flight, although not exclusively. Males of all sizes participated in the nuptial flight and larger males were preferred as a mating partner by all females, which is in agreement with results from other ant species (Davidson 1982; Wiernasz et al. 1995; Abell et al. 1999). Nevertheless, I found that size assortative mating occurs in *M. ruginodis*, which seems to be caused indirectly by mostly macrogyenes participating in the nuptial flight and preferring larger males as a mating partner (Fig. 5). Furthermore, I found no sign of temporal isolation between size morphs.

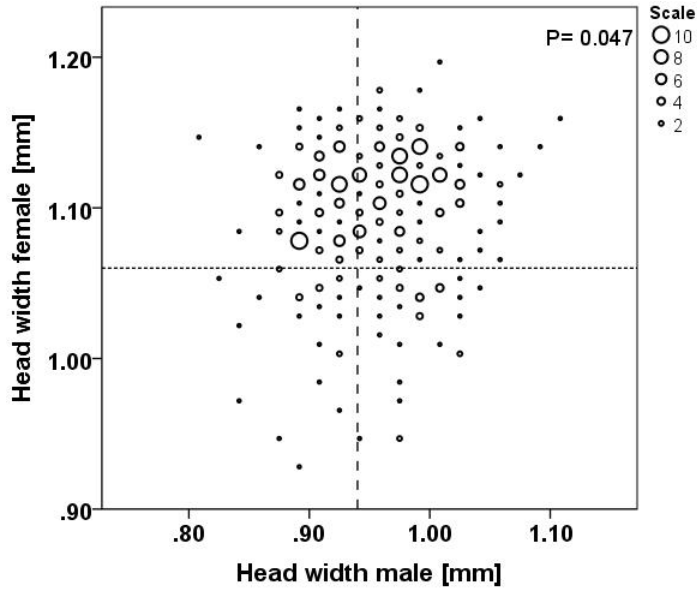


Fig. 5 Head width of females and their mating partners collected from the nuptial flights ($n = 315$). The dotted line divides females into macrogynes and microgynes (1.065 mm) and the dashed line divides the males into two categories according to Elmes (1991; 0.94 mm). Note that the size of the symbols reflects the number of identical data points on a continuous scale from 1 to 10.

V. Genetic differentiation of the morphs

In the previous chapters, I described different causes for QSD and demonstrated that, especially in ants, it is not a stable state, but a transitional process with intraspecific polymorphism as a starting point (Chapter I). I showed that reproductive strategies of the two morphs differ and create incomplete mating isolation between them, even when occurring in sympatry (Chapter IV). Subsequently, I tested for potential genetic differentiation between the morphs in Chapter V.

I used several populations and two sets of genetic markers (DNA microsatellite and allozymes) in two alternative hierarchies to verify if populations or morphs explain the amount of genetic variation between macrogynes and microgynes best. I showed that populations are the most appropriate explanation for genetic differences. Differentiation among morphs was only significant when nested within populations and only in the DNA microsatellite data. Furthermore, I performed a pairwise comparison of F_{ST} values, which revealed that allopatric microgynes have the highest values, which is in accordance with their lower dispersal behaviour (Chapter II and IV).

I used several populations and two sets of genetic markers (DNA microsatellites and allozymes) and tested two alternative hierarchies to explore if populations or morphs explain best the distribution of genetic variation in the study system. I showed that a hierarchy where morphs are nested within populations is the most appropriate explanation for the distribution of genetic variation, although differentiation among morphs was also significant in the DNA microsatellite data in this hierarchy. Controlling for nest effects did not change our results. Furthermore, comparison of pairwise F_{ST} values revealed that allopatric microgynes have the highest values.

Additionally, genetic and geographic distances (IBD) did not correlate with each other. Probably dispersing males and macrogynes (Chapter IV) provide enough gene flow between populations, which has also been speculated to occur in another size dimorphic species (Rüppel et al. 2001b).

Finally, Bayesian clustering analysis (BAPS) did not cluster the queens into either morphs or populations. This is probably due to the quite low F_{ST} values, i.e. low genetic differentiation which precluded separation of the groups. Overall, populations are clearly more differentiated from each other than sympatric occurring morphs. However, differentiation among morphs when nested within populations may facilitate speculations about the potential for sympatric speciation in this species. Nevertheless, this result is not significant in the allozyme markers and the very low pairwise F_{ST} values also show that a differentiation of morphs is at best very small.

Conclusions

This thesis explores the phenomenon of queen size dimorphism in social insects in general, and also investigates QSD in a palaeartic ant species – *Myrmica ruginodis*. The addition of case studies allows a comprehensive analysis of many factors involved in the existence of queen size dimorphism, which will be a valuable source in other studies of size dimorphism in social insects or even unrelated dimorphic taxa.

With this thesis, I set out to deepen our knowledge of the causes, occurrence and prevalence of QSD as well as to identify evolutionary processes involved in it. This thesis depicts how QSD occurs most often in ants and stingless bees, with very different mechanisms behind it. Stingless bees develop into miniature queens due to selfish larvae development, while QSD in ants is connected to speciation related

hypotheses. Here, intraspecific polymorphism, connected to alternative reproductive strategies is the starting point and morphs might evolve from an ancestral unimodal species into two separate species – often via inquilinism. The evolutionary process is facilitated by high levels of (predominantly) obligate polygyny, which only occurs in ants, and provides the necessary prerequisites to develop from intraspecific polymorphism into further transitional stages.

In the focus studies of *M. ruginodis*, morphs could be associated with the classic life-history traits suggested for them, i.e. social colony structure and queen recruitment, although a large amount of variation between individual nests or populations occurred. The suggested parasitic relationship between macrogynes and microgynes, namely inquilinism, was not valid in this species. Both morphs contributed to sexual and worker production and larger queens produced generally more female offspring, probably due to a higher fecundity.

Furthermore, gynes were different in their reproductive strategies, with macrogynes dispersing mostly by flight and microgynes on the ground. This caused a spatial separation between the morphs, and caused size assortative mating with incomplete mating isolation between the two morphs. Investigation of genetic differentiation showed that size morphs are less differentiated from each other than populations.

In conclusion, by using a multimodal approach, I showed that macrogynes and microgynes in the ant *M. ruginodis* represent an intraspecific polymorphism with alternative reproductive strategies, but without inquilinism involved in it. Incomplete spatial isolation between the two morphs seems to create, at best a very early transitional stage from intraspecific polymorphism along the divergence continuum. It is unclear if selection pressure will drive the morphs further towards an incipient stage of divergence or if gene flow, provided predominantly by males, will counterbalance this effect. Generally, studying queen size dimorphism stimulates our understanding of evolutionary steps towards social parasitism and speciation processes and studying size dimorphic ants provides an interesting approach to this concept.

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