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Do ants (Hymenoptera: Formicidae) need conservation and does ant conservation need genetics?

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Abstract

Ants are in the centre of terrestrial environment, and losing ant biodiversity would be a global ecological experiment without match. As genetic diversity is an integral part of biodiversity and extinction is likely to be influenced also by genetic factors, all genetic information that can be used to illuminate their population biology is directly relevant for their conservation. My aim here is to review what kind of genetic information is available for evaluating the conservation status of ants and to consider what kind of information is still lacking. According to the data on endangered species, ants are just as vulnerable as any other group of organism. Some of the idiosyncrasies of social hymenopterans are likely to expose ants to genetic and demographic threats, for instance the effective population sizes (N_e) in ants are a tiny fraction of the total numbers of ants we see. The genetic effects of polygyny and polyandry are also smaller than they seem to be at first sight, suggesting that the number of nests is often the most important determinant of N_e. Thus, it seems that many ant populations have problems with respect to the "50 / 500" rule for N_e to escape extinction. Rather than the number of breeding queens, connectivity of populations seems to be decisive for the amount of genetic variation in ant populations, with small and isolated populations being depauperate of genetic diversity. Female philopatry characterizes spatial population structure in many ants, causing increasing problems for colonizing new habitats and localities if habitats remain fragmented. Finally, the decreasing allelic diversity in the complementary sex determination locus is a direct threat to shrinking ant populations, because it can induce a strong inbreeding depression in the form of diploid male production.

Key words: Ants, biodiversity, conservation genetics, effective population size, genetic diversity, inbreeding, review, spatial population structure.

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Introduction

Conservation genetics is a science that applies genetic methods with the aim to prevent or minimize the risk of species and populations to go extinct, and to retain their evolutionary potential to cope with potential and often unforeseen environmental changes (e.g., FRANKHAM & al. 2002, ALLENDORF & LUIKART 2007). This is a demanding request in a world that seems to be changing in front of our eyes, where potential has suddenly become probable and unforeseen can be envisioned far too well already today. Extinction is a demographic process that is likely to be influenced by genetic factors under some circumstances (ALLENDORF & LUIKART 2007), and an important goal in conservation genetics is to try to identify which circumstances those are. I will review what kind of genetic information relevant for evaluating the conservation status of ants is available today, and consider what is still lacking.

This review concentrates on ants. Part of the questions touched upon are general conservation issues relevant to any organisms, but many are also idiosyncratic to social insects. Social hymenopterans (ants, bees and wasps) share two features that make their population biology stand out from most other animal groups. First, they are social which in most cases means sedentary life in a nest, and second, they are male haploid. Ants differ from bees and wasps in that their colonies are always perennial and iteroparous (HÖLLDOBLER & WILSON 1990), whereas in many bees (MICHENER 1974) and wasps (ROSS & MATTHEWS 1991) colonies are annual and semelparous. This can have important bearing on the population dynamics in these groups, as perennial species may react more slowly to changing environment compared to annual ones. On the other hand, long-lived colonies can also act as a buffer against environmental perturbations, as large and healthy ant colonies are not doomed if they fail to reproduce in any given year.

Genetic topics relevant to ant conservation include (I) description of the so far unknown biodiversity, including resolving various taxonomic uncertainties; (II) assessing effective population size, including important aspects of ant social biology such as colony queen numbers and queen mating frequencies; (III) assessing the amount of genetic variation in populations, both in neutral marker loci and non-neutral genes; (IV) assessing spatial population structure and the extent of dispersal and gene flow and (V) detecting inbreeding and the effects of inbreeding depression. Allozymes and DNA microsatellites are the most commonly used nuclear marker systems in such studies, while maternally inherited mitochondrial variation is studied either by direct sequencing or by fragment analysis, such as restriction fragment length polymorphisms (RFLP) or single strand conformation polymorphism (SSCP). So far, only a handful of papers have specifically focused on genetic issues of ant conservation but there is a wealth of published literature that can be useful from the conservation genetics angle as well.

Do ants need conservation?

Ants are many, they are important, but they are not particularly species rich. Edward O. Wilson, the foremost proponent of ant biodiversity has in many occasions strongly stressed the dominance of ants in terrestrial ecosystems (e.g., WILSON 1990, 1992). Based on studies made in the Amazonian rainforests (e.g., BECK 1971, FITTKAU & KLINGE 1973) and on his remarkable personal experience, Wilson has suggested that ants and termites are responsible for at least half of the insect biomass and close to one third of the global animal biomass (WILSON 1990). Many ants are important ecological engineers by moving soil and vegetation. They are principal herbivores, granivors, scavengers, seed dispersers, and invertebrate predators in many areas, and they have a great impact on the invertebrate community by managing their foraging area and controlling the numbers and abundances of other species. Ants also engage in unique and fascinating symbiotic and parasitic relationships with a large number of other species ranging from bacteria and fungi to other social insects (HÖLLDOBLER & WILSON 1990).

The ecological dominance of ants does not correspond with particularly high species richness, though. The number of described ant species approaches 12,400 (May 2008, www.antbase.org), but has been estimated to be only about 2 % of the global insect fauna (WILSON 1990). Based on the rate of discovery of new species, HÖLLDOBLER & WILSON (1990) suggested that the total number of extant ant species is in the order of 20,000. This means that more than one third of ant biodiversity still awaits discovery and many of them may in fact go extinct without us ever learning that they existed.

Molecular phylogeny and taxonomy have proved useful for resolving evolutionary history of the species as well as identifying new ones for some time now (e.g., HILLIS & al. 1996), including ants (SMITH & al. 2005, BRADY & al. 2006, OULLETTE & al. 2006). Recently, sequencing a standardized mitochondrial sequence, called DNA barcoding, has been suggested as a solution for resolving many taxonomic problems (e.g., HEBERT & GREGORY 2005), but this approach has been criticised strongly for its rigidity for accommodating varying degrees of intraspecific variability in DNA sequences (e.g., MEIER & al. 2006, ELIAS & al. 2007). However, SMITH & al. (2005) applied DNA barcoding to the Madagascar ant fauna and showed that it can be accurate and efficient in assessing biodiversity in ants, too. They even suggested that it could replace time-consuming morphological analysis (SMITH & al. 2005), but it can hardly replace the morphology-based approach in alpha-taxonomy, because in most instances taxonomically correct names can be allocated to the taxa defined by molecular genetic methods only through morphological analysis of type specimens (SCHLICK-STEINER & al. 2007).

The correct identification of species is not always straightforward in ants, even with an abundance of variable genetic markers. For instance, the five mound-building wood ant species in the so-called *Formica rufa* group (*F. rufa*, *F. polyctena*, *F. aquilonia*, *F. lugubris* and *F. paralugubris*) have been notoriously difficult to identify (e.g., VEPSÄLÄINEN & PISARSKI 1981) with overlapping morphological characters (COLLINGWOOD 1979, SEIFERT 1996, CZECHOWSKI & al. 2002). Small genetic divergence as measured using three classes of genetic markers – mtDNA (GOROPASHNAYA & al. 2004), DNA microsatellites (GYLLENSTRAND & al. 2004) and allozymes (P. Pamilo, pers. comm.) – can be the reason for the incomplete phenotypic distinction between the species. Taxonomic confusion in the *F. rufa* group is further escalated by species being able to hybridize (SEIFERT 1991, GOROPASHNAYA & al. 2004), and CZECHOWSKI & RADCHENKO (2006) even suggested that they can form mixed colonies, with queens from more than one species breeding in the same colony.

If ants are so ubiquitous, do they need conservation in the first place? Globally, IUCN red lists 148 ant species (March 2008, IUCN 2008), which is ~ 1.2 % of the described ant species and as much as ~ 16 % of all insect species listed. Three of the species are known from just one or a few locations and are therefore classified as critically endangered. The bulk of the species have a restricted distribution or a restricted number of known locations and are classified as vulnerable. All these are social parasites, including inquilines, slave makers, guest ants and temporary parasites. The rest are six mound-building *Formica* species, which are considered near threatened (IUCN 2008). Locally, the proportion of endangered species can be much higher, though. In Finland, for instance, an area where the local ant fauna is relatively well-known, 7 - 8 % of the species are considered worth attention (RASSI & al. 2001). However, it is quite likely that the number of endangered species is much larger than what we can read from various lists today. A large proportion of species still remain to be discovered, and our knowledge of the distribution and abundance of both known and unknown species is often rather poor (CHAPMAN & BOURKE 2001).

The bad ant and the good ant. An issue directly linked to the species diversity and survival is the spread of the invasive species, as species that are successful in colonizing new areas are sometimes very good competitors and a threat to the native ant fauna. The best studied invasive species are the fire ant *Solenopsis invicta*, and the Argentine ant, *Linepithema humile*, both natives of South America, but many other ant species also thrive in new environments (MCGLYNN 1999). Today, *S. invicta* has a wide distribution throughout southern USA (USDA 2007), but it has been recently found also in other parts of the world, e.g., Australia (MCCUBBIN & WEINER 2002, HENSHAW & al. 2005) and Taiwan (CHEN & al. 2006, YANG & al. 2008). *Solenopsis invicta* is an extremely able competitor, and it can have a devastating effect on the native ant species. For instance, it eradicated 70 % of the native ant species after colonizing a Texan prairie habitat (PORTER & SAVIGNANO 1990), but the generality of this observation has been doubted (TSCHINKEL 2006). *Linepithema humile* has been even more successful. It has a current distribution in Mediterranean habitats throughout the world (MCGLYNN 1999, SUAREZ & al. 2001) and in coastal California, for instance, it displaced practically all native ant fauna (HOLWAY & al. 2002, HOLWAY & SUAREZ 2006).

Ants many, populations small

The effective population size (N_e) can be defined as the number of independent individuals in a population that has the same genetic properties as the one observed. In ants, the most important factors reducing N_e compared to the census population size are those that are characteristic to social Hymenoptera: haplo-diploid genetic system and reproductive division of labor, i.e., evolution of sterile worker caste.

Haplo-diploidy reduces the effective population size compared to diploid organism. In diploid systems, N_e can be calculated as:

$$N_{e-D} = \frac{4N_m N_f}{N_m + N_f} \qquad [1]$$

and in haplo-diploid systems as:

$$N_{e-HD} = \frac{9N_m N_f}{4N_m + 2N_f}, \qquad [2]$$

where N_m and N_f refer to the number of breeding males and females, respectively (WRIGHT 1969). Thus, with equal breeder sex ratio, N_e in haplo-diploids is 75 % of that in diploids. Only with unrealistically strongly female biased sex ratios (~ 7 : 1) can N_e be larger in haplo-diploids than in diploids, but in ants the breeder sex ratios are typically equal or slightly male biased (see below). Workers are sometimes also able to produce males from unfertilized eggs, which adds another step of generations and consequently increases drift and slightly decreases N_e (CROZIER 1979).

Only breeding individuals count. Effective population size in social insects is determined by the number of nests in the population and the number of breeding individuals in each nest (PAMILO & CROZIER 1997). Polygyny (multiple queens in nests) and polyandry (multiple mating by queens) commonly occur in ants (BOURKE & FRANKS 1995, CROZIER & PAMILO 1996) and increasing queen number and queen mating frequency increase N_e. Queen numbers can sometimes be extremely high, as in the mound-building wood ants *Formica aquilonia* and *F. polyctena* ("hundreds", ROSENGREN & al. 1993). Genetic relatedness among worker nestmates is also an indication of the queen number in the nests (QUELLER 1993, ROSS 1993, SEPPÄ 1994). Low relatednesses and consequently high queen numbers have been measured in a number of species in at least some populations (e.g., SEPPÄ & WALIN 1996, SUNDSTRÖM & al. 2005). High queen mating frequencies (QMF) have evolved in a few distinct ant lineages: Leaf cutters (VILLESEN & al. 2002), harvester ants (RHEINDT & al. 2004, WIERNASZ & al. 2004), *Cataglyphis* (PEARCY & al. 2004), and army ants (DENNY & al. 2004, KRONAUER & al. 2006).

The effective population sizes are often surprisingly low in ants, however, as counting queens and their matings is not enough to determine how many genetically independent individuals colonies and populations represent. The genetic effects of breeding queens and males are best seen as effective number of queens (N_Q, QUELLER 1993, ROSS 1993) and effective QMF (M_e, STARR 1984, PAMILO 1993). N_Q and M_e are mostly affected by three factors. First, the queen number and queen mating frequency usually vary strongly across nests and across queens, respectively, and the genetic effects of the varying queen number and QMF are best represented by their harmonic means (WADE 1985). Thus, the effective numbers are most affected by the smallest classes. Second, reproduction among coexisting queens or multiple mates of a single queen are never exactly equal, and increasing reproductive skew decreases effective numbers. Third, coexisting queens (but not males) are typically related in secondarily polygynous nests (CROZIER & PAMILO 1996), which decreases the effective queen numbers compared to unrelated queens (QUELLER 1993, ROSS 1993).

Diversity counts

Genetic diversity is the prerequisite for a population to adapt to future environmental changes. Neutral genetic variation is a good proxy also for adaptive and detrimental variation when genetic drift determines the amount of genetic variation, i.e., when populations are small (HEDRICK 2001). At short time scales, neutral variation is largely determined by how quickly genetic drift decimates variation from the populations, with migration simultaneously introducing new genetic variation from other populations. In general, social hymenopterans have less genetic variation than diploid insects, probably due to both haplo-diploidy and small effective population sizes in general (HEDRICK & PARKER 1997). Whether this is directly bad for them is not clear, because comparisons of different genetic systems and selection regimes are not straightforward (see below).

Comparing genetic variation across species, populations and marker loci in a meaningful way is difficult, and only a few studies have systematically compared the levels of variation in con-specific populations in different situations. Most extensively, the level of variation has been studied in the wood ant *Formica lugubris* in a series of studies. First, GYLLENSTRAND & SEPPÄ (2003) compared genetic variation in two separate population systems. One of the study systems (England) was an isolated group of closely located populations, where the total number of nests was in hundreds and all nests were highly polygynous. The other system (Finland) was not isolated, and it had less than twenty mainly monogynous nests in total. However, the larger numbers of nests and breeding queens in the English population did not translate into more genetic variation in DNA microsatellites (GYLLENSTRAND & SEPPÄ 2003). In another study, MÄKI-PETÄYS & BREEN (2006) studied small populations of *F. lugubris* in Ireland, isolated from each other and any other known *F. lugubris* population. Their microsatellite variation was clearly lower than in England or Finland (MÄKI-PETÄYS & BREEN 2006). Finally, a group of Russian populations somewhat isolated from each other showed intermediate microsatellite variation between the ones mentioned above (MÄKI-PETÄYS & al. 2005).

Resembling *F. lugubris*, the level of allozyme variation in *Myrmica sulcinodis* was not always affected by the number of breeding queens (PEDERSEN & BOOMSMA 1997). Populations of *Odontomachus rixosus* and *Pheidole annexa* in the rainforests of northern Borneo harbored a larger amount of variation in RAPD markers in large patches (~ 40,000 ha) than in smaller ones (20 - 4,000 ha) (BICKEL & al. 2006). In *Formica cinerea* populations across Northern Europe, DNA microsatellite diversity was significantly lower in isolated populations compared to those with continuous distribution (GOROPASHNAYA & al. 2001), and in

Formica exsecta least DNA microsatellite diversity was found in a small and isolated population in England (Goropashnaya & al. 2007).

Footprints of past demographic events, such as a temporary dip in the effective population size (genetic bottleneck) can also be embedded in the patterns of genetic variation, and can be important information about the past population sizes. Genetic bottleneck can be recognized as a mismatch between the observed and expected allelic diversity distributions based on mutation-drift equilibrium (Cornuet & Luikart 1996). Mäki-Petäys & al. (2005) found a significant bottleneck signal in a Russian population of the wood ant *Formica aquilonia* near Moscow, and could associate it with fragmentation and decline of the population during three decades prior to sampling.

A founder effect means founding of new populations by very few individuals, but its genetic effects are similar as in bottlenecking (Hedrick 2005). A significant bottleneck signal has been found in some populations of *Formica truncorum* (Elias & al. 2005), *F. cinerea* (Goropashnaya & al. 2001), *F. lugubris* (Gyllenstrand & Seppä 2003), *Petalomyrmex phylax* (Dalecky & al. 2007), *Linepithema humile* (Tsutsui & al. 2000, Buczkowski & al. 2004) and *Solenopsis invicta* (Yang & al. 2008), and it seems likely that the underlying demographic event in all these cases is a founder effect.

Invasive species provide a convenient opportunity to study the genetic effects of founding events. As expected, introduced populations have low levels of genetic variation compared to their native range in *Linepithema humile* (USA: Tsutsui & al. 2000, 2001, Buczkowski & al. 2004; Europe: Krieger & Keller 1999, Giraud & al. 2002; New Zealand: Corin & al. 2007a, b) and *Solenopsis invicta* (USA: Ross & al. 1993, Ross & Shoemaker 1997, Ross & al. 1997, 2007, Caldera & al. 2008; Taiwan: Yang & al. 2008), but it seems that introductions have not caused major qualitative changes in their genetic constitution. *Linepithema humile* forms extensive supercolonies in the introduced range (e.g., Tsutsui & al. 2000, Giraud & al. 2002), and Tsutsui & al. (2000) suggested that the reduced genetic variation in the recognition cue diversity caused by the founder effect has allowed the development of unicolonial social organization. However, unicolonial populations exist also in the native range (Pedersen & al. 2006), meaning that rather than initiating, introductions have probably merely boosted the scale of the unicolonial populations. A prominent feature of the social biology in *S. invicta* is the marked social polymorphism (e.g., Tschinkel 2006) associated with polymorphism in the *Gp-9* gene (e.g., Keller & Ross 1998). The polygynous form was first thought to have arisen in the introduced populations in the USA (Ross & Shoemaker 1997), but polygynous colonies and populations with the same genetic background also exist in the native range (Mescher & al. 2003).

Adaptive and detrimental variation. There are two factors that modify the patterns of non-neutral variation in haplo-diploids. First, recessive alleles causing a fitness difference in traits expressed in both sexes are not hidden from selection as heterozygotes in haploid males. This speeds up the response to selection and particularly the frequency of detrimental alleles should be lower in haplodiploids compared to diploids due to the purging selection in males (Hedrick & Parker 1997). Second, the relative strength of selection (s) and genetic drift (N_e) is determined by their product, so that allele frequency changes are largely determined by genetic drift when Ne * s < 1 (Hedrick 2005). Since endangered populations are small by definition, selection coefficients must be considerable for selection to have a marked effect in them.

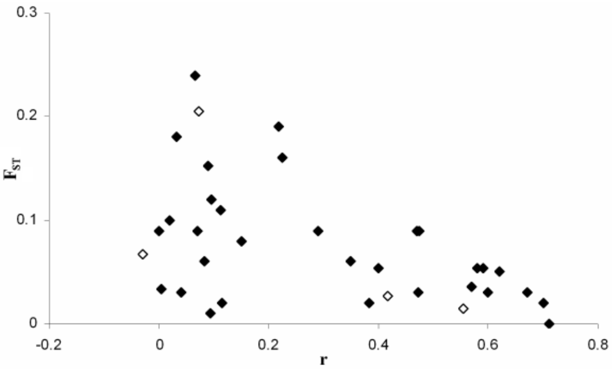

Fig. 1: Association between social type measured as genetic relatedness between worker nestmates (r) and population differentiation (F_{ST}) in *Formica* and *Myrmica*. Data from *Formica* (closed diamonds) comes from Sundström & al. (2005). Data from *Myrmica* (open diamonds) comes from *M. rubra* (one study), *M. ruginodis* (two studies) and *M. sulcinodis* (one study). Relatedness values were corrected for inbreeding (Pamilo 1985) to remove the possible effect of spatial structure for relatedness. References for *Myrmica*: Seppä (1992, 1994, and unpubl.), Seppä & Pamilo (1995), Seppä & al. (1995), Seppä & Walin (1996), Pedersen & Boomsma (1997, 1999), Walin & Seppä (2001), van der Hammen & al. (2002). The actual data are available from the author on request.

We are on the brink of a major revolution in studying molecular details of genes involved in important ecological and evolutionary traits, as genomic resources are quickly becoming available also for non-model organisms (e.g., Hudson 2008), but so far there are not many examples where non-neutral molecular variation has been studied in detail in ants. One of the first studies on non-neutral genes was made on butterflies, where different alleles of the allozyme locus *Pgi* are associated with different dispersal ability (Haag & al. 2005). Since many ants have extensive dispersal polymorphism, these kinds of findings are likely in ants, too.

The best characterised example of non-neutral variation in social Hymenoptera is the gene responsible for the complementary sex determining system (CSD). In CSD, sex is determined by heterozygosity in one or more sex determining loci, so that heterozygotes develop into females and homozygotes and hemizygotes (haploids) to males (Heimpel & de Boer 2008). Thus, homozygotes in the CSD locus develop to males, which are diploid and almost always inviable or sterile (Heimpel & de Boer 2008). In the honey bee, a single sex determining locus (*csd*) was isolated recently (Beye & al. 2003), but it is not yet clear whether the same or a similar single-locus system is to be found in ants as well. It seems likely, however, that single-locus CSD can not be universal in ants, as there are several examples of ants with regular inbreeding (Hölldobler & Wilson 1990). In *Cardiocondyla*, for instance, males are ergatoid and stay and mate in the natal colony, often with their sisters. Under single-locus CSD, this should result in high diploid male production, but since none have been detected, *Cardiocondyla* must either have a multilocus CSD with a high number of loci involved, or they have a different mechanism for sex determination (SCHREMPF & al. 2005, 2006). I will discuss conservation significance of CSD below.

Most ants have wings but many do not reach very far

Dispersal propensity, ability and the success of its undertakers are crucial factors when new areas are colonized. Successful colonization of new populations is an important aspect of ant conservation as many habitats are continuously becoming more and more fragmented due to human activities. Spatial structure of populations is the manifestation of dispersal patterns and subsequent gene flow, traditionally assessed as Wright's F_{ST} (WRIGHT 1969). Gene flow is determined both by how frequently individuals disperse, and how well dispersing individuals survive the founding stage and manage to breed in the target population. In monogynous species, dispersal occurs during a nuptial flight, where both sexes gather from large areas to mate, whereas polygynous species can have local matings and restricted dispersal (HÖLLDOBLER & WILSON 1990). Thus, stronger gene flow is expected in monogynous than in polygynous species based on the dispersal propensity of sexuals (SEPPÄ & PAMILO 1995), but the detailed gene flow patterns are determined by ecological factors as well.

Dispersal in ants can be affected by morphological, physiological, behavioural and social factors. Sexuals in most ants have normal wings and have a potential to disperse long distances. However, wingless or short-winged females are known particularly in poneromorph subfamilies and the Myrmeciinae (PEETERS & ITO 2001), and wingless males are common in the genus *Cardiocondyla* (HEINZE & al. 2005). Furthermore, in many poneromorph species, queens have been replaced by gamergates, mated wingless workers (PEETERS & ITO 2001). Not having wings obviously restricts dispersal and strong population differentiation, usually more pronounced in the mitochondrial than in the nuclear genome, has been found in *Nothomyrmecia macrops* (short-winged queens, SANETRA & CROZIER 2003), *Proformica longiseta* (apterous queens, SEPPÄ & al. 2006), and three ponerinae with gamergates: *Diacamma cyaneiventre* (DOUMS & al. 2002), *Rhytidoponera metallica* (CHAPUISAT & CROZIER 2001) and *Rhytidoponera* sp. 12 (CROZIER & al. 1984). Another group with mostly wingless sexuals are the permanent social parasites, but genetic studies on their spatial population structure are largely awaiting (but see STEINER & al. 2006).

Social organization of the species may affect both the propensity of sexuals to disperse and their success in nest founding after the dispersal. In *Formica truncorum*, sexuals maturing in monogynous colonies are better equipped for dispersing than sexuals maturing in polygynous colonies, and females also for founding new colonies (SUNDSTRÖM 1995). Furthermore, both classes of colonies and both sexes have dispersal polymorphism, with some females and males being more willing to disperse than others (SUNDSTRÖM 1995). Accordingly, population differentiation is an order of magnitude stronger between polygynous than between monogynous populations in *F. truncorum* (SUNDSTRÖM 1993, GYLLENSTRAND & al. 2005).

The traditional dichotomy of monogynous and polygynous species mentioned above does not actually reflect well the changes in the dispersal patterns observed in genetic studies (SUNDSTRÖM & al. 2005). Moreover, many species cannot be classified this way because they are socially polymorphic, with both monogynous and polygynous populations. A more useful way is to divide population types into monogynous and / or weakly polygynous populations (M type) on the one hand and populations that are highly polygynous (P type) on the other hand. In *Formica*, populations of the P type are significantly more structured than those of the M type (SUNDSTRÖM & al. 2005), and this holds well also in the few studies made in *Myrmica* (Fig. 1). Exceptions to this rule come from systems where the association between social organization and dispersal patterns does not hold. For example, *Cataglyphis cursor* is a monogynous species but it founds new colonies by fission (LENOIR & al. 1988), and has a strong spatial population structure as a consequence (CLÉMENCET & al. 2005). In *Plagiolepis pygmea*, both sexes are philopatric which results in strong inbreeding and population structure at local scale (TRONTTI & al. 2005).

Dispersal is sex biased in many ants, with females usually as the philopatric sex (CROZIER & PAMILO 1996). Restricted female dispersal can be observed at three levels of social organization: within nests, within polydomous colonies and between populations. First, in secondarily polygynous ants, coexisting queens are typically related, which indicates that nests recruit their own daughters as new queens (CROZIER & PAMILO 1996). Second, in a polydomous colony, new nests are founded by budding, with one or more queens leaving the old nest and starting a new one (ROSENGREN & PAMILO 1983). This leads to viscose population structure, evidenced by clustering of maternally inherited mitochondrial haplotypes (e.g., *Leptothorax acervorum*, studied by STILLE & STILLE 1993), but has been identified from nuclear data as spatially closely located nests being also genetically more similar than average nests in the population (e.g., SEPPÄ & PAMILO 1995, CHAPUISAT & al. 1997, PEDERSEN & BOOMSMA 1999, GOROPASHNAYA & al. 2001).

Third, female philopatry results in stronger spatial structure in the mitochondrial than in the nuclear genomes. In a few cases, spatial population structure has been studied by using nuclear and mitochondrial markers in parallel in a system where populations are located within potential dispersal distance of sexuals. Because the effective population size in the mitochondrial genome is smaller compared to the nuclear genome, the ratio of mitochondrial (F_{ST-MT}) to nuclear differentiation (F_{ST-NUC}) exceeding three indicates male-biased gene flow (SEPPÄ & al. 2004). Mitochondrial differentiation usually exceeds this threshold (Tab. 1), and the ratio is considerably lower in the M type systems (median 7.6) than in the P type or mixed systems (median: 14.3 and 30.3, respectively). A large part of this difference comes from the introduced populations of the fire ant *Solenopsis invicta*, where there is no nuclear differentiation between P type populations (ROSS & SHOEMAKER 1997, ROSS & al. 1997). Sometimes female philopatry is evident from strong mitochondrial differentiation alone, as in *Temnothorax rugatulus* (RÜPPEL & al. 2003).

Tab. 1: Genetic differentiation estimated from nuclear ($F_{ST\text{-}NUC}$) and mitochondrial ($F_{ST\text{-}MT}$) markers in the same populations when the populations are within a potential dispersal distance. "M systems" and "P systems" refer to differentiation between monogynous / weakly polygynous populations and between highly polygynous populations, respectively, and "Mixed system" include both types of populations. References: [1]FOITZIK & HERBERS (2001), [2]CLÉMENCET & al. (2005), [3]DOUMS & al. (2002), [4]SEPPÄ & al. (2004), [5]ROSS & al. (1997), [6]ROSS & SHOEMAKER (1997), [7]GYLLENSTRAND & SEPPÄ (2003), [8]GYLLENSTRAND & al. (2005), [9]SEPPÄ & al. (2006).

Species	**Number of populations**	**Scale (km)**	$F_{ST\text{-}NUC}$	$F_{ST\text{-}MT}$	$F_{ST\text{-}MT}$ / $F_{ST\text{-}NUC}$	**Notes**
M systems						
Protomognathus americanus[1]	3	~ 0.1	0.03	0.27	10.1	
Cataglyphis cursor[2]	5, 6	0.5 - 1	0.05	0.75	15.1	Average of two regions
Diacamma cyaneiventre[3]	3	< 0.5	0.02	0.33	16.9	Wingless gamergates
Diacamma cyaneiventre[3]	2 - 3	"a few"	0.15	0.75	5.0	
Formica exsecta[4]	4	~ 3 - 7	0.00	0.06	–	
Solenopsis invicta[5]	3, 6	> 5	0.03	0.10	3.5	Native range, average of two regions
Solenopsis invicta[6]	3	~ 12 - 30	0.00	0.00	–	Introduced range
P systems						
Formica exsecta[4]	6	0.2 - 9	0.10	0.46	4.7	
Formica lugubris[7]	5	1 - 3	0.03	0.53	15.6	
Solenopsis invicta[5]	5, 5	> 5	0.02	0.30	13.0	Native range
Solenopsis invicta[6]	6	3 - 8	0.00	0.50	>> 100	Introduced range
Mixed systems						
Formica exsecta[4]	10, 11	0.2 - 9, 2 - 23	0.06	0.27	4.4	Between social types, average of two regions
Formica truncorum[8]	2	< 3	0.12	1.00	8.0	Between social types
Proformica longiseta[9]	5	0.1 - 1.5	0.03	0.63	20.3	Queens wingless
Solenopsis invicta[5]	8, 11	> 5	0.01	0.18	22.0	Native range, between social types, average of two regions
Solenopsis invicta[6]	9	3 - 30	0.00	0.25	>> 100	Introduced range, between social types

Inbreeding – a family affair or just shrinking populations?

Inbreeding is one of the central questions in conservation genetics, as it leads to the loss of genetic variation and fitness due to increased homozygosity exposing recessive detrimental alleles to selection, i.e., inbreeding depression. The negative effects of inbreeding depression are well known from natural populations and can contribute to the vulnerability of species (KELLER & WALLER 2002), but among ants regular inbreeding causing fitness loss has been reported only in *Cardiocondyla obscurior* so far (SCHREMPF & al. 2006). The hymenopteran sex determining system is a further inbreeding concern for ants, as it is a straightforward mechanism for inbreeding to affect population survival (ZAYED & PACKER 2005).

The association between the social type and dispersal leads to different expectations about the mating structure as well. In monogynous species, matings take place during nuptial flights (HÖLLDOBLER & WILSON 1990), and mating is generally expected to be random. However, the probability of mating by relatives is determined by how many nests simultaneously release sexuals to the mating swarm. A single mating swarm typically includes sexuals from numerous nests, but the only genetic study on the composition of a mating swarm suggests that the number of nests participating can in fact be very small (HASEGAWA & YAMAGUCHI 1995). On the other hand, many polygynous species do not have massive nuptial flights, but females mate locally near or even on or in the nests (HÖLLDOBLER & WILSON 1990), which has also been shown as elevated inbreeding coefficient in some species (e.g., CHAPUISAT & KELLER 1999, TRONTTI & al. 2005, SCHREMPF & al. 2005, 2006). The actual effects of local matings depend on male dispersal and on the degree of polygyny, as nestmates in highly polygynous nests may be only distantly related (SUNDSTRÖM & al. 2005).

A negative association between inbreeding coefficient (F) and the degree of polygyny in the population, measured as nestmate relatedness, was found in *Formica* ants

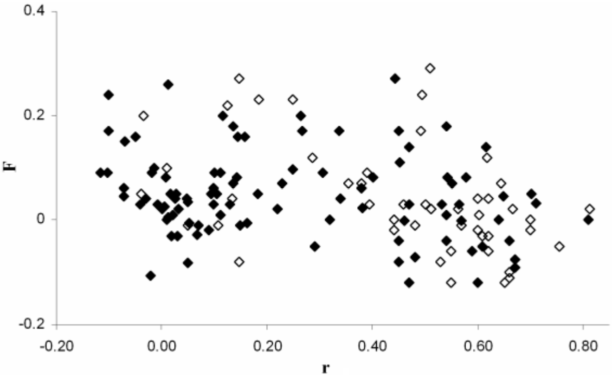

Fig. 2: Association between social type measured as genetic relatedness between worker nestmates (r) and inbreeding (F) in *Formica* and *Myrmica*. Data from *Formica* (closed diamonds) comes from Sundström & al. (2005). Data from *Myrmica* (open diamonds) comes from *M. rubra* (11 populations), *M. ruginodis* (28 populations) and *M. sulcinodis* (11 populations). Relatedness values were corrected for inbreeding (Pamilo 1985) to remove the possible effect of spatial structure for relatedness. References for *Myrmica*: Seppä (1992, 1994), Seppä & al. (1995), Seppä & Walin (1996), Pedersen & Boomsma (1997, 1999), Walin & Seppä (2001), van der Hammen & al. (2002). Actual data available from the author on request.

(Sundström & al. 2005), but it is not perfect and other factors affect population mating structure as well (Fig. 2). The association was significant when a single species with an unusual association of monogyny and restricted dispersal was removed from the analysis (*F. exsecta*, Sundström & al. 2003). Similar association is also apparent in three *Myrmica* species (*M. rubra*, *M. ruginodis*, *M. sulcinodis*, Fig. 1), with an overall significant negative correlation between inbreeding and relatedness ($r_S = -0.46$, $p < 0.001$, $n = 50$). In *Myrmica*, inbreeding coefficients are significantly higher in the highly polygynous populations ($F = 0.088 \pm 0.106$ SD) than in weakly polygynous or monogynous populations ($F = -0.0001 \pm 0.108$ SD; $t = 2.80$, $P = 0.007$, $df = 48$).

A positive inbreeding coefficient cannot be taken directly as evidence that mating between close relatives constitutes an important element of the breeding system, though. There are numerous adaptations for avoiding inbreeding (Pusey & Wolf 1996), with sex-biased dispersal (see above) and specialization to produce just one sex in a nest (split-sex ratio, Pamilo & Rosengren 1983) serving as such in ants. An elevated inbreeding coefficient can be a result of hidden population structure, i.e., the Wahlund Effect (Wahlund 1928), and sampling covering more than one differentiated population is probably the reason for the elevated inbreeding coefficient in many of the studies mentioned above.

The complementary sex determining system can be a direct threat to population persistence (Zayed & Packer 2005). Heterozygosity in the sex locus is determined by its allelic diversity, and diploid males are expected to occur when population sizes are reduced, for instance due to bottlenecking or when isolation and small population sizes lead to non-random mating. Diploid male production has detrimental effects for the colony fitness (Pamilo & al. 1994), and there is a strong balancing selection for increased allelic diversity in the sex locus, favoring immigration. In the "diploid male vortex", any perturbation that causes the population size to decrease also decreases allelic diversity in the CSD locus. This in turn increases diploid male production, which will decrease the growth rate of the population. Finally, the circle ends with reduced growth rates decreasing population sizes even further (Zayed & Packer 2005). The diploid male vortex is a model for a closed population (Zayed & Packer 2005), however, and any immigrants bearing new allelic diversity would alleviate it.

Diploid males have been found in a few ant species, but in varying amounts. In the polygynous form of *Solenopsis invicta*, diploid male production is frequent in the introduced populations but not in the native ones, probably due to a bottleneck and a collapse in the number of alleles in the sex determining locus (Ross & al. 1993). In *Formica*, the proportion of nests producing diploid males can be as high as 50 %, but the fraction of males that are diploid can be very small (Pamilo & al. 1994, Mäki-Petäys & Breen 2006), possibly because diploid males are effectively destroyed during development (Pamilo & al. 1994). Interestingly, an isolated and genetically depauperate *F. lugubris* population in Ireland produces a considerable amount of diploid males (Mäki-Petäys & Breen 2006), which may contribute negatively to its welfare. Diploid male production has been only rarely reported in others ants (e.g., Hammond & al. 2001, Fernández-Escudero & al. 2002).

Conclusions and perspectives

Do ants need conservation? Losing any biodiversity is irreversible and regrettable, but losing ant biodiversity would be a global ecological experiment without match. Ants strongly dominate most terrestrial ecosystems (Wilson 1990), but they are not immune to the current extinction wave and the effects of losing such a central group of animals can only be anticipated. The data on endangered species show that ants are no less vulnerable than any other group of organism. On the contrary, the number of ant species recognized as threatened is higher than in other insects (IUCN 2008), and we still only have a poor understanding of the global biodiversity and distribution of ants.

Apart from the few species encountered only for a few times, there are two groups of ants that are particularly in the risk zone. First, species that have the most specialized life style, permanent social parasites (inquilines), are all classified as vulnerable (IUCN 2008). Since their life is confined to the nests of their host, their true abundance and distribution is poorly known, but such specialized parasites must always be relatively rare compared to their hosts. Another vulnerable group are the species with unusually weak dispersal, such as those with wingless or short-winged sexuals. Furthermore, in many polygynous species female dispersal is restricted. If habitat destruction and fragmentation continues, colonizing new suitable habitat patches can become increasingly difficult, making these strong candidates for becoming the next generation of threatened species.

Idiosyncrasies of social Hymenoptera are likely to expose ants to some unique genetic and demographic threats. The sheer numbers of ants are simultaneously both spectacular and deceiving. Reproductive division of labour means that the effective population sizes (N_e) are but a tiny fraction of the total numbers of ants we see. Polygyny and polyandry occur commonly in ants, but the effective queen numbers and queen mating frequencies are seldom extreme, and as a consequence, the genetic effects of polygyny and polyandry are often smaller than seems at first sight. Furthermore, a large fraction of ant species are monogynous (BUSCHINGER 1974) and queens in most species studied so far mate only with a single male (STRASSMANN 2001). This suggests that the number of nests is often the most important determinant of N_e.

According to the "50 / 500" rule (see FRANKHAM & al. 2002), N_e should exceed 50 in order to avoid extinction due to immediate detrimental effects of inbreeding depression, and N_e should exceed 500 in order to populations not to lose genetic diversity in the mutation-drift balance and retain the potential to adaptively respond to the potential future changes. Since the effective population size is often around 10 % of the census population size (queens and their mates) (FRANKHAM & al. 2002), it is clear that N_e is not even close to those numbers in many monogynous and / or territorial ants.

Rather than the number of breeding queens, connectivity of populations seems to be decisive for the amount of genetic variation in them. Small and isolated populations harbour less genetic diversity in neutral marker loci than larger and better connected populations, but a few studies also suggest that a high queen number does not always increase genetic diversity in the population. Thus, small N_e should lead to low levels of variation, but migration can very effectively introduce new genetic variation to the populations.

The direct fitness effects of inbreeding can be envisaged at many levels in social insects, for individual queens, their colonies and the whole populations, but such studies are largely awaiting. An obvious candidate for instigating inbreeding depression is the complementary sex determination system (CSD) of hymenopterans. The decreasing allelic diversity in the sex locus is a direct risk to shrinking ant populations, because it conveys a strong fitness loss in the form of diploid male production. However, there are many ants whose mating system includes regular inbreeding, such as inquiline parasites, and it seems likely that their sex determination must be based either on CSD with multiple loci involved, or some completely other mechanism.

Does ant conservation need genetics? Some conservation genetics topics are concerned with issues like identification of individuals and their source populations for forensic purposes, and management and reintroduction of captive populations (FRANKHAM & al. 2002, ALLENDORF & LUIKART 2007), which are less relevant for ants. Many other topics are, however, general population genetics issues highly relevant for any species with reduced population sizes. Apart from taxonomy, the ones I took up here were effective population size, the amount of genetic variation, spatial population structure and inbreeding. As one of the most important goals of conservation genetics is to recognize when species' evolutionary potential becomes compromised, all these are core issues in conservation genetics.

One attempt to answer the question of the importance of genetics in conservation is to reformulate the question: What important information would we have missed if genetics had not been not used for studying population biology in endangered species? Generally, the whole concept of conserving the evolutionary potential relies on the knowledge of the genetic constitution of populations, their effective population size and the factors affecting it, such as breeding and dispersal biology. Very few of the studies reviewed here were originally designed with conservation in mind, but studies dealing with the issues above are valuable information for assessing evolutionary potential of the study system in question. For instance, without genetic information we might not understand how small ant populations often are in terms of genetically independent individuals, what the perils are of the small population size due to inbreeding depression and particularly complementary sex determination system, or what the problems are that polygynous ants may face when colonizing new areas.

Thus, it is quite clear that genetic studies have a lot to give for conservation biology. The impressive operations for securing the short-term survival of the last of a given species will probably never take place in ants, but genetics can and must be seen as an important part of the toolbox when the goal is to care for the long-term potential of the species to adapt to unpredictable environmental changes. Furthermore, the different emphasis of geneticist and other conservation biologists is probably irrelevant, as the real challenge is to convince political and economical decisions makers that ants are worth conserving.

What else should we know? There are several lines where additional research would benefit conservation of ants. First, genetic information is currently available of maybe 0.5 per cent of the known ant species, and it would be unrealistic to imagine that the number will increase strongly in the near future. Rather, genetic studies should adopt the model organism approach and concentrate on studying a few representative species in detail, and generalize the results thereafter. This practice is partly in use already, as a large proportion of genetic studies have been made in just a few well-known species or groups of species, such as *Solenopsis invicta* or *Formica* spp. A better taxonomic balance is desirable, however, as many ant subfamilies still await the first studies on their breeding systems or spatial population structure. Second, the haplo-diploid genetic system coupled with social life style decreases the amount of neutral genetic variation in ant populations, but what this means in terms of their future fitness prospects is not clear. Furthermore, the "50 / 500" rule refers to diploid populations, and how haplo-diploids cope with inbreeding depression and the loss of genetic variation, has not been worked out yet theoretically. Third, invasive ants are an increasing problem, and all information is welcome to assess their potential threat to the native species. Fourth, as the price for genomic studies goes down, we will soon start seeing many studies on non-neutral genes. Of those with a conservation connection, first in line will probably be the gene for complementary sex determination system and those related to dispersal. Finally, taxonomic studies will be topical as long as one third of the ant fauna awaits discovery and description, and a large fraction of the rest remain in turmoil.

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Zusammenfassung

Ameisen spielen eine zentrale Rolle in terrestrischen Lebensräumen, und die Biodiversität von Ameisen zu verlieren wäre ein globales ökologisches Experiment unvorstellbarer Dimension. Da genetische Diversität eine wichtige Komponente der Biodiversität ist und das Aussterben von Arten wahrscheinlich auch von genetischen Faktoren bedingt wird, ist jegliche genetische Information, die zur Klärung der Populationsbiologie einer Art dient, auch direkt relevant für ihren Schutz. Mein Ziel hier ist es, einen Überblick zu bieten darüber, welche genetische Information für die naturschutzbiologische Evaluierung von Ameisen vorhanden ist, sowie zu umreißen, welche Information noch fehlt. Gemäß den Rote-Liste-Daten sind Ameisen genau so verletzlich wie jede andere Organismengruppe auch. Einige Eigenheiten sozialer Hymenopteren setzen Ameisen wahrscheinlich besonderen genetischen und demographischen Bedrohungen aus; beispielsweise ist die effektive Populationsgröße (N_e) bei Ameisen nur ein winziger Bruchteil der Gesamtzahl der Ameisen, die wir sehen. Die genetischen Konsequenzen von Polygynie und Polyandrie sind ebenfalls geringfügiger als sie auf ersten Blick zu sein scheinen, was folglich nahe legt, dass die Zahl der Nester oft die wichtigste Einflussgröße auf N_e ist. Insgesamt dürften somit viele Ameisenpopulationen die "50 / 500" Regel für N_e kaum erfüllen, wie es generell als nötig erachtet wird, um einem Aussterben langfristig zu entgehen. Statt der Zahl der Nestköniginnen dürfte die Vernetzung von Populationen ausschlaggebend sein für das Ausmaß genetischer Variation in Ameisenpopulationen: Kleine und isolierte Populationen sind genetisch verarmt. Philopatrie von Weibchen kennzeichnet die räumliche Populationsstruktur vieler Ameisenarten, was naturgemäß die Probleme verstärkt, die mit dem Besiedeln neuer Lebensräume verbunden sind, wenn die Lebensräume fragmentiert sind. Schlussendlich ist die abnehmende Allelvielfalt am Locus für die komplementäre Geschlechtsbestimmung eine direkte Bedrohung für schrumpfende Ameisenpopulationen, weil sie eine starke Inzuchtdepression durch die Produktion diploider Männchen bedingen kann.

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