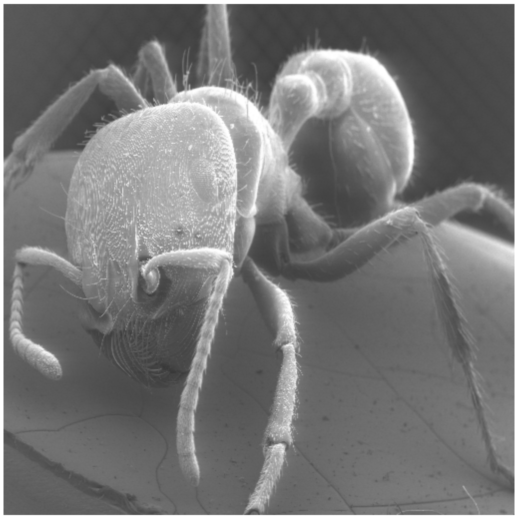
Evolution of Colony Characteristics in The Harvester Ant Genus Pogonomyrmex



Dissertation zur Erlangung des naturwissenschaftlichen Doktorgrades der Bayerischen Julius-Maximilians-Universität Würzburg

vorgelegt von

Christoph Strehl

Nürnberg

Würzburg 2005

Eingereicht am: ....

Mitglieder der Prüfungskommission:

Vorsitzender: ....

Gutachter : ....

Gutachter : ....

Tag des Promotionskolloquiums: ....

Doktorurkunde ausgehändigt am: ....

1. Index

2. General Introduction and Thesis Outline

"For such is the way of it: to find and lose, as it seems to
those whose boat is on the running stream"

(Tolkien 1991, p.399)

The selective forces of evolution created a manifold life with impressive forms in shaping not only the morphology of species, but also their behavior. An extraordinary example for the evolution of behavioral characters is the clumping of individuals into one single group that collectively performs social tasks. Its apex is an altruistic self restraint, as found in impressive ways among colonies of ants or bees (eusocial Hymenoptera): their eusocial colonies are not only characterized by overlapping generations and cooperative brood care by the adults, but also by a reproductive division of labor implementing the presence of non-reproductive helpers as "workers" (Michener 1969; Wilson 1971; Alexander 1974; Michener 1974; Hölldobler and Wilson 1990; Ratnieks and Anderson 1999).

The altruistic behavior of eusocial Hymenoptera was for a long time explained by the perception of natural selection not only acting at the level of single organisms, but also at the *family-level* (Darwin 1859). Later, this was mathematically formulated by Hamilton in his theory of kin selection (Hamilton 1964), which is now the key concept for explaining the evolution of group living behavior. The Hymenopteran sex determination system hits the underlying genetic assumption of Hamilton's formula ($br-c>0$; b = benefits connected to behavior, r = relatedness of actor to recipient, c = costs involved for actor), because unfertilized eggs become males and fertilized eggs normally females (relatedness "asymmetry") (Hamilton 1964; Trivers and Hare 1976). By this the cost to benefit ratio for 'altruistic' behaviors decreases for females, favoring their accumulation in eusocial groups (colonies).

Group living is quite divers, and might even be lost again if no longer favorable, depending on internal and external needs (Heinsohn and Legge 1999; Gadagkar 2002; Krieger and Ross 2002; Roux and Korb 2004). Basic rules generating these social phenotypes are pertinent across taxa, and they produce ubiquitous patterns of social organization, like mass action responses, division of labor and social hierarchies (Fewell 2003). The tremendous variety of group living among Hymenoptera (bees, ants, wasps) has its greatest diversity among ants (Formicidae), making them especially suitable in studying the characteristics of social colonies (Hölldobler and Wilson 1990).

In the present work analyses on the evolution of colony characteristic in the harvester ant genus *Pogonomyrmex* (Hymenoptera: Formicidae) will be presented and discussed. In the following chapters of this introduction in a first step the general characteristics of an ant colony will be described, relatedness as a major component driving the evolution of colony characteristics is introduced, and the phenomena of polyandry, polygyny and morphological polymorphism are emphasized. Subsequently the studied genus *Pogonomyrmex* and the aims of this study are introduced.

1.1 The characteristics of an ant colony

Several characters are prominent in constituting ant-colonies (e.g.: Alexander 1974; Gordon 1984b; Hölldobler and Wilson 1990; Gordon 1995; Ratnieks and Anderson 1999; Sanders and Gordon 2000; Anderson and McShea 2001; Fewell 2003): (1) number of individuals (colony size); (2) cohesion (nest, odor, age); (3) nest architecture; (4) organization of colony members (e.g. reproductive division of labor, task allocation, information transfer); (5) robustness (e.g. defense, disease and parasite transmissibility, territoriality in space and time); (6) efficiency (e.g. resource use, reproductive output); (7) relatedness among colony members.

These single colony characteristics are influenced by various factors. For illustration, task performance and division of labor, which constitute a wide field of recent research, are effected among other things by colony size (Murakami et al. 2000; Gautrais et al. 2002), circadian rhythmic (Gordon 2002; Wuellner and Saunders 2003), information transfer (Gordon 1989; Tofts and Franks 1992; Franks et al. 2001; Fewell 2003), presence or absence of queens (Vienne et al. 1998; Backen et al. 2000; Heinze and Keller 2000; Kikuchi and Higashi 2001), or genetic variability (Stuart and Page 1991; Myerscough and Oldroyd 2004). Analogous several other factors exist, which influence group living behavior in ants.

In the main, colony characteristics are influenced by extrinsic environmental and intrinsic nest factors (e.g.: Alexander 1974; Fewell 2003). For example changes of the external environment are influencing the colonies efficiency and robustness, and can be managed via a flexible response of the whole colony, e.g. by information transfer in the "social environment" between colony members (Fewell 2003). Territoriality on the other hand, i.e. the ability of colonies to obtain and maintain a certain foraging area within their population, is strongly shaped by several intrinsic factors like behavior, aggressiveness, or growth rate of colonies (Hölldobler 1974; Hölldobler 1979; Cole and Wiernasz 1999; Cole et al. 2001).

Additionally single colony characteristics can be reciprocally influenced by others. For example, pathogen resistance as important intrinsic factor is shaped by the colonies efficiency and organization, because it is most importantly influenced by the cleanliness of the whole colony (maybe improved by task specialists), or by the recognition of medically parasitized individuals and their exclusion / elimination or their cure of parasitation (Moore 1995; Julian and Cahan 1999; Bot et al. 2001; Hart and Ratnieks 2001; Moore 2002).

The benefits of group living are various and general items for the benefits of group living, which might also be applicable to a social colony might be (1) a lowered susceptibility to predation, e.g. via an aggressive group defense or using the group as cover, and (2) reaching a profitable utilization of food sources, e.g. if these are scattered (Alexander 1974). Therefore, the robustness and efficiency of ant colonies might be regarded as being predominantly in the focus of natural selection. Particularly, the efficient mechanisms of division of labor, "in particular series-parallel operation and transfer of information among group members" are enabling ant colonies to interact with an unpredictable environment (Krieger et al. 2000). These direct benefits are also mirrored in non-Hymenopteran societies, and even robotic systems can be enhanced by implementing such "ant-inspired algorithms" (Krieger et al. 2000).

However, "there is no automatic or universal benefit from group living" (Alexander 1974) and there are also costs involved to living in a colony (Heinsohn and Legge 1999). For example social parasitism evolved several times in ants to exploit existing colonies (Hölldobler and Wilson 1990; Parker and Rissing 2002; Savolainen and Vepsalainen 2003). Accordingly, the spread of medical parasites and pathogens might be favored in eusocial groups, especially under the condition of high genetic uniformity due to a high relatedness among group members, like in ants or bees. Additionally, colony characteristics by themselves can have negative feedback (Fewell 2003; Johnson

2003). For instance, there is a relationship between group size and efficiency, such that the benefits of group living decrease in larger groups, most probably because of interference during foraging (Krieger et al. 2000).

1.2 Relatedness as a major component driving the evolution of colony characteristics

Although it is hard to determine characters which are applicable in explaining the connections that occur between any two individuals within a colony, and much harder the connections across the whole colonies society (Fewell 2003), at least the relatedness among its members is representing such a key factor. The relatedness among its members is influencing the various characteristics of a colony. For instance, in *Camponotus yamaokai* a decrease in relatedness is affecting the cooperation of group members, because the frequency of food exchange between workers (trophallaxis) drops and the number of foraging workers rises (Sanada et al. 1999). Or, there might be drawn a direct relation between colony kin structure and size (Sundström 1995; Bourke 1999), e.g. in fungus-growing ants (Attini) where a positive correlation of mating frequency with colony size is reported, and "some" correlation between mating frequency and caste complexity (Murakami et al. 2000).

Relatedness is, beside costs and benefits of group living behavior, the major component driving the evolution of colony characteristics among colony members, according to Hamilton’s formula for kin selection (Hamilton 1964). Moreover, nowadays relatedness measurements are easily made accessible due to the rapidly developing molecular methods based on PCR (Saiki et al. 1985; Sambrook et al. 1989; Simon et al. 1994; Loxdale and Lushai 1998; Hwang et al. 2001), making it a leading tool in social insect research. Moreover, molecular markers are helping to determine skews in the kin structure of colonies, e.g. if paternity is unequally distributed between the mates of a queen (Boomsma and Ratnieks 1996).

Relatedness among colony members is determined by the colonies number of matrilines, i.e. number of queens, and the number of patrilines, i.e. number of matings per queen(s), and altered via a change in the number of matrilines and / or patrilines. Consequently, polygyny (several queens) and multiple mating of queens (polyandry) are main sources for genetic heterogeneity of colonies of social insects (Bourke and Franks 1995; Crozier and Pamilo 1996; Gadau et al. 1998; Heinze and Keller 2000). However, in ants low genetic heterogeneity is caused for the most part by polygyny, whereas multiple mating is behaviorally and genetically observed in only four ant genera: *Atta*, *Acromyrmex*, *Eciton* (observation only) and *Pogonomyrmex* (Strassmann 2001). Additionally, genetic variability in ants can be increased by slavery (Hölldobler 1979; Bourke and Franks 1995; Foitzik and Herbers 2001; Lenoir et al. 2001) or by brood raids from neighboring colonies (Kronauer et al. 2003).

1.3 The evolution of polyandry: ultimate and proximate causes

The mating behavior of Hymenopteran species is different from that of many other insects in that mating normally takes places only once in the lifetime of sexually reproductive individuals, whose lifetimes differ with sometimes extreme values (e.g. 2-3 months for males vs. 14-30 years for queens). For the evolution of multiple mating several hypotheses have been stated (for reviews see for example (Page and Metcalf 1982; Crozier and Page 1985; Boomsma and Ratnieks 1996; Yasui 1998; Strassmann 2001), and are best tested for the polyandrous honeybee.

By mating multiple times the queens of the honeybee (*Apis mellifera*) are creating high genetic diversities among her colonies workers (e.g.: Haberl and Tautz 1998; Franck et al. 2000; Kraus et al. 2003). This is valuable for the whole honeybee colonies, as they can respond better to changes in the availability and search for resources because of diversity in worker thresholds for foraging (Oldroyd et al. 1993; Fuchs and Moritz 1998; Page and Erber 2002; Pankiw et al. 2002; Fewell 2003). Moreover, a higher genetic diversity is beneficial to honeybee colonies by preventing severe infections with pathogens (Palmer and Oldroyd 2003; Tarpy 2003), but not parasites (Neumann and Moritz 2000; Woyciechowski and Krol 2001). Similar preventive effects of increased genetic diversity are reported for bumble bees (*Bombus terrestris*: Baer and Schmid-Hempel 1999; Baer and Schmid-Hempel 2001) and might also hold true for ant colonies (Schmid-Hempel and Crozier 1999). For example, in the harvester ant *Pogonomyrmex occidentalis* higher growth rates of colonies are connected to lower relatedness, i.e. higher genetic variability due to polyandry (Cole and Wiernasz 1999; Cole and Wiernasz 2000; Wiernasz et al. 2004). These differences in the colonies efficiency are resulting in a 35-fold fitness increase (Cole and Wiernasz 1999) by rising "the probability of colony (queen) survival, longevity or reproductive effort, ultimately yielding higher lifetime reproductive success" (Wiernasz et al. 2004). Increased growth due to decreased relatedness in *Pogonomyrmex occidentalis* might be caused either by lowered pathogen loads, or by an increase of the colonies efficiency, e.g. in foraging or brood care (Wiernasz et al. 2004). Similar to honeybees, also in ants task specialists might be assumed which can increase the colonies efficiency and inherit a genetic component (Stuart and Page 1991). Such task related genetic lineages are likely as in one of the other multiple mating ant genera, for the leaf-cutting ant *Acromyrmex echinatior*, worker caste development was found to have a significant genetic component, not linked to rare or exceptional circumstances, e.g. interspecific hybridization (Hughes et al. 2003).

Similar to the above hypotheses of increased disease and parasite resistance (Hamilton 1987; Schmid-Hempel 1994), or about the facilitation of task differentiation (Starr 1984), the origins of polyandry were also explained via a beneficial increase in intracolonial genetic diversity in the hypothesis of prevention of negative inbreeding effects due to diploid male production (Page 1980). Incompatibility avoidance hypotheses for explaining multiple mating have a widespread use across animals (e.g.: Zeh and Zeh 1996; Hosken and Blanckenhorn 1999; Newcomer et al. 1999; Zeh and Zeh 2001). But, due to their haplodiploid sex determination systems, inbreeding is implementing for colonies of eusocial Hymenoptera an additional risk of producing diploid males, which are unfertile non-helpers; this threatening of the viability of brood is especially dangerous during the founding stage of colonies (Page 1980).

Additionally, hypotheses have been stated, explaining the origins of polyandry with an minimization of existing kin conflicts between members of the colonies (queens and workers) over reproduction and allocation of resources into female- and male-production (Bourke and Franks 1995; Crozier and Pamilo 1996), which are present because of the relatedness asymmetry among them (Hamilton 1964; Trivers and Hare 1976). Polyandry then can be seen as favoring the interests of the queen, e.g. by preventing worker reproduction (Woyciechowski and Lomnicki 1987), or facultative biasing of sex ratios (Sundström 1994).

Another hypothesis stated that polyandry might be a result of the queen’s sperm need (Cole 1983), assuming that a single male cannot provide enough sperm to completely fertilize a female. This was reviewed several times and could not yet convincingly be confirmed (Page 1986; Boomsma and Ratnieks 1996).

Most of the above listed appreciable hypotheses for the evolution of polyandry in eusocial hymenoptera review this phenomenon from an ultimate perspective. However, polyandry also might have evolved on proximate causes: "The most important evolutionary causes of polyandry are likely to be male-provided direct benefits (resources)." (Yasui 1998)

Moreover, multiple mating in the light of the “convenience” hypothesis is seen proximately as a result of male dominance during copulation (Alcock et al. 1978): male-male competition may have led to strong male dominance, and, for females, “convenient” multiple mating. However, in ants this hypothesis is an unlikely explanation for polyandry, because females need to move their sting aside in order to copulate (Strassmann 2001).

1.4 Polygyny and slavery

Although natural selection might lead to monogynous species with queens founding their nests alone (haplometrotically) and without foraging (claustrally), still polygyny is frequently found in ant colonies (Hölldobler and Wilson 1990). The coexistence of several egg-laying queens might have been secondarily selected and maintained in ants because it provided more genetic variability (e.g. in the ponerine ant *Gnamptogenys striatula* (Blatrix et al. 2000)). Therefore, most of the above introduced hypotheses for explaining the evolution of polyandry via an increase of genetic variability also can be used for ultimately explaining the existence of multiple queens in ant colonies.

Proximately, benefits of cooperation represent major causes for the socialization of multiple queens. For instance in *Messor pergandei* the selective advantage of co-founding new colonies (pleometrosis) "may derive directly from the ability of multiple foundresses to produce a larger brood raiding force" (Rissing and Pollock 1991). Or, in locally restricted polygynous forms of *Pogonomyrmex californicus* the number of queens is positively affecting queen survival and brood production (Johnson 2004). Even (artificial) groups of founding queens from different species (*Pogonomyrmex barbatus* and *P. californicus*) can benefit from cooperating, although here a negative relationship between excavation activity and foundress survival is implemented for one of the two species (Fewell and Page 1999). Therefore, despite its positive effect in incipient queen groups, division of labor also can be negatively influenced by within-group selection due to a cost skew among group members (Cahan and Fewell 2004), emphasizing that “the evolution of social behaviour, rather than promoting cooperation, is driven by the evolution of competitive strategies designed to exploit group members and reduce the risk of being exploited by others” (Helms-Cahan 2001). Accordingly, either monogyny or secondary polygyny are favored by some “ecological prime movers” like competitive pressure or variable resource supply (Hölldobler and Wilson 1977; Hölldobler and Wilson 1990), e.g. in *Temnothorax* species where queen numbers are depending on the limitation of nest sites (Herbers 1986; Foitzik and Heinze 1998).

The increase of genetic variability via an accumulation of several matrilines is not only achieved via a summation of the present queens families, but might be also realized by a forced addition of alien individuals, in ants particularly via common intra- and interspecific slavery (e.g. (Hölldobler and Wilson 1990; Heinze and Keller 2000). Slavery in ants is ranging from occasionally collecting foreign brood as a side-product of intraspecific territorial fighting, to highly specialized and obligate intraspecific slavery (Hölldobler and Wilson 1990). The occurrence of slavery is facilitated in ants as nestmate recognition is based on pheromones, learned by individuals during their ontogeny; therefore, individuals growing up in foreign colonies are learning their new colonies odor as their own (e.g.: Lenoir et al. 2001), acting as if they were in their natal nests.

1.5 Morphological polymorphisms between workers and queens

Many examples are known in ants, where individuals have become specialized for functions such as feeding, cleaning or defense, leading to the fine-tuned task specialization of often thousands of individuals in colonies (Wilson 1978; Hölldobler 1984; Hölldobler and Wilson 1990; Dorit et al. 1991; Hart and Ratnieks 2001). This might be achieved firstly via a temporal division of labor following the needs of the colony (for examples see: Tschinkel 1998; Tschinkel 1999a; Antonialli-Junior and Giannotti 2003; Johnson 2003), a mechanism which is often also called “age polyethism” (Hölldobler and Wilson 1990) or “temporal polyethism” (Tofts and Franks 1992). In contrast a form of “physical polyethism” might exist (Tofts and Franks 1992), i.e. morphological polymorphism predicting the specialization of forms for the division of labor. Many colonial species are characterized by such forms (Dorit et al. 1991).

The morphological differentiation of females into one reproductive caste (queens) and its mostly non-reproductive helpers (workers) is characteristic for the Hymenopteran’s eusociality and related to their different roles in the colony. This type of polymorphism seems to be a widespread phenomenon, as it is found also in other animal orders, e.g. termites (Wilson 1971), or even vertebrates (O'Riain et al. 1996). For ants (Formicidae) size differences between queens and their helpers are higher among independent nest founding species compared to dependent nest founders (Stille 1996).

Therefore, size differences between ant queens and their helpers seem to play a role for their strategies in founding new colonies.

Additionally, distinct morphological differences between queens might be found. For example, in *Atta texana* for this genus unusual small queens are found which initiate nests via primary polygyny and not like most congeners via monogyny. This is adaptive as the multiple-queen strategy results in higher survival rates of *A. texana* founding queens compared to single foundresses (Mintzer 1987). Therefore, a link between gyny-level (number of queens) and queen-size differences might be hypothesized. Moreover, such fundamental changes in breeding systems and life history often are connected to changes in relatedness asymmetry (Chapuisat and Keller 1999). For example in *Hypoponera opacior* colonies were found, headed by single, dealate queens, which did not rear wingless sexuals but presumably reproduced through winged reproductives that mate in nuptial flights; the social structure of those colonies contrasted with nests containing wingless reproductives, which were highly polygynous and polydomous (Foitzik et al. 2002).

The size and morphology of queens is primarily adapted to advance reproductive abilities (Stille 1996). In contrast, workers are adapted to their roles in improving colony efficiency, e.g. nest maintenance, food processing, or defense (Hölldobler and Wilson 1990). In ants these employments sometimes are visible if additional morphological differentiations were developed, e.g. bigger soldiers in *Pheidole* (Wilson 1975; Wilson 1976) or seed millers in *Solenopsis* (Wilson 1978). Morphological caste determination in ants, but also bees and wasps was long teached to be regulated solely “by the presence or absence of certain substances provided in the immature stages by other members of the colony” (Dorit et al. 1991, p.761). However, there are indications that worker caste development can in some ant species also involve a genetic component (Hughes et al. 2003), possibly specifying an individual’s threshold to environmental cues that control caste fate (Page et al. 1989; Hölldobler and Wilson 1990; Wheeler 1991). Behavioral polymorphisms are realized in several social insects for the division of labor, likely involving a genetic component (Page et al. 1989; Rissing et al. 1989; Fewell and Page 1999; Fewell and Page 2000; Kolmer and Heinze 2000; Beshers and Fewell 2001; Fewell 2003), and might represent a first step in the evolution of first genetically and later also morphologically distinguishable specialists.

1.6 Introduction to the study genus *Pogonomyrmex*

The approx. 60 described species of the harvester ant genus *Pogonomyrmex* (Hymenoptera: Formicidae, Myrmicinae) are distributed throughout the arid and semiarid regions of the New World (Taber 1998) (Fig. 1). *Pogonomyrmex* species are known as scavengers and specialists in utilizing the often only temporarily abundant grass seeds (Fig. 2), giving them some ecological and economic importance (MacKay 1990; MacMahon et al. 2000).

Figure 1:

Typical habitat of *P. rugosus* at Coon Bluff Rec. Area near Tempe, AZ (USA)

Figure 2:

Workers of *Pogonomyrmex rugosus* harvesting grass seeds at McCartney Rd.-population near Tempe, AZ (USA) in August 2002.

The genus *Pogonomyrmex* is predisposed for analyzing the evolution of colony characteristics, due to the exceptionally well known biology of several species, in combination with a rare lucidity in species numbers promising good sampling efforts for phylogenetic analyses. Moreover, *Pogonomyrmex* species are covering a broad variance of life history forms, allowing comparative analyses, and tests on their distribution and evolution. Especially the North-American representatives of this genus were subject to extensive, but also quite diverse studies (e.g.: Gordon 1943; Hermann and Blum 1967; Gordon 1984a; Gordon and Hölldobler 1987; MacKay et al. 1992; Gordon 1999; Heinze 1999), and are taxonomically and sociobiologically well analyzed (Cole 1968; Hölldobler and Wilson 1990; Taber 1998). In the following a quick overview of this genus will be given; for additional information it is referred to the books of Cole (1968) or Taber (1998).

The ant genus *Pogonomyrmex* evolved within the ant subfamily Myrmicinae about 30 Mio. years ago in the drought phase of the Oligocene (Kusnezov 1951; Burnham 1978; MacKay 1990; Lattke 2003; Morgan and Czaplewski 2003), in parallel with diversifying deciduous trees, grasses and herbs (Lehmann 1992). Morphologically, most females of the *Pogonomyrmex* harvester ants show adaptations for collecting and processing grass seeds as their main food source, evident in the characteristic big-headed look (Figs. 3 and 4) caused by strong mandibles and mandibular muscles, which are used for processing the collected seeds. Additionally, in most species psammophores are present, i.e. long hairs below the head (Fig. 4) used for gathering small seeds, but additionally helping to increase the efficiency of the transportation of fine sand and pebbles during nest construction (Spangler and Rettenmeyer 1966; Wheeler and Wheeler 1986; Porter and Jorgensen 1990), or to carry eggs (Nagel and Rettenmeyer 1973). The presence of psammophores gave *Pogonomyrmex* its name related to the Greek meaning for “bearded” ant (Taber 1998).

Figure 3:

Workers of *Pogonomyrmex barbatus* at the nest entrance of their colony at Coon Bluff Rec. Area near Tempe, AZ (USA).

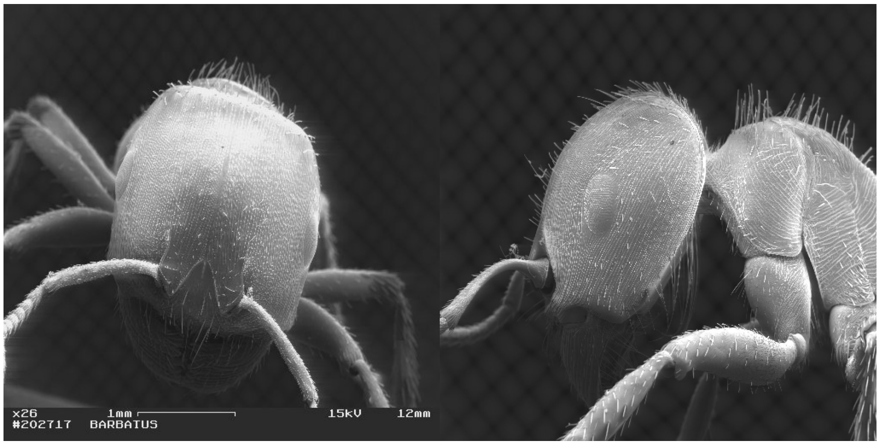


Figure 4:

Pogonomyrmex barbatus, SEM of head of worker (left: frontal, right: lateral)

Several other characters separate the genus *Pogonomyrmex* and its sister genera *Myrmica* and *Hylomyrma* (Taber 1998) taxonomically, from which most strikingly are (Cole 1968): (1) a 12-segmented antennae with a club in females (workers, queens), but a 13-segmented antennae without a club in males; (2) the presence of tibial spures at the mid and hind legs, which are finely pectinate in females but strongly pectinate in males; (3) the absence of a distinct meso-epinotal impression. Queens usually are bigger sized than workers, in *P. occidentalis* leading to a queen-worker thorax volume ratio of 12 (Cole and Wiernasz 2000). Males are variable in their size (MacKay 1980; Davidson 1982; Abell et al. 1999)

Cole (1968) deplored in his revision of the North-American species of the genus “an apparent lack of satisfactory definitive diagnostic characters” in *Pogonomyrmex* “also because of the very puzzling nature of the known distributional patterns” (Cole 1968, p.4). In my work I used the classification systems of Cole (1968) and Taber (1998) with two subgenera, *Pogonomyrmex sensu stricto* and *P. Ephebomyrmex*, with *P. mayri* as the single representative of the former third subgenus *P. Forelomyrmex* (Wheeler 1902) being regarded as member of the subgenus *Ephebomyrmex*. Main differences are evident between the two subgenera (Taber 1998) *sensu stricto* and *Ephebomyrmex* (Cole 1968): In the subgenus *sensu stricto* psammophores usually are bigger, stronger developed, ≤ 7 mandibular teeth are present, and colony size spans up to 10,000 workers, whereas in the subgenus *Ephebomyrmex* psammophores usually are smaller, weaker developed, ≤ 6 mandibular teeth are present, and the colony size spans only ≤ 200 workers.

Due to their sizes, colonies of *Pogonomyrmex sensu stricto* usually constitute prominent landmarks, e.g. those of the western harvester ant *P. occidentalis* (Fig. 5), or of *P. rugosus* (Fig. 6). In contrary, those of *Pogonomyrmex Ephebomyrmex* are inconspicuous, e.g. those of *P. pima* (Fig. 7).

Figure 5:

Prof. Dr. Jürgen Gadau collecting workers from a typical nest-mound of *Pogonomyrmex occidentalis* near Seligman, AZ (USA)

Figure 6:

Nest entrance of *Pogonomyrmex rugosus* (in front) surrounded by a cleared disc area (∅ = approx. 5 m) at Chiricuahua Mts. near Rodeo, NM (USA).

The nests of *P. sensu stricto* colonies consist of a central tunnel system to a depth of about two meters, ramified into several vertical tunnels and chambers in larger nests (e.g.: Lavigne 1969; MacKay 1981; Cole 1994; Gaglio et al. 1998; Tschinkel 2004). Inside nests *Pogonomyrmex* species are storing seed, which might be defended using potent venoms in the sting apparatus (Bernheimer et al. 1980; Schmidt and Schmidt 1989) but see (Brown et al. 1997). Stings of *Pogonomyrmex* usually are painful for humans (Schmidt 1994; Vetter and P.K. 1998) and were ritually used by native Americans, such that its venoms are constituting ”the first well-documented ethnoghraphic example of an hallucinogenic agent of insect origin” (Groark 1996).

Figure 7:

Inconspicuous nest entrance of a *Pogonomyrmex* (*Ephebomyrmex*) *pima* colony (in the center of this picture) at McCartney Rd.-population near Tempe, AZ (USA)

Nest architecture and colony organization seem to be influence by one another. In *P. badius* (Tschinkel 1999a) nest depth increases “dramatically” with colony size, ranging from 35 cm for incipient colonies to 2.5 m for largest colonies. In parallel with a colonies age chamber numbers and areas increase, with more than half of the total chamber area in the top third of the nest. Most seeds (up to 300,000) are stored in the upper parts of the nest, whereas the main brood mass is found in the lower parts of the nest, where the queen is also found (Tschinkel 1999a; Tschinkel 1999b), and which is typical also for other species than *P. badius* (MacKay 1981; Gordon 1992). The mean age of workers increases in a linear fashion between the nest bottom and the surface, suggesting that workers constantly move upward with age (Tschinkel 1999a). This kind of age polyethism is caused by a preference of young workers for high carbon dioxide concentrations, accompanied by a tendency for workers to dig more under low carbon dioxide concentrations, additionally explaining the top-heaviness of the nest's architecture in *P. badius* (Tschinkel 2004). Additionally, as in *P. badius* major and minor workers are produced in constant rates regardless of a colonies size (Tschinkel 1999b), an additional form of physical polyethism with underlying genetic components might be thinkable.

The structure of the nest's surface is an adaptation for achieving comfortable temperatures for foragers (McCook 1882) and brood (Anderson and Munger 2003), and maybe also achieving a comfortable humidity as it is significantly affecting water infiltration after rainfalls (Blom et al. 1994). Nest architecture is even believed to have protective functions against ants of other species, e.g. in *P. badius* where charcoal is used as a deterrent (Gordon 1984a). As nest size below surface, also surface structures like cones or cleared disc areas are positively correlated with the colonies size and age (Gordon 1992; Wiernasz et al. 1995; Cole and Wiernasz 1999).

Food is transformed in worker fat reserves for over-wintering, and for producing the next year's sexuals (MacKay and MacKay 1984; Tschinkel 1998). Seeds are therefore not stored as readily available resources, but moreover seem to represent a “protection against predation, especially by spiders” (MacKay and MacKay 1984). This might explain why in *P. desertorum* neither present levels of predation nor scarcity of food limit reproductive output (Munger 1992 1217). Selection therefore might be subjected to the toughness of colonies as their ability to use and store collected food resources (seeds), but also to hold their foraging fields against competitive neighbouring colonies and predators. The last is evident for several *Pogonomyrmex* species, where frequent encounters between foragers set up a marked and well analyzed territoriality (e.g.: Hölldobler 1974; Hölldobler 1976b; Davidson 1977; Davidson 1980; Harrison and Gentry 1981; Gordon 1984a; Gordon 1984b; Gordon 1993; Schilder 1994; Gordon and Kulig 1996), with pheromones playing an important and well known role (e.g.: Hölldobler and Wilson 1970; Hölldobler 1971; Blum 1974; Lindauer 1991, p.147f; Ruano et al. 2000; Hölldobler et al. 2001; Tissot et al. 2001; Liu and Liu 2002; Greene and Gordon 2003). However, predispositions influencing intercolonial encounters can differ for colonies (Fig. 8), e.g. caused by the social environment via differences in the stimulation of nestmates (Haefner and Crist 1994), or by the colonies age and size (Gordon and Kulig 1996), modified by intracolonial uniformity (Cole and Wiernasz 1999).

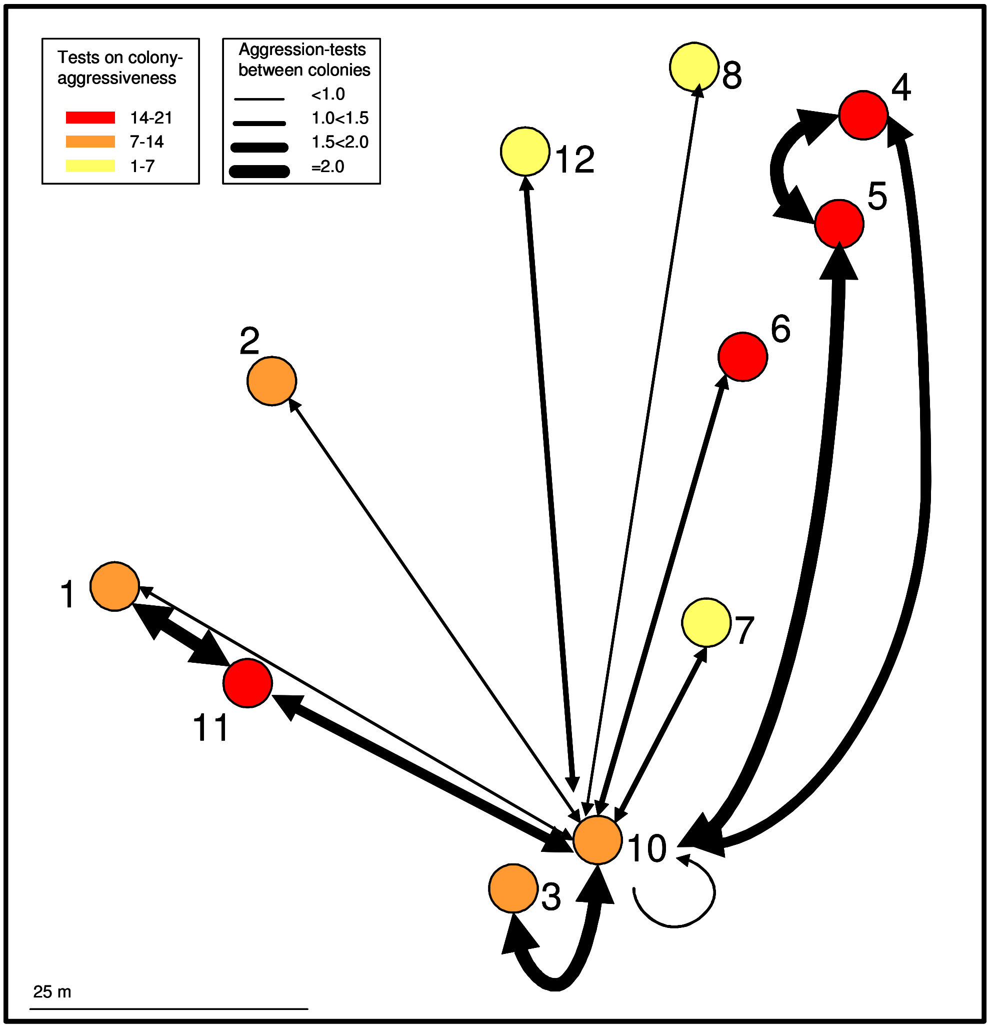


Figure 8:

Pogonomyrmex rugosus colonies (numbered circles) vary in their levels of aggressiveness: Preliminary results of aggression tests for measuring predispositions and intercolonial outcomes at Coon Bluff Rec. area near Tempe, AZ (USA) obtained from students during a practical course in August 2002 (*unpublished*; boxes: increasing numbers indicate increasing levels of aggressiveness, derived from categorized inter-individual interactions, and from measurements of a general alarm response of the colony against disturbance with a wet leather belt).

The distances between nests are regulated by competition for food (Ryti and Case 1984), and so the recruitment of new colonies is more depending on locations of already established colonies than colony densities (Schooley and Wiens 2003). Whereas in *P. sensu stricto* species an independent colony founding by single queens (primary monogyny) seems to be the normal case (Nagel and Rettenmeyer 1973; Hölldobler and Wilson 1990; Fewell and Page 1999; Cole and Wiernasz 2000; Julian and Gronenberg 2002), in *P. Ephebomyrmex* species the existence of multiple, sometimes "intermorphic" queens, i.e. intermediates between workers and queens (Heinze 1998), might be an indication of a distinct founding strategy (Creighton 1956; Kugler 1978; Heinze et al. 1992; Schilder 1994). Two different modes of a dependent colony founding tactic are thinkable: (1) dispersal of queens in a stable environment via budding of established nests, (2) dispersal in a temporal variable environment by (re-)adoption of queens into established nests (Peeters and Ito 2001). Both, budding and adoption will converge in both types of environments to the occurrence of a smaller, energy-saving queen morph. Therefore, the phenotypic variability of queen morphs, as in *P. Ephebomyrmex* species, is symptomatic for species with dependent colony founding.

Despite the knowledge on the colony characteristics of *Pogonomyrmex* species in North America, by start of the present study in 2000 only some have been sociogenetically analyzed (Schilder 1994; Cole and Wiernasz 1999). For those species where it is known, hypotheses on the intracolonial relatedness can be made by the number of queens inside colonies and by behavioral observations of their mating behavior (e.g.: Hölldobler and Wilson 1990; Crozier and Pamilo 1996; Taber 1998). However, given the importance of relatedness for understanding the evolution of the various characteristics of this ecologically important genus, precise data on the expression, extent and abundance of genetic variability are needed; current knowledge on this topic is summarized in the following paragraphs.

Monogyny is reported for several *Pogonomyrmex* species of the North-American *sensu stricto* species (Cole and Wiernasz 2000), whereas polygyny is rarely observed for *sensu stricto* species (Cole 1968). An effective share of egg-laying (functional polygyny) of multiple queens is confirmed only for localized populations in the Californian harvester ant, *P.* (*s.str.*) *californicus* (Johnson 2004). In contrast, multiple queens were observed several times in the subgenus *Ephebomyrmex* (Kusnezov 1951; Kugler 1978; Heinze et al. 1992; Heinze 1998). However, it was not tested if this polygyny in *Ephebomyrmex* species is leading to functional polygyny and thus increases intracolonial genetic variability.

Mating behavior in *Pogonomyrmex* is known for the North-American representatives of the subgenus *sensu stricto*, which is exceptional for eusocial Hymenoptera because males are unusually large, retain strong mandibles and show a promiscuous mating behavior. Typically they establish treetop mating aggregations (Michener 1960) or huge mating leks on the ground (e.g. (Willard and Crowell 1965; Hölldobler 1976a) to which the females fly and mate (but see: *P. anergismus*; Johnson 1994), *P. colei*: (Johnson et al. 1996) and *P. badius*: (Van Pelt 1953; Harmon 1993). At the mating leks strong competition is reported between males (Davidson 1982), occasionally resulting in fatalistic injuries of females during mating, for which females have evolved a specific "liberation" signal to terminate copulation (Hölldobler 1976a; Markl et al. 1977). Observations of matings at the leks point to extremely high mating frequencies. Recently, effective multiple mating of females was confirmed by the use of molecular markers for the North-American species *P. barbatus* (Volny and Gordon 2002), *P. occidentalis* (Cole and Wiernasz 2000; Wiernasz et al. 2004), and *P. huachucanus* (J. Gadau and C.-P. Strehl, *unpublished*), and for the South-American species *P. inermis* (J. Gadau and R. Pol, *unpublished*).

For two of the species with proofed effective queen multiple mating also hybridization is reported: *P. barbatus* (hybridizing with *P. rugosus* (Helms-Cahan et al. 2002; Julian et al. 2002) and *P. occidentalis* (hybridizing with *P. maricopa*: Cole 1968; Anderson 2002). In *P. barbatus* a broad zone of overlap with *P. rugosus* exists, where hybridization is well documented by both behavioral observations at mating leks and molecular markers (RAPD, allozymes, microsatellites, mtDNA). Three independent studies of this hybrid zone at a site in southeastern Arizona/USA (Fig. 6) found that all queens and sexuals were homozygous for species-specific markers whereas workers from the same colonies were exclusively heterozygous for the same markers (Helms-Cahan et al. 2002; Julian et al. 2002; Volny and Gordon 2002), suggesting a system of genetic caste determination.

However, an obligate production (Helms-Cahan and Keller 2003) of sexuals due to this genetic caste determination system would bear a clear fitness-cost to small founding colonies for hybridizing *P. barbatus* and *P. rugosus*. At this critical phase in a colony's life competition with neighboring colonies is highest and growth of the colony (Cole and Wiernasz 1999) is the key to its survival. Therefore, the detrimental production of sexuals in founding colonies has to be costly prevented. Although in laboratory reared *P. rugosus* colonies from central Arizona/USA (Fig. 1) female sexuals were raised in rare numbers after 8-9 months (own observations), in fact early founding colonies of hybridizing *P. barbatus* and *P. rugosus* were so far not observed producing sexuals in nature. This absence of sexuals in early *P. barbatus* x *P. rugosus* colonies might be due to selective infanticide of homozygous brood, or ontogenetically driven due to differential survival of reproductive and non-reproductive brood (Strehl et al. 2003). In founding colonies from hybrid populations homozygous individuals do occur among the brood, but are lacking among the first adult workers and seem to be physically eliminated by the workers, the queen, or both (Strehl et al. 2003).

1.7 Outline of the thesis

The genetic composition of colonies due to multiple mating and polygyny are considered to be main elements driving the evolution of several colony characteristics. For understanding the origins, distribution and outcomes of low intracolonial relatedness in the ant genus *Pogonomyrmex*, three species were analyzed: *P. rugosus*, *P. badius* and *P.* (*Ephebomyrmex*) *pima*. They were chosen for exceptional knowledge of their life histories (e.g. *P. rugosus*), unique distributional patterns (*P. badius*), distinct features (e.g. in *P. badius*: worker polymorphism and mating on top of the colony), or accessibility (e.g. *P. pima*). Additional samples covering a broad taxonomic range from several *Pogonomyrmex* species were collected to construct a gene-tree, and to integrate the known subgenera and complexes into this phylogenetic hypothesis. Several studies were conducted to solve the following questions:

1. Is *Pogonomyrmex* a monophyletic group, or should *P. Ephebomyrmex* and *P. sensu stricto* species alternatively be placed into separate genera?
2. How do the sociogenetic structures vary in the genus *Pogonomyrmex* (sensu Cole 1968), e.g. in *P. badius* due to worker polymorphism, in *P. rugosus* as typical representative of the subgenus *sensu stricto*, and in *P.* (*E.*) *pima* as representative of the subgenus *Ephebomyrmex*? It therefore was asked:
    - To what extend is polygyny found in populations of the subgenus *Ephebomyrmex*?
    - Is polyandry a plesiomorphic trait for the whole genus *Pogonomyrmex*?

  - What is the effective mating frequency of *Pogonomyrmex* queens?

3. Are all patrilines evenly distributed among workers of colonies in polyandric *Pogonomyrmex*, and is there a genetic influence of distinct patrilines on worker phenotypes in *P. badius*?
4. Has restricted dispersal and inbreeding in *P. badius* a noticeable influence on its colonization pattern?
5. Are intermorphic queens in the subgenus *P. Ephebomyrmex* produced as a morph for short-range dispersal?

These questions are addressed in the following chapters, which are organized as independent manuscripts.

Particularly, in chapter 9 results of the phylogenetic analyses are presented, the monophyly of the genus *Pogonomyrmex* is discussed, and multiple mating is recognized as possibly invented during the evolution of the *sensu stricto* clade(s). Chapter 6 is focusing on the biogeography of *Pogonomyrmex badius* and is discussing the impact of the mating behavior of this species for its colonization pattern.

Colony structure, the extent of polygyny and the presence of queen dimorphism in *P.* (*Ephebomyrmex*) *pima* are documented in chapter 7. To analyze the extent of polyandry throughout *Pogonomyrmex* this species was also studied sociogenetically, which is documented in chapter 8. In both chapters the impact of intracolonial organization on reproductive output and dispersal strategies is discussed.

In chapters 3 and 4 high effective multiple mating of queens is shown for two *Pogonomyrmex sensu stricto* species, *P. badius* and *P. rugosus*. For both species significant differences among males in successfully fathering offspring are also documented. Occasional brood raids as an additional determinant of intracolonial relatedness in *P. rugosus* are introduced in chapter 3. Finally, for *P. badius* it is shown in chapter 5 that the patrilineal inheritance of a genetic component is determining worker polymorphism.

The present set of analyses was planned to reveal some evolutionary mechanisms which have led through the river of time to the current colony characteristics of *Pogonomyrmex* harvester ants. The results hopefully will give also insights into factors influencing the colonial organization of other ant species.

1.8 Publications

At the date of this thesis delivery chapters 3-9 are either published, or submitted, or intended for publication as follows:

Chapter 3: Gadau J, Strehl C-P, Oettler J, Hölldobler B (2003) Determinants of intracolonial relatedness in *Pogonomyrmex rugosus* (Hymenoptera; Formicidae): mating frequency and brood raids. Molecular Ecology 12:1931-1938

Chapter 4: Rheindt FE, Gadau J, Strehl C-P, Hölldobler B (2004) Extremely high mating frequency in the Florida harvester ant (*Pogonomyrmex badius*). Behavioral Ecology and Sociobiology 56:472-481

Chapter 5: Rheindt FE, Strehl C-P, Gadau J (2005) A genetic component in the determination of worker polymorphism in the Florida harvester ant *Pogonomyrmex badius*. Insectes Sociaux 52:1-6

Chapter 6: Strehl C-P, Gadau J (2004) Cladistic Analysis of Paleo-Island Populations of the Florida Harvester Ant (Hymneoptera: Formicidae) Based Upon Divergence of Mitochondrial DNA Sequences. Florida Entomologist 87(4): 576-581

Chapter 7: Johnson RA, Strehl C-P, Gadau J (submitted) Colony Structure and Morphometrics in the Queen Dimorphic Harvester Ant *Pogonomyrmex pima*. Insectes Sociaux

Chapter 8: Strehl C-P, Johnson RA, Gadau J (in prep.) Colony Structure and Sociogenetics of the Queen Dimorphic Harvester Ant, *Pogonomyrmex* (*Ephebomyrmex*) *pima*.

Chapter 9: Strehl C-P, Feldhaar H, Gadau J, Johnson RA, Hölldobler B (in prep.) Phylogeny of the Harvester Ant Genus *Pogonomyrmex* (Hymenoptera: Formicidae) in North-America and its Relation to South-American *Pogonomyrmex* species.

1.9 Literature

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3. Determinants of Intracolonial Relatedness in *Pogonomyrmex rugosus* (Hymenoptera; Formicidae): Mating Frequency and Brood Raids

Published in: Molecular Ecology (2003) 12, 1931–1938

Authors: Jürgen Gadau, Christoph-P. Strehl, Jan Oettler, Berthold Hölldobler
(Jürgen Gadau and Christoph-P. Strehl contributed equally to this publication)

3.1 Abstract

The genus *Pogonomyrmex* is one of three ant genera with an *effective mating frequency* (m_e) > 2.0. We developed microsatellites to determine me for *P. rugosus* because mating frequency of *P. rugosus* was known only from observational data which do not allow an estimate of m_e. We genotyped 474 workers from 20 colonies for two microsatellite loci. Observed mating frequencies ranged from 3 to 12 and me for *P. rugosus* was 4.71. Observed patriline frequencies were significantly different from the expected patriline frequencies generated with a simulated data set under the assumption of equal patriline representation. The available mating frequency data and phylogenetic information of the genus *Pogonomyrmex* suggest that multiple mating is the ancestral state in the North American *Pogonomyrmex sensu stricto*. Established *P. rugosus* colonies raid and destroy smaller conspecific colonies. During these raids ant workers were observed carrying pupae and larvae from the raided colony into the nest of the raiding colony. However, it was not clear whether raided brood emerged in the raiding colony and were subsequently recruited into the work force (intraspecific slavery) or were used as food (predation). Our analyses indicate 6 of 14 field colonies contained foreign *P. rugosus* workers (43%). The range of the intracolonial frequency of foreign workers collected directly from the nest entrance was between 4 and 28%.

3.2 Introduction

Multiple mating by ant queens was long believed to be more common than single mating (e.g. 2/3 of the ant species listed in Hölldobler & Wilson 1990). However, this view shifted completely with the application of highly variable molecular markers and the concept of effective mating frequency (m_e) (Boomsma & Ratnieks 1996). Effective mating frequency or effective paternity frequency is defined as the reciprocal of the sum of the squared proportional paternities. This value is especially important in social insects because it represents average relatedness between workers within a colony. Intracolonial worker relatedness may vary considerably if paternity is unequally distributed between the mates of a queen. For example, if a queen mates twice, the effective mating frequency will be 2 if both males contribute equally to the offspring, but 1.22 if one male sires 90% of the offspring. Differences in *me* and its impact on within-colony genetic conflicts provided a rich source for the investigation of kin selection theories (Hamilton 1964; Ratnieks 1988; Bourke & Franks 1995; Crozier & Pamilo 1996; Strassmann 2001). For example, when workers control sex allocation and realize split sex ratios in response to their mother's mating frequency (Boomsma & Grafen 1990), an evolutionary conflict between queens and males over mating frequency arises because males favour a more female-biased sex ratio than queens because males gain no fitness through male offspring due to the haplo-diploid sex determination system.

Boomsma & Ratnieks (1996) found that only one highly derived eusocial ant taxon, *Atta*, had paternity frequencies > 2. Since then, multiply mated colonies with an *me* > 2.0 have also been demonstrated for *Acromyrmex* (the sister taxon of *Atta*, Bekkevold *et al*. 1999), *Pogonomyrmex occidentalis* and *P. barbatus* (Cole & Wiernasz 1999; Volny & Gordon 2002). Army ant queens of the genus *Eciton* (Rettenmeyer 1963) also appear to mate with multiple males (1–5) but this is based only on behavioural data and needs genetic confirmation.

The life history of *Pogonomyrmex* differs significantly from that of other genera with regular multiple mating, in that *Atta*, *Acromyrmex* and *Eciton* have huge colonies (up to a couple of million individuals), a high degree of worker polymorphism and polyethism, very specialized and derived food sources, fungus growing and very effective mass predation, respectively (see Hölldobler & Wilson 1990; chapters 16 and 17 for details). In contrast, *Pogonomyrmex* has medium-sized colonies (range 603–12 358 individuals, Table 3-2 in Hölldobler & Wilson 1990) and only 2 of the 60 described species in this genus show worker polymorphism (Taber 1998). Most *Pogonomyrmex* species live in dry or semidry areas and are dominant seed harvesters of the North American desert and grassland habitats. *P. rugosus* is widespread in the deserts and grasslands of the south-central and southwestern USA (from central Texas to California) and Northern Mexico

(Johnson 2000). Like most other species of *Pogonomyrmex*, *P. rugosus* is strictly monogynous (MacKay 1981), i.e. one queen per colony, and typically occurs in dense and overdispersed populations.

Molecular markers were used to estimate *me* in two *Pogonomyrmex* species *P. occidentalis me* = 6.8 (BJ Cole & Wiernasz 2000), and *P. barbatus me* = 3.34 (Volny & Gordon 2002). Hölldobler (1976a) observed 4–5 matings per queen in *P. rugosus*. The observed variance in mating frequency within and between *Pogonomyrmex* species raises the possibility of analysing the proximate causes and consequences of high and low mating frequencies and testing several proposed ultimate mechanisms for the evolution of multiple mating. In order to do this we need exact intracolonial estimates of the number and frequency of patrilines. Therefore, we developed microsatellites for *P. rugosus* and genotyped 20 colonies to determine the number and frequency of intracolonial patrilines and estimated *me* for *P. rugosus*. Six of these colonies were derived from a single founding queen and reared in the laboratory. We also tested whether patrilines were equally represented in workers because we knew that mating time differed significantly between consecutive matings (Hölldobler 1976a).

Hölldobler & Markl (1989) observed 10 interspecific mixed colonies (*P. rugosus* and *P. barbatus*) that contained 3–11% foreign workers. In all 10 colonies, *P. barbatus* workers were incorporated into *P. rugosus* colonies. Hölldobler & Markl (1989) suggested that these interspecific mixed colonies originated from territorial raids. Adult workers from intraspecific raids cannot be distinguished morphologically, but we suspected that the percentage of mixed colonies and the frequency of raided workers in *P. rugosus* colonies was significantly higher than the estimates derived from interspecifically mixed colonies. To detect intraspecific slavery we analysed workers collected directly from the colony entrance of 14 field colonies.

3.3 Materials and methods

Samples

Specimens were collected in 1999 (workers from 3 field colonies) and in 2000 (workers from 11 field colonies and 6 laboratory-reared colonies, derived from founding queens) from a single population near Tempe, AZ (USA) (Coon’s Bluff; N 33°32’40.7’’, W 111°38’3.6’’). Workers were collected directly in 95% ethanol from trunk trails (1999) or directly from nest entrances (2000). Founding queens were brought into the laboratory and raised under constant temperature (30 °C) and a 12:12 h light/dark regime.

DNA analysis

DNA was isolated using phenol–chloroform extraction following Landry *et al.* (1993) for the specimens collected and analysed in 1999. For all other individuals a modified version of the Puregene Kit protocol (Gentra Systems/Biozym, Germany) was used.

For the Puregene method individual workers (without gaster) were ground in liquid nitrogen. Then 100 µL cell lysis solution DNA (Biozym) was added and incubated at 65 °C for 1 h. After incubation, 34 µL of protein precipitation solution was added, the combined solution was briefly vortexed and kept on ice for 5 min. After centrifugation (21910 *g* for 5 min) the solution was transferred to another vial and the pellet discarded. DNA was precipitated with isopropanol/ethanol, dried by vacuum centrifugation and dissolved in 50 µL low TE buffer.

Microsatellite development

Microsatellites were isolated using a nonradioactive detection method developed by Toonen [Robert J. Microsatellites for Ecologists: Non-Radioactive Isolation and Amplification Protocols for microsatellite markers, web document available from the author or from http://biogeek.ucdavis.edu/Msats/(IP: 169.237.66.129)].

Polymerase chain reactions/gel electrophoresis

Polymerase chain reaction (PCR) amplifications were performed in 25 µL reaction volumes at heating rates of 5 °C/s (thermocycler of Biometra, Germany). The reaction mix contained approx. 4 ng genomic (DNA), 2.5 µL 10xPCR buffer [750 mm Tris–HCl, 200 mm (NH4)2SO4, 0.01% Tween 20], 0.4 mm of each dNTP, 1.25 mm MgCl2, 0.2 µL *Taq* DNA polymerase (5 U/µL, MBI Fermentas) and 0.5 µM of each primer. For the analysis two newly developed microsatellite loci (*PR1* and *PR2*) and one locus developed for *Leptothorax acervorum* (*LxA GT1*) (Bourke *et al.* 1997) were used (Table 1). All loci were amplified using the following parameters: 33 cycles of 30 s at 94 °C, 1 min at the annealing temperature (Table 1), and 1 min (plus 1 s per cycle) at 72 °C; an initial denaturation step of 3 min at 94 °C and a final elongation step of 1 min at 72 °C was added.

PCR products in the analyses conducted in 1999 by Jan Oettler at the Arizona State University Tempe (three colonies labelled JO, Table 2) were radioactively labelled, separated and genotyped on denaturing polyacrylamide gels (4%). These colonies were used only to derive the intracolonial number and frequency of patrilines and not to calculate heterozygosities and regression relatedness.

PCR products for the colonies collected in 2000 (*n* = 17) were analysed using a submerged gel electrophoresis system from Elchrom Scientific AG. For the separation Elchrom®-Spreadex® EL 600 or 800 precast gels were used. Gels were stained with SYBR®-green I Nucleic Acid Gel Stain (BMA/BioWhittaker Molecular Applications).

Alleles were named relative to each other by running all putative alleles of each locus at least once side by side on a single gel.

Estimating mating frequency

Two methods were used to calculate the effective mating frequency. First, from the worker genotypes a minimum number and frequency of patrilines per colony were deduced (the pedigree effective mate number, Pedersen & Boomsma 1999; patrilines in Table 2). Patriline frequencies (p_i) were then used to calculate the effective number of matings for each colony [$m_e = 1/\Sigma(p_i)2$; Starr 1984]. Average worker–worker relatedness for each colony was determined assuming that fathers were unrelated [with $g_{ww} = 1/4 + (0.5*(1/ m_e)$; Pamilo 1993]. We excluded foreign workers and then calculated the mean and SD of the relatedness values across all colonies. Second, average intracolonial worker relatedness (the genetical effective mate number, Pedersen & Boomsma 1999) was calculated using relatedness 4.2c (Queller & Goodnight 1989; Goodnight 2001). Average intracolonial relatedness was then used to calculate m_e using the above formula for g_{ww}. We also used this program to calculate allele frequencies and an inbreeding coefficient (F_{IS}). The standard error of the estimate was obtained by jackknifing over colonies and was used to test whether the estimated F_{IS} was significantly different from 0 using a *t*-test (Sokal & Rohlf 1995; Box 7.2). Expected and observed heterozygosity for each locus was determined using the program gda (Lewis & Zaykin 2001).

Test for equal distribution of patrilines

To estimate the effect of sampling error on the paternity bias observed in our colonies, we compared our data with a random data set generated using statistica 5.5

(StatSoft Inc.) and analysed using the same program and Microsoft Excel 97 (Microsoft Corporation). The procedure was as follows:

1. Subsequent blocks of 2000 numbers ('workers') were assigned to five classes (five 'patrilines'). This generated a data set with 10 000 numbers ('colony' with 10,000 'workers').

2. Then 1000 data sets were generated by drawing 20 numbers (workers) between 1 and 10 000 using a random number generator implemented in statistica 5.5.

3. In these 1000 data sets patrilines were ranked according to their frequency, and mean ±SD were calculated for each of the five patriline classes.

4. A Mann–Whitney *U*-test was used to compare the observed and simulated patriline classes. Significant differences between the observed and simulated patrilines frequencies in each patriline class indicate that the observed skew in patriline distribution is a characteristic of the mating behaviour in *Pogonomyrmex rugosus* and not caused by sampling error.

Detecting foreign workers

Excavations of numerous mature colonies of *P. rugosus* (B. Hölldobler unpublished data and MacKay 1981) did not find multiple queens (see also discussion for further information). Hence, we assumed that *P. rugosus* is strictly monogynous. Also, workers are able to reproduce, they produce only male offspring because they cannot mate. Therefore, foreign workers were defined as workers with genotypes that cannot be explained by a single mother. For example, three worker genotypes, each homozygous for three different alleles, cannot be explained by a single heterozygous mother. We analysed all worker genotypes using this method and whenever we found worker genotypes incompatible with a single matriline, workers from the lowest frequency matriline were defined as foreign workers.

3.4 Results

The sequences of the two newly developed microsatellitesare available in GenBank (Accession nos AF521193, AF521194). All three microsatellites used were highly variable with observed heterozygosity (H_O) levels > 0.78 (Table 1). Most colonies (n = 16) were analysed with two loci (the remaining four colonies were analysed using a single microsatellite locus). Consequently, the nondetection probability of a second male because he has the same allele as the first male was < 5% [$P_{\text{non-det}} = (1 -$

$\mathrm{Het}_{\mathrm{exp/Pr1}})(1 - \mathrm{Het}_{\mathrm{exp/Pr2}})] = 0.035$). However, because we have multiple patrilines, absolute nondetection probability is > 0.035.

Nevertheless, the average decrease of the intracolonial relatedness between workers is only slight, from 0.42 to 0.375 ($r = 1/2m_e + 0.25$) with the addition of a fourth patriline (the minimum number of patrilines we detected in our samples was three). Therefore, even if we overlooked an additional patriline due to nondetection, we can neglect its effect on intracolonial worker–worker relatedness.

Mating frequency

We genotyped 474 *Pogonomyrmex rugosus* workers from 20 colonies (Table 1), 6 laboratory (started from single founding queens) and 14 field colonies, to calculate the effective mating frequency (m_e). In addition, frequency of foreign workers was analysed in these 14 field colonies. The intracolonial worker relatedness estimates derived from the minimum patriline estimates ($g_{\mathrm{ww}} = 0.373$; Table 2) and calculated using Relatedness 4.2 ($R = 0.384$; Table 2) were almost identical. Based on these relatedness estimates, m_e was 4.71 (Table 2). Mating frequency ranged between 3 and 12. Mating frequency was not significantly different ($U = 37.5$, $p = 0.71$; Mann–Whitney U-test) between the laboratory-reared ($n = 6$) and field colonies ($n = 14$).

The observed frequency distribution of the five most frequent patrilines within colonies is shown in Fig. 1. Only the first five patriline classes were significantly different from each other (Mann–Whitney U-test; Fig. 1) supporting our interpretation of an unequal patriline representation. All comparisons between the observed and simulated patrilines within each patriline class were significantly different (all $P < 0.01$, Mann–Whitney U-tests), meaning that the differences between the observed and simulated patrilines are not due to sampling error.

Inbreeding

None of the used microsatellite loci showed an inbreeding coefficient significant different from 0.

Foreign workers

Six of fourteen field colonies contained, according to our definition, foreign workers (43%, Table 2). The range of the intracolonial frequency of foreign workers was between 4 and 28% with an average of 12.8%.

3.5 Discussion

Mating frequency in Pogonomyrmex rugosus

Hölldobler (1976a) reported, based on mating observations, that *Pogonomyrmex rugosus* queens mate with 4–5 males. We corroborated multiple mating but found up to 12 patrilines in individual colonies (Table 2). Effective mating frequency (m_e) derived from intracolonial worker relatedness was calculated to be 4.71 ± 2.02 (Table 2). With an average of 24 analysed workers the effect of sampling error, i.e. not sampling rare patrilines, seems to be limited, according to simulations for an effective paternity frequency of $n = 5$ in honeybee colonies (Tarpy & Nielsen 2002). Multiple mating seems to be obligatory in *P. rugosus* because none of our queens mated with only one male. Thus, together with *P. occidentalis* and *P. barbatus*, *P. rugosus* is the third *Pogonomyrmex* species with a confirmed $m_e > 2.0$. This result also suggests that the observational data on mating frequency in *Pogonomyrmex* reviewed in Hölldobler & Wilson (1990; p. 156) are reliable and that probably four additional *Pogonomyrmex* species (*P. desertorum, P. californicus, P. badius* and *P. maricopa*) are multiply mated. This means, that in each species complex of the North American subgenus *P. sensu stricto* with 24 described species (AC Cole 1968; Taber 1990, 1998), is at least one species that is highly polyandrous. So, multiple mating seems to be a plesiomorphic character for the North American subgenus *P. sensu stricto*. It will be interesting to see whether *P. huachucanus*, as the basal taxon of the North American *sensu stricto* species or the whole subgenus *Ephebomyrmex (*for phylogenies see Taber 1998; Parker & Rissing 2002), is mono- or polyandrous as this would allow an exact estimate when multiple mating evolved in *Pogonomyrmex*.

A comparison between *Pogonomyrmex* and the higher leafcutter ants *Acromyrmex* and *Atta (*the only other proven monophyletic ant genera with an effective mating frequency > 2.0) reveals striking differences between these genera. *Pogonomyrmex* has no worker polymorphism (with the exception of *P. badius* in North America), smaller colonies and probably less complex division of labour (Hölldobler & Wilson 1990). However, all three genera are food specialists and rely heavily on a variety of plant-derived food sources, leaves in the case of the leafcutters *Acromyrmex* and *Atta*, and seeds in the harvester ant genus *Pogonomyrmex*. Most plants contain a high number of secondary compounds, some of which need to be detoxified. Consequently, organisms feeding on these plants might actually benefit from a higher genetic variability. If this is true, then we would expect that other harvester ants such as *Messor* or *Pheidole* species should also have a higher intracolonial genetic diversity, either through polygyny or polyandry.

Copulation time of *P. rugosus* males decreases with mating number, i.e. the first male copulates on average for 12.4 ± 5.5 min (n = 9), second male 7.2 ± 5.2 min (n = 9) and third male 5.8 ± 3.7 min (n = 7) (Hölldobler 1976a). This result correlates well with our finding that patrilines are not equally represented within colonies. The most frequent patriline had a mean representation of 34% which gradually decreased to approx.10% in the fourth patriline (Fig. 1). The significant differences between the observed and simulated data sets (which were generated using an unbiased worker population) in patriline representation suggest that the earlier observed bias in mating time (Hölldobler 1976a) is also reflected in the observed biased patriline representation of our data (Fig. 1). This might explain the fierce competition between *P. rugosus* males to remain in copula with the female as long as possible. However, to demonstrate firmly that copulation time is correlated with patriline representation requires sampling individual males which mated with a queen, timing their copulations, then determining patriline representation in workers produced by this queen. An alternative explanation could be sperm clumping. To test this possibility we would need to resample the same colonies over time. This will be tested in the future using the laboratory colonies. Sperm clumping seems very unlikely over the long-term, and evidence for this is not present/not convincing in a well-studied social insect, the honeybee (e.g. Haberl & Tautz 1998).

Mated queens of *P. rugosus* contain an average of 2.8 million sperm. In contrast, *P. rugosus* males have an average of 11 million sperm (R. A. Johnson unpublished). Because male spermatogenesis is terminated after maturation in most ants there are two possibilities to explain this finding. Either males inject all of their sperm but the queen discards most of it, or males inject only a small portion of their sperm, and try to mate again with a different queen. The first hypothesis is unlikely because *P. rugosus* males do not die immediately after copulation like honeybee drones and have been suspected to mate multiply (Hölldobler 1976a; Hölldobler & Bartz 1985). Therefore, the mating system of *P. rugosus* is most likely promiscuous and probably very similar to the system described for *Acromyrmex versicolor* (Reichardt & Wheeler 1996). Nevertheless, because queens mate on average with 4-5 males ($m_e = 4.71$) there is a possibility of sperm competition. In the future, we need to look for other proximate and ultimate mechanisms of the unequal patriline representation.

There are three main hypotheses for multiple mating in social Hymenoptera. First, the sperm limitation hypothesis (Cole 1983), a single male cannot provide enough sperm to completely fertilize a female. Second, the convenience hypothesis, females mate multiply because it is forced on them by males (Alcock et al. 1978). Third, the genetic diversity hypothesis, an increase in intracolonial genetic diversity is beneficial for a colony because of either an increase in disease resistance (Hamilton 1987; Schmid-Hempel 1994), facilitatation of task differentiation (Starr 1984), or prevention of negative inbreeding effects due to diploid male production (Page 1980).

Why is *Pogonomyrmex* multiple mated? As argued above, multiple mating seems to be an ancestral character of the North American subgenus *Pogonomyrmex*, and probably evolved early in the evolution of this genus. Therefore, the ultimate cause for the evolution of multiple mating in this group is hidden in the past. However, *Pogonomyrmex* might be a suitable genus to explore the adaptive significance of multiple mating because the species seem to vary in their average mating frequency (Hölldobler & Wilson 1990). This variation, in combination with a phylogeny of the genus and ecological and life history analysis, may reveal the selective forces leading to an increase or decrease in mating frequency in *Pogonomyrmex*.

We can exclude the 'sperm limitation hypothesis' as a valid explanation, because males have approximately three times more sperm than a female can store (R. A. Johnson unpublished). The 'convenience hypothesis' might explain some of the observed variance in mating frequency, but does not explain the absence of singly mated females among successful foundresses if queens do not want to mate multiply. Also, because hymenopteran females need to move their sting aside in order to copulate, the 'convenience hypothesis' is an unlikely explanation for multiple mating in Hymenoptera in general, and *P. rugosus* in particular (Strassmann 2001). Most likely, one or more variations of the genetic diversity hypothesis is/are good candidate/s. In *P. occidentalis* a lower intracolonial relatedness is positively correlated with colony growth rates (BJ Cole & Wiernasz 1999). If the frequency of intraspecific brood raiding in *P. occidentalis* is comparable with *P. rugosus* (see below), then this correlation may not be a selective advantage (BJ Cole & Wiernasz 1999), but may be an artefact of the higher raiding frequency of larger and more successful colonies. Finally, we know nothing about the parasite load of these species which might be an important factor influencing colony size and intracolonial relatedness and should be analysed in the future.

Intraspecific slavery

There is not a single report of a polygynous colony in *P. rugosus*, although multiple researchers have excavated numerous colonies. During 1971-94 we collected a total of 168 founding colonies of *P. rugosus* in the Rodeo Desert (Rodeo, AZ). Furthermore, during the same period we excavated 42 *P. rugosus* nests of different sizes, at least 8 of which had the size of a mature colony. In 36 colonies we found a single queen. In the other colonies we were unable to detect any queen. We probably missed the resident queen. We found a single queen in five of the eight large colonies. In three of those colonies we failed to capture the queen. Interestingly, these three colonies were among the first we excavated; we probably lacked experience and had not yet developed our screening technique sufficiently. Excavations conducted later were more successful in detecting the queen. MacKay (1981) reported the excavation of 20 mature *P. rugosus* nests. He found a single queen in 16; no queen was detected in the remaining 4. We excavated an additional 187 founding colonies in 2000 and 2002 from the same population used in this study on mating frequency (Coon's Bluff, AZ). All colonies had one queen.

Also, all attempts to rear multiple queen colonies in the laboratory have failed (R. A. Johnson & K. Anderson unpublished data).

Therefore, polygyny is unlikely to explain the occurrence of a second matriline in our field colonies and this second matriline is more readily explained by brood raiding.

Although intra- and interspecific raids including brood raiding have been described for *P. rugosus* (Hölldobler & Markl 1989) it was not clear how this changes the genetic composition of individual colonies, and whether it is a significant factor influencing the genetic structure of *P. rugosus* colonies. We found it surprising that 43% (6/14) of our field colonies contained foreign workers because in contrast to *Myrmecocystus mimicus* (Hölldobler 1976b, 1981), *P. rugosus* has not developed special behavioural adaptations for intraspecific competition.

It is not clear whether our results are representative of the whole colony, because we collected workers only from the entrance which are predominantly foragers. Because raided workers may be over-represented in the foraging caste, our estimated frequency of foreign workers may be too high.

Although change in genetic structure due to presence of foreign workers is transient, foreign workers gradually die out, this may have a short-term impact on colony sex ratio. For example, Evans (1995) added foreign brood to colonies of *Myrmica tahoensis* which shifted the colony sex ratio of the reproductive caste towards males, as sex ratio theory predicts. The intracolonial frequency of foreign workers found in our colonies was sometimes high, reaching 28% in one colony (mean, 12.8%; range 4-28%). To illustrate the effect of foreign workers on the worker-worker relatedness of a colony, we calculated intracolonial worker relatedness with and without the foreign workers. In two colonies (JG35 and JG40, Table 2) we identified 15% foreign workers, a ratio that came closest to the average percentage of foreign workers (12.8%) found in all tested colonies. Average intracolonial worker relatedness calculated (using RELATEDNESS 4.2c) with and without these workers decreased from 0.372 to 0.294 and 0.270 to 0.212, respectively. This is a significant decrease in intracolonial relatedness compared with the relatedness differences in *P. occidentalis* given in BJ Cole and Wiernasz (1999; Fig. 3) where most intracolonial relatedness values were between 0.2 and 0.4. Therefore, the presence of foreign workers in *P. rugosus* can temporarily influence the outcome of intracolonial conflicts and might also have an effect on the colony recognition system.

The ant genus *Pogonomyrmex* is exceptional within the Formicidae with respect to its high m_e . However, this is not the only factor influencing the intracolonial relatedness in this genus. Owing to the high population densities in some species, intraspecific competition is very intense and brood raiding including the adoption of the raided brood might be an important aspect both for the energy budget and the genetic composition of a colony. Therefore, any correlation of genetic diversity with other important life history factors in *Pogonomyrmex* needs to be checked for the influence of brood raiding to avoid artifacts.

3.6 Acknowledgements

We thank R. A. Johnson for his support during long hours of field work, his comments on this manuscript and allowing us to use his unpublished data on sperm counts in males and queens. JG thanks Rob Toonen for his help developing the microsatellites at the UC Davis. CPS thanks Michaela Strätz (Regensburg) for support in microsatellite analysis. We thank two anonymous referees and K. Anderson for comments on an earlier version of our manuscript. This study is supported by grants of the Deutsche Forschungsgemeinschaft GA 661/1-2 and SFB 554-TP C-5. JG and BH acknowledge the Santa Fe Institute (SFI) for providing the opportunity to discuss these issues in a working group of SFI.

3.7 Tables

(following pages)

Table 1

Summary of the characteristics of the used microsatellite loci. The population-wide F_{IS} for both loci is 0.09 ± 0.05

Locus	**F primer/R primer (5' → 3')**	**Annealing temp. (°C)**	**No. alleles (bp)**	**H_O**	**H_E**	**F_{IS} ± SE**	**Individuals**	**Colonies**
PR1	AATCTCAGCAGGCGAGAAAG/	54	9					
	GAGAGCGTAGACGGAAATGC		(355-431)	0.790	0.800	0.021 ± 0.041	286	19
PR2	ACATTCGGCATACAAATGGG/	55	8					
	AGTGCGACGTTTTTCATTCC		(222-249)	0.726	0.824	0.151 ± 0.086	270	15
LxAGT1	Bourke et al. 1997	55	6					
			(256-293)	1.000	0.822	-	24	2

F/R primer = sequences of forward/reverse primers used to amplify the loci (5'→3' direction); # alleles = number of detected alleles among the genotyped individuals (range of allele sizes); H_O= observed heterozygosity; H_E= expected heterozygosity; F_{IS} ± SE = inbreeding coefficient plus/minus SE (RELATEDNESS 4.2c) both values were not significantly different from 0 (*t*-test).

Table 2 (following page):

Summary of the microsatellite analysis of 20 *Pogonomyrmex rugosus* colonies. In colonies with two matrilines the workers of the second matriline were omitted in all further relatedness calculations (R, patrilines, and m_e, respectively). Six colonies (given in italics) were reared in the laboratory from single founding queens collected in 2000.

Colony	**CPS44**	**CPS47**	**CPS48**	**CPS49**	**CPS59**	**JO3**	**JO4**	**JO5**	**JG35**	**JG36**	**JG38**	**JG39**	**JG40**	**JG41**	***56 -3***	***56 -4***	***56 - 15***	***56 - 18***	***56 - 27***	***56 - 29***	**mean**
g_{ww} *	0.513	0.355	0.405	0.377	0.401	0.304	0.381	0.454	0.338	0.343	0.373	0.334	0.370	0.300	0.364	0.401	0.339	0.337	0.395	0.382	0.37 ± 0.05
R †	0.541	0.362	0.427	0.362	0.410	-	-	-	0.372	0.357	0.247	0.481	0.270	0.163	0.282	0.593	0.454	0.532	0.628	0.291	0.38 ± 0.04
matrilines	1	1	1	2	1	2	2	2	2	1	1	1	2	1	1	1	1	1	1	1	-
patrilines	4	6	6	5	5	11	5	3	6	6	5	8	5	12	7	4	7	7	4	4	6.00 ± 2.27
P_{maj} ‡	0.70	0.35	0.50	0.33	0.42	0.13	0.33	0.44	0.21	0.25	0.35	0.29	0.29	0.15	0.37	0.41	0.24	0.27	0.38	0.33	0.34 ± 0.13
m_e §	1.90	4.76	3.23	3.95	3.31	9.24	3.81	2.45	5.67	5.41	4.08	5.98	4.16	10.00	4.38	3.32	5.64	5.75	3.46	3.79	4.71 ± 2.02
Pr1	3	3	5	3	3	4	5	4	4	5	5	4	6	6	5	4	-	4	5	4	4.32 ± 0.95
Pr2	5	4	4	3	3	9	-	-	3	4	5	4	4	4	4	-	6	5	-	-	4.47 ± 1.51
LxAGT1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	2	-	3.00 ± 1.41
Worker analysed	20	20	20	20	19	47	50	19	20	20	20	21	20	20	19	23¶	39	31¶	14	12	23.7 ± 10.13

*g_{ww}, average genetic relatedness among workers (pedigree relatedness), calculated under the assumption of unrelated fathers using m_e and formula 4.16 of Crozier & Pamilo (1996; p. 102). †R, intracolonial relatedness, calculated using RELATEDNESS 4.2c (genetic relatedness), with allele frequencies weighted by colonies (confidence interval of the mean was estimated by jackknifing over colonies). ‡P_{maj}, contribution of the most frequent patriline. § m_e , effective mating frequency, calculated using patriline frequencies, following Starr (1984). ¶Including queen of the colony.

3.8 Figures

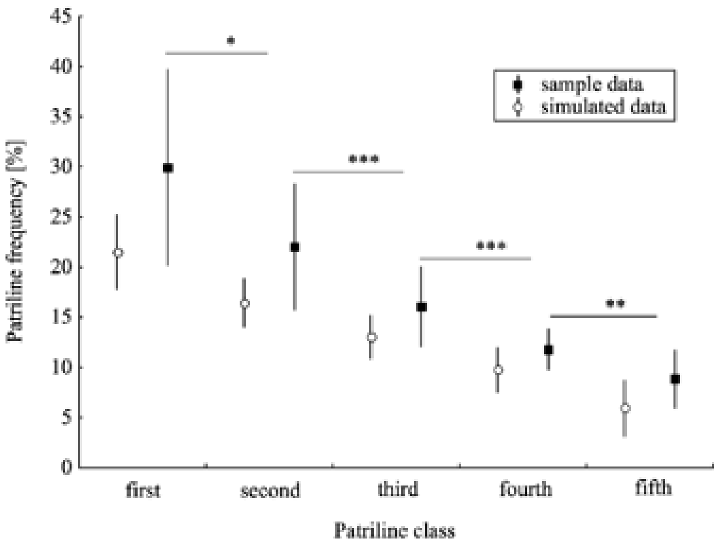

Figure 1:

Frequency of patriline classes 1-5 over colonies containing five or more patrilines. Patrilines were ranked into classes according to their frequency. Patriline frequencies were then averaged for all colonies (observed or simulated). (▪) Mean ± SD of field and laboratory-reared colonies ($n = 15$); (○) mean ± SD of simulated data. Asterisks indicate significant differences (Mann-Whitney *U*-test) in patriline frequency between neighbouring classes of the observed patrilines (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$). Patriline classes six and higher are not shown, but differ not significantly from patriline class five. Patriline frequencies in each patriline class differ significantly between sampled and simulated data (all $P < 0.01$, Mann-Whitney *U*-tests).

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This paper is part of our research on the evolution of mating frequency in the genus *Pogonomyrmex* and comprises part of Christoph-Peter Strehl's PhD and Jan Oettler's diploma thesis. Jürgen Gadau is interested in evolutionary genetics with an emphasis on social insects. Bert Hölldobler is an evolutionary biologist with a broad interest in the sociobiology and behavioural ecology of ants.

4. Extremely High Mating Frequency in the Florida Harvester Ant (*Pogonomyrmex badius*)

Published in: Behavioral Ecology and Sociobiology (2004) 56: 472-481

Authors: Frank E. Rheindt, Jürgen Gadau, Christoph-P. Strehl, Berthold Hölldobler

4.1 Abstract

High effective multiple queen mating is a rare but taxonomically widespread phenomenon in eusocial Hymenoptera that has arisen convergently in several taxa. In ants, high effective mating frequencies have been confirmed in only two clades: the higher leaf-cutters (*Atta, Acromyrmex*) and the *Pogonomyrmex* seed harvesters. We analysed polyandry in *Pogonomyrmex badius*, which has a life-history unique within the genus, and report the highest numerical mating frequencies thus far recorded in ants. We also show that *P. badius* is characterized by one of the highest effective mating frequencies hitherto found in ants. It is now clear that all major sub-clades of *Pogonomyrmex sensu stricto* exhibit high levels of polyandry. Therefore, multiple mating must have arisen early in the evolution of the genus, and may have constituted a mechanism to increase offspring variability for queens that were confronted with increasingly complex levels of organization. Too few congeners have been investigated by the same method to be certain that polyandry in *P. badius* is really higher than in the rest of the genus. If so, research should concentrate on a possible link between high queen mating frequency and the distinct caste system found in *P. badius*.

4.2 Introduction

Ever since the Hamiltonian revolution in evolutionary biology (Hamilton 1964a, 1964b), there has been a surge of scientific interest in polyandry, its implications for offspring relatedness and its possible origins in eusocial insects. Despite considerable costs in terms of energy expenditure and predation risk (Bourke and Franks 1995), multiple mating was confirmed to be widespread in ants—long before the advent of molecular techniques—by means of simple observation and/or sperm counts in males and queens (Hölldobler and Wilson 1990). Boomsma and Ratnieks (1996), however, showed that effective paternity frequency is grossly overestimated by these methods and that reliable estimates of paternity can only be derived from genetic analyses. Even if a queen can be shown to mate with more than one male, the level of relatedness among her offspring may still be close to that in a monandrous species if the paternity share of one male is dominant over that of the others. In such a case, while the numerical mating frequency (*m*) of a queen is well above 1, her effective mating frequency (m_e i.e. the minimal number of males required to generate a given intracolonial relatedness) may still be close to 1.

Meanwhile, an effective mating frequency of 2 or more has been recorded in only two ant clades, namely a leaf-cutter ant complex comprising the two sister genera *Atta* and *Acromyrmex* (Boomsma and Ratnieks 1996; Bekkevold et al. 1999; Boomsma et al. 1999; Villesen et al. 1999; Fjerdingstad and Boomsma 2000; Murakami et al. 2000), as well as the seed harvester ants of the genus *Pogonomyrmex* (Cole and Wiernasz 1999, 2000; Helms Cahan et al. 2002; Volny and Gordon 2002a; Gadau et al. 2003). Reviewing the incidence of polyandry across all social hymenopterans, Strassmann (2001) made it clear that effective multiple queen mating should be considered an uncommon but widespread phenomenon that must have evolved convergently on several occasions within the social insects.

A plethora of hypotheses have been advanced to explain the evolution and maintenance of polyandry in hymenopterans (e.g. Crozier and Pamilo 1996): the "sperm supply hypothesis" (Cole 1983; Boomsma and Ratnieks 1996; Fjerdingstad and Boomsma 1998) contends that multiple mating entails a gain in female fecundity. It has been pointed out, however, that this hypothesis provides no explanation for the lack of directional selection towards larger male ejaculate size (Crozier and Page 1985; Keller and Reeve 1994). Moreover, multiply mating queens in leaf-cutter and harvester ants store only the equivalent of a single male's sperm or considerably less (Boomsma et al. 1999; Gadau et al. 2003).

The "convenience hypothesis" (Alcock et al. 1978) envisages polyandry as an active mating strategy of the male and explicitly relegates it from the domain of the queen. As such, multiple mating is exercised by males (and forced on females) to preclude the transmission of their entire sperm store to a single female (Boomsma 1996). According to Strassmann (2001), however, coerced copulation is unlikely in social hymenopterans, a group of insects in which most females have to move aside their sting for copulation to occur, though it is by no means inconceivable in, for instance, several western

Nearctic *Pogonomyrmex* species that are characterized by huge mating aggregations (Hölldobler and Wilson 1990).

In a seminal study on *Pogonomyrmex occidentalis*, Cole and Wiernasz (1999) found a correlation between mating frequency and colony fitness, indicating that selectional processes at the colony level (rather than the individual level) are likely to play a major role in explaining the occurrence of polyandry. This result provided a strong hint that multiple mating may ultimately be linked to favorable effects accrued by an increase in intracolonial genetic diversity and/or low relatedness. Hypotheses that adopt the notion of genetic diversity or low within-colony relatedness to account for polyandry had been presented decades earlier and continue to be discussed today.

One of them, the "split sex ratio hypothesis", is based on the observation that a monogynous species with a mating frequency of 1 will encounter conflicts between workers and queens of a colony over the preferred sex ratio (Macevicz 1979; Craig 1980; Page and Metcalf 1984; Page et al. 1993; Tsuji and Yamauchi 1994; Hasegawa and Yamaguchi 1995). By increasing the number of mating partners, a queen may thus seek to reduce genetic relatedness among her worker offspring and consequently shift her daughters' preferred sex ratio closer to her own (Starr 1984; Moritz 1985; Queller 1993; Moritz et al. 1995; Ratnieks and Boomsma 1995). If this is the case, workers must be able to assess the genetic diversity of their colony and facultatively adjust the sex ratio against the queen's preferences. Recent studies on different genera have shown conflicting results, demonstrating that workers either can or cannot regulate sex allocation in response to patriline representation and/or queen mating frequency (Fjerdingstad et al. 2002; Boomsma et al. 2003).

Diploid males have been reported repeatedly in ants (Crozier and Pamilo 1996). The "diploid male hypothesis" builds on the fact that a single matched mating at the sex-determining locus results in the production of non-viable diploid male offspring that constitute a burden to the colony. The variance of diploid male production in a species can be greatly reduced by the adoption of multiple mating, which may be advantageous under certain life-history scenarios (Page 1980; Page and Metcalf 1982; Crozier and Page 1985; Pamilo et al. 1994).

Finally, many authors have come to regard intracolonial genetic diversity itself as a beneficial trait (Crozier and Page 1985). Genetically more diverse colonies may perform better because of wider and more efficient resource utilization (Keller and Reeve 1994) or because they are more resistant to pathogens (Hamilton 1987; Sherman et al. 1988; Shykoff and Schmid-Hempel 1991; Schmid-Hempel 1994; Liersch and Schmid-Hempel 1998; Baer and Schmid-Hempel 1999). The high exposure of leaf-cutter ants and their fungus gardens to pathogens of all kinds (Van Borm et al. 2002) led Boomsma et al. (1999) to believe that the “pathogen hypothesis” best explains polyandry in ants. Meanwhile, other studies both supported this view (Boomsma and Ratnieks 1996; Hughes et al. 2002; Brown and Schmid-Hempel 2003) and rejected it as the sole explanation (Villesen et al. 1999; Fjerdingstad and Boomsma 2000; Murakami et al. 2000).

Boomsma et al. (1999) were, of course, unaware then that effective mating frequencies comparable to those in leaf-cutters were going to be found in *Pogonomyrmex* (Cole and Wiernasz 1999, 2000; Volny and Gordon 2002a; Gadau et al. 2003), a genus of harvester ants with an entirely different life-history (Johnson 2000). Harvester ants are denizens of dry to semi-arid environments and do not engage in fungal symbiosis. Hence, they are possibly much less prone to pathogen infestation. Pathogen resistance does seem to play a crucial role in leaf-cutters, but it would be premature to rule out that in harvester ants an increased intracolonial genetic variability additionally conveys other (potentially more important) benefits, such as improved resource utilization or competitive ability.

The genus *Pogonomyrmex* is widespread throughout open habitats from Patagonia to southwestern Canada (Taber 1998). The North American subclade, which has so far received most attention, is centred around the deserts and sub-deserts of the southwestern United States and northern Mexico (Johnson 2000). To date, three *Pogonomyrmex* species have been genetically investigated with respect to multiple mating (*P. occidentalis*, Cole and Wiernasz 1999, see also Cole and Wiernasz 2000; *P. barbatus*, Helms Cahan et al. 2002; Volny and Gordon 2002a; *P. rugosus*, Gadau et al. 2003), all from this area, and all featuring effective mating frequencies unsurpassed among ants.

We here investigate mating frequency and within-colony relatedness in *P. badius*, a species unique among the North American subclade of *Pogonomyrmex*, in that it is allopatric to all congeners, solitarily occupying a distributional range that stretches from Louisiana to Florida and North Carolina (Johnson 2000). The peculiar mating behaviour of *P. badius*, in which females wait at their nest entrance and mate with any male that may be passing by from potentially nearby and closely related colonies (M. Deyrup, personal communication) or even with males from the same nest (Van Pelt 1953; Harmon 1993), differs substantially from the well-studied mating aggregations of most of its congeners (Hölldobler and Wilson 1990). Moreover, *P. badius* is the only North American representative that exhibits a pronounced worker polymorphism. Owing to this distinctness, we were curious to see how *P. badius* compares to its congeners and other ant groups in terms of queen mating frequency.

4.3 Methods

Collecting

We investigated workers from 15 colonies that had been collected across 3 different localities in peninsular Florida (Table 1) in August and September 2001. Five colonies were located around the Archbold Biological Station (N27°11', W81°20'), six colonies within Withlacoochee State Forest (N28°48', W82°29'), and the remaining four at Ocala National Forest (N29°16', W81°49'). Specimens were immediately put in 100% ethanol upon collection.

DNA extraction

Workers were taken out of the ethanol, quickly dried in air and their gasters removed. The workers were placed in tubes of liquid nitrogen and thoroughly ground. We used the same phenol/chloroform DNA extraction protocol as Gadau et al. (1996). The DNA was dissolved in 100µl low TE storage buffer (10 mM Tris-HCl, 1 mM EDTA; pH 7.6) and kept frozen at –23°C.

Gel electrophoresis

The success of amplification was monitored by minigel electrophoresis. The DNA products of successful amplifications were subjected to Spreadex gel electrophoresis (Elchrom, Switzerland), which enables us to distinguish between fragment length differences of 2–4 bp. We used Elchrom Scientific SPREADEX EL 800 precast gels that were loaded with 3–5 µl of a mixture of 16-µl PCR product and 10 µl of ×10 gel loading dye and run at 120 V for 75 min. Gels were stained with SYBR-green I Nucleic Acid Gel Stain (BMA/Bio Whittacker Molecular Applications, USA) for 20–180 min and watered overnight to reduce blurriness of the resulting bands under UV light.

Microsatellites and PCR reaction

Mating frequency was determined using three microsatellite loci. We tested all microsatellite primers developed for other *Pogonomyrmex* species by Volny and Gordon (2002b; *n*=10) and by Gadau et al. (2003; *n*=2), but found that only two and one of them, respectively, exhibited an instructive level of allele variability in *P. badius* (Pb5 and Pb6 of Volny and Gordon 2002b as well as PR2 of Gadau et al. 2003). The remaining loci were either not variable at all (*n*=6) or were so to a very low degree (*n*=3) within our study colonies. The PCR reaction mix contained 2µl of the genomic DNA dissolved in low TE. In each of the loci, PCR reaction conditions followed either Volny and Gordon (2002b) or Gadau et al. (2003), respectively.

Table 1

Location of colonies, the minimum number of patrilines detected by using each of the three loci, as well as by combining all three (=multi-locus analysis), number of individuals successfully genotyped, effective mating frequency m_e and effective mating frequency m_{eqc} corrected for sampling effort (*ABS* Archbold Biological Station, *WSF* Withlacoochee State Forest, *ONF* Ocala National Forest; *colonies in which we detected one worker that could not be explained by a single mother, see Results); 95% confidence intervals are given for the average effective mating frequencies

Colony number and location	**Minimum number of patrilines detected using**				**Individuals successfully genotyped: Pb6, Pb5, PR2**	**m_e as inferred from paternity shares (q)**	**m_{eqc} corrected for sampling effort**
	Locus Pb6	**Locus Pb5**	**Locus PR2**	**Multi-locus analysis**			
ABS-29	2	2	4	7	18,19,19	5.08	6.46
ABS-33	1	1	4	4	14,11,20	3.18	3.59
ABS-37	3	1	2	5	19,19,18	3.92	4.63
ABS-42	3	4	4	8	20,17,20	3.47	3.99
ABS-73	2	4	6	12	38,36,38	7.02	8.23
ONF-63	4	3	0	7	17,13,0	5.90	7.94
ONF-65	6	4	6	15	40,33,38	6.96	8.21
ONF*-62	4	4	3	9	19,16,19	6.56	9.28
ONF-69	5	3	4	10	18,13,18	7.41	11.18
WSF-54	4	2	8	12	19,18,20	10.53	21.11
WSF-56	4	3	5	7	39,32,34	4.17	4.54
WSF*-	3	7	9	27	39,39,39	11.08	14.93

Colony number and location	Minimum number of patrilines detected using				Individuals successfully genotyped: Pb6, Pb5, PR2	m_e as inferred from paternity shares (q)	m_{eqc} corrected for sampling effort
	Locus Pb6	**Locus Pb5**	**Locus PR2**	**Multi-locus analysis**			
51							
WSF-55	5	8	3	16	39,38,39	5.76	6.55
WSF-53	7	7	6	19	33,38,38	13.12	19.03
WSF-52	4	4	5	7	17,17,17	5.71	7.60
Average	3.8	3.8	4.6	11.0		6.66 (±1.61)	9.16 (±2.95)

Estimation of mating frequency

The numerical mating frequency of each colony was determined by individually interpreting the band-sharing patterns exhibited by all workers of a colony in the most parsimonious fashion and by making the assumption of monogyny. Polygyny has never been reported in the genus and/or in the species despite extensive fieldwork (Taber 1998; Tschinkel 1998; Cole and Wiernasz 2000; Gadau et al. 2003). All alleles of each locus were carefully homologized across colonies by running most of them multiple times on the same gel side by side. In rare instances, the queen's genotype could not be unequivocally determined. In those cases, she was assigned the most conservative genotype, i.e. that which yielded the least amount of mating partners. Equally, the most parsimonious genotypes were assigned whenever male genotypes were equivocal due to identical heterozygous genotypes of queen and worker. In two colonies, we found single workers that required the assumption of an additional mother (see Results). These workers were removed from the dataset used for subsequent analyses. Allele information of all three loci was merged and each individual assigned to a combination of three paternal alleles (one from each locus), which it had to share with other individuals in order to be considered a member of the same patriline (multi-locus fingerprint). This practice boosted the numerical mating frequencies of most colonies (Table 1), indicating that the utilization of a single locus would have seriously underestimated multiple mating.

A possible source of underestimating the real number of patrilines in a colony is non-detection due to limited genetic variability of the microsatellite loci employed. All in all, a maximum of 2,688 different patrilines [the product of the number of alleles (Appendix) of each of the loci investigated=16×14×12] could have potentially been detected with our 3 markers. Despite such a high total, it is still possible that some fathers were missed because their multi-locus genotype was identical to that of another male. To estimate the magnitude of this non-detection error, we determined the sum of squares of all male multi-locus haplotypes (Σq_z^2; Pamilo 1993).

We calculated effective mating frequency (m_e) by quantifying the paternity shares (q) of each multi-locus patriline (z) and by entering them into the following equation: $m_e = 1/\Sigma q_z^2$ (Pamilo 1993). When using this approach, one can additionally correct for the non-detection of rare patrilines due to limited sampling by applying the expression $m_{eqc} = 1/[(N\Sigma q_z^2 - 1)/(N-1)]$ developed by Pamilo (1993), where N equals the number of offspring analysed per colony.

4.4 Results

Sampling effort and locus properties

Four hundred and twenty individuals from 15 colonies were genotyped for 3 microsatellite loci (PR2; Pb5; Pb6; Table 1). Six of the colonies that had seven or more patrilines after a first screen were given a higher sampling effort to reduce sampling error (Table 1). Locus-specific levels of observed heterozygosity (i.e. the sums of each colony's proportion of heterozygous workers divided by the number of colonies) were 0.949, 0.738 and 0.652 for PR2, Pb5 and Pb6, respectively. These values were well within the range of heterozygosity rates previously reported from the species for which the markers were developed initially (Pb5=0.60; Pb6=0.95 Volny and Gordon 2000a; PR2=0.824, Gadau et al. 2003).

Mating frequency and detection errors

Table 1 shows the numerical mating frequency of each colony—i.e. the minimum estimate of the number of patrilines—as assessed with each of the three loci, as well as by combining all three loci in a multi-locus analysis. Numerical mating frequencies as assessed by multi-locus analysis ranged from 4 to 27, with a mean of 11.0 (Table 1). At two of the field localities, numerical mating frequencies were mostly below 11, whereas at the remaining locality (Withlacoochee State Forest), the majority of colonies investigated exhibited mating frequencies greater than 11. This pattern may weakly suggest geographical differences in mating frequencies, but the sample size was too low for statistical confirmation.

Two workers were found that could not be explained by a single mother (Table 1). They may have resulted from intraspecific raids (Gadau et al. 2003), collecting errors or polygyny. Both workers were removed from further analyses.

All in all, 165 fathers were inferred. Based on the sum of squares of population-wide patriline frequencies (Σq_z^2; Pamilo 1993), there was an average probability of 0.008 that one of the patrilines actually represented two fathers with identical multi-locus alleles. This translates into an estimated non-detection of 1.38 fathers for our entire study, which may be conservative, given that potential geographical structuring (see above) reduces the probability of occurrence of many possible multilocus genotypes. Yet the actual number of non-detected fathers may be slightly higher, because it is particularly those fathers with a small paternity share that are most likely to go unnoticed.

Average effective mating frequency (±95% confidence intervals) was $m_e = 6.66$ (±1.605; Table 1). When correcting for the non-detection of rare patrilines via the relationship $m_{eqc} = 1/[(N\Sigma q_z^2 - 1)/(N-1)]$ (Pamilo 1993; see Methods), the effective mating frequency (±95% confidence intervals) rose to $m_{eqc} = 9.16$ (±2.948; Table 1). The relative contributions of the different patrilines to the sampled worker offspring of each colony are depicted in Fig. 2.

4.5 Discussion

High numerical mating frequency in Pogonomyrmex badius

The numerical mating frequencies of some of our study colonies (e.g. m=27) are extreme, as is the mean numerical mating frequency averaged over all colonies (m=11.0): Within social insects, comparable or higher levels of polyandry have only been found in *Apis* bee queens (Estoup et al. 1994; Moritz et al. 1995; Strassmann 2001; Tarpy and Nielsen 2002). This is all the more astonishing when considering that a numerical mating frequency such as that of 27 is likely an underestimate, as the sample size for that particular colony (n=40) was only 48% higher than the number of detected fathers.

High effective mating frequency in Pogonomyrmex badius

The Florida harvester-ant colonies sampled by us displayed an extremely high average effective mating frequency at m_{eqc} =9.155 (corrected for sampling bias; see Methods). *P. badius* queens thus range among the hymenopterans with the highest known effective mating frequency, second only to *Apis* bees (m_e =9.1–25.65; Estoup et al. 1994; Moritz et al. 1995; Strassmann 2001; Tarpy and Nielsen 2002; Fig. 1; note that not all the studies referenced by these authors corrected for sampling bias). This mating frequency of m_{eqc} =9.155 is considerably higher than that of the two ant species for which the next two highest effective mating frequencies among ants have been calculated by the same method (Boomsma et al. 1999; Gadau et al. 2003), namely *P. rugosus* and *Acromyrmex octospinosus* (Fig. 1). Note, however, that Cole and Wiernasz (1999) found a fairly high effective mating frequency of m_e =6.76 for *P. occidentalis*, but they used a non-homologous method, so their results are not comparable to ours.

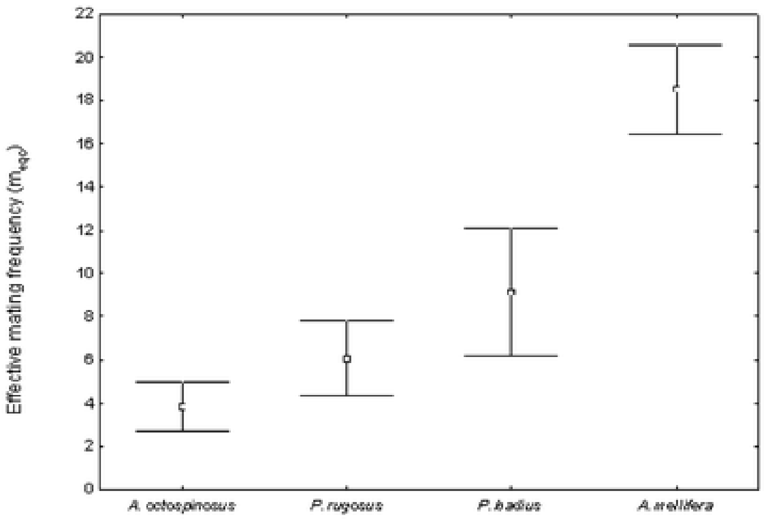

Fig. 1

Average effective mating frequencies (m_{eqc} ±95% confidence intervals) across study colonies (*n*) of *Pogonomyrmex badius* (present study; *n*=15), *P. rugosus* (Gadau et al. 2003; *n*=20), *Apis mellifera* (Neumann et al. 1999; Neumann and Moritz 2000; *n*=41) and *Acromyrmex octospinosus* (Boomsma et al. 1999; n=9) determined by the paternity shares (*q*) of each intracolonial patriline (*z*) and the relationship $m_{eqc} = 1/[(N\Sigma q_z^2 - 1)/(N-1)]$ (Pamilo 1993). Pamilo's (1993) correction was not applied by Gadau et al. (2003), so their values needed to be corrected for this study. The sources of the data for *Apis mellifera* (Neumann et al. 1999; Neumann and Moritz 2000) were selected from 17 honeybee studies reviewed by Tarpy and Nielsen (2002) because they were the only ones in which effective mating frequency was calculated by the same method across several colonies (Pamilo 1993)

Boomsma et al. (1999) reported that paternity skew in *Acromyrmex octospinosus* tended to increase with the number of fathers per colony. Similarly, in *P. badius*, it seems that colonies with a low degree of polyandry have a far more equal patriline representation than those with a high degree of polyandry (Fig. 2). However, this observation requires statistical confirmation with a higher sampling effort.

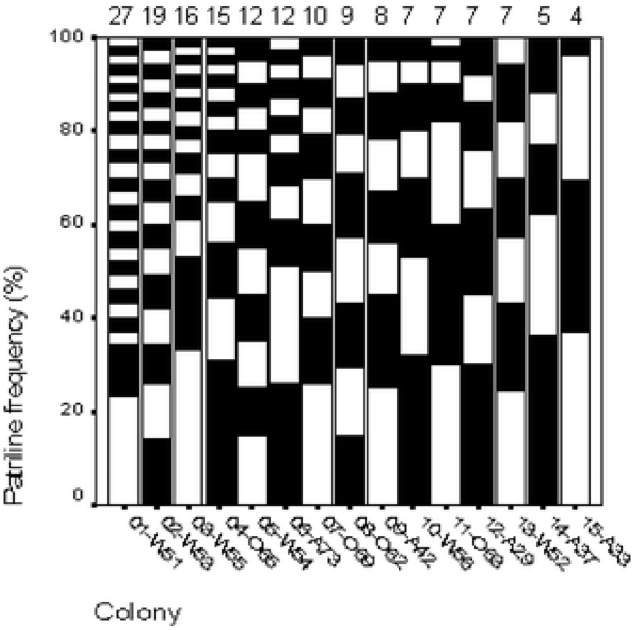

Fig. 2

Patriline representation among colonies ranked according to frequency. Patrilines are represented by *alternate shading*. Colonies (label below *bar*) are in descending order of the number of detected fathers per colony (given *above bars*). Colony labels: *W* Withlacoochee State Forest, *O* Ocala National Forest, *A* Archbold Biological Station; number before letter indicates m_{eqc} rank, number after letter indicates colony label as given in Appendix

Monogyny and brood raiding in Pogonomyrmex

Our estimates of effective mating frequency are based on the assumption of monogyny, so any incidence of polygyny would be a possible source of inflation. Two out of 420 workers could not be explained by a single mother, which may either arise from collecting errors, polygyny or brood raiding. All ants were collected at nest entrances or in their immediate vicinity, making collecting errors unlikely. Polygyny has never been reported in the genus and/or in the species despite extensive fieldwork (Taber 1998; Tschinkel 1998; Cole and Wiernasz 2000; Gadau et al. 2003). Hence, the two individuals in question are probably recruited from intraspecific nest raids.

Raided workers may go unnoticed in genotyping if they happen to share an allele with the mother of the raiding colony (and thus mimic an additional patriline). Generally, this chance event should be sufficiently rare, such that most raided workers ought to be detected. However, if queens of neighbouring colonies are related, the incidence of undetectable raided workers may rise, concomitantly inflating the number of rare patrilines in the colony. In any case, intraspecific raiding may play some role in *P. badius*. However, the low number of unambiguously detected foreign workers despite our high detection probability suggests that this role should be minor. Gadau et al. (2003) found intraspecific raiding to be more common in *P. rugosus*, but their population was characterized by a much higher colony density than ours (personal observation).

Evolution of polyandry in *Pogonomyrmex badius*

With this study as the final contributor, it has now been shown that members of all four major Nearctic species groups of *Pogonomyrmex sensu stricto* are polyandrous (*P. occidentalis*, Cole and Wiernasz 1999, 2000; P. *barbatus*, Helms Cahan et al. 2002; Volny and Gordon 2002a; *P. rugosus*, Gadau et al. 2003; see Taber 1998 on taxonomic grouping). Therefore, multiple mating has probably arisen early in the evolution of this group and might have been linked to the development of seed harvesting (Gadau et al. 2003), though studies on many more species and other seed-harvesting genera (e.g. *Pheidole, Messor*) will have to be conducted before this conclusion can be reached with certainty. The development of large colony size and a high level of organization always seem to go along with polyandry in hymenopterans, though not necessarily vice versa (Strassmann 2001). Multiple queen mating, then, might have constituted a mechanism to increase offspring variability for queens that were confronted with increasingly complex levels of organization, whether driven by high pathogen exposure, as has been proposed for leaf-cutters (Boomsma and Ratnieks 1996; Boomsma et al. 1999; Hughes et al. 2002; but see also Villesen et al. 1999; Fjerdingstad and Boomsma 2000; Murakami et al. 2000), or by the need to refine foraging techniques, resource utilization and/or competitive ability, as appears more plausible in *Pogonomyrmex*.

P. badius queens exhibit effective mating frequency estimates that are well within the generic average and may even supersede most other *Pogonomyrmex* species investigated (and for that matter, most other ants investigated to date). It is too early to engage in serious speculation about reasons for a potential rise in effective mating frequency in *P. badius*, since too little is known about effective mating frequency in most of its congeners. Future research may demonstrate that other species in the genus are characterized by similar or even greater effective (and/or numerical) mating frequencies. However, mating frequencies of other *Pogonomyrmex* species may not reach the high level of *P. badius*, the sole North American representative that both displays geographic isolation from all congeners and exhibits a morphological worker caste system. If this is the case, we suggest future research concentrates on the role that the morphological caste system of *P. badius* may play with regard to mating frequency.

4.6 Acknowledgements

Karin Möller deserves warm thanks for assistance with laboratory work. We are indebted to Annett Endler and Jürgen Liebig for helping us procure specimens in the field. Mark Deyrup and Walter Tschinkel are acknowledged for fruitful discussion. Three anonymous reviewers provided invaluable help for the improvement of the manuscript. We would like to thank the Archbold Biological Station for providing facilities during fieldwork. This work was funded by the SFB 554-TP-C5. The treatment of ants complied with current German and U.S. laws.

4.7 Appendix

Table 2 (following pages)

Scoring table with allele inferences for all three microsatellite loci (PR2, Pb5, Pb6) and across all 420 individuals genotyped; location: 1 Archbold Biological Station, 2 Withlacoochee State Forest, 3 Ocala National Forest; individual label: m minor, M major; caste: 1 major, 2 minor; "x" denotes missing alleles

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
1	29	m1	2	i/h	f/c	h/f
1	29	m2	2	a/i	c/c	j/g
1	29	m3	2	i/h	f/c	h/f
1	29	m4	2	a/i	c/c	j/f
1	29	m5	2	a/h	f/c	h/f
1	29	m6	2	a/h	f/c	g/g
1	29	m7	2	a/i	c/c	j/g
1	29	m8	2	a/h	f/c	g/g
1	29	m9	2	a/i	c/c	j/f
1	29	M10	2	a/i	c/c	j/g
1	29	M1	1	i/h	f/c	g/g
1	29	M2	1	x/x	f/c	g/g
1	29	M3	1	a/h	f/c	h/f
1	29	M4	1	a/i	c/c	j/f
1	29	M5	1	i/h	f/c	h/f
1	29	M6	1	i/i	c/c	j/g
1	29	M7	1	a/h	f/c	g/g
1	29	M8	1	a/h	f/c	g/g
1	29	M9	1	a/h	f/c	g/g
1	33	M1	1	n/l	f/d	f/f
1	33	M2	1	n/f	f/b	f/f
1	33	M3	1	o/f	x/x	f/f
1	33	M4	1	o/l	d/d	f/f
1	33	M5	1	o/n	f/e	f/f
1	33	M6	1	o/f	x/x	f/f
1	33	M7	1	o/f	f/d	f/f
1	33	M8	1	n/h	x/x	f/f
1	33	M9	1	o/f	f/d	f/e

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
1	33	m11	2	o/n	f/d	f/f
1	33	m1	2	o/l	f/d	f/f
1	33	m2	2	n/f	f/b	f/f
1	33	m3	2	o/n	f/d	f/f
1	33	m4	2	n/l	d/d	f/f
1	33	m5	2	o/h	f/d	f/f
1	33	m6	2	o/f	f/d	f/e
1	33	m7	2	o/n	d/d	f/f
1	33	m8	2	o/n	d/a	f/g
1	33	m9	2	o/l	f/d	f/f
1	33	m10	2	o/n	f/a	g/g
1	73	M1	1	b/b	f/e	h/h
1	73	M2	1	d/b	f/e	h/h
1	73	M3	1	i/l	e/c	g/g
1	73	M4	1	b/b	c/c	h/h
1	73	M5	1	l/b	e/c	g/g
1	73	M6	1	i/l	e/c	h/g
1	73	M7	1	d/b	e/c	h/h
1	73	M8	1	d/b	e/c	g/g
1	73	m11	2	f/b	j/c	h/h
1	73	m12	2	i/f	j/c	h/h
1	73	m1	2	x/x	e/c	h/h
1	73	m2	2	x/x	f/e	h/h
1	73	m3	2	l/b	e/e	g/g
1	73	m4	2	i/f	j/c	h/h
1	73	m5	2	d/b	e/c	h/h
1	73	m6	2	d/b	e/e	h/h
1	73	m7	2	d/b	e/f	h/h
1	73	m8	2	b/b	e/c	h/h
1	73	m9	2	i/m	e/c	h/h
1	73	m10	2	i/f	e/e	h/h
1	73	m21	2	d/b	x/x	h/h
1	73	m22	2	d/i	c/c	x/x
1	73	m23	2	i/b	e/f	h/h
1	73	m24	2	d/i	f/c	h/h
1	73	m25	2	d/i	e/c	h/h
1	73	m26	2	i/b	e/c	h/h

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
1	73	m27	2	i/b	e/c	h/h
1	73	m28	2	c/b	f/c	h/h
1	73	m29	2	d/i	e/e	g/g
1	73	m30	2	i/f	e/e	h/h
1	73	m31	2	d/b	f/c	h/h
1	73	m32	2	d/b	x/x	h/h
1	73	m33	2	f/b	e/c	x/x
1	73	m34	2	m/b	e/f	h/h
1	73	m35	2	i/d	f/c	h/h
1	73	m36	2	i/d	f/c	h/h
1	73	m37	2	d/b	e/e	h/h
1	73	m38	2	d/b	x/x	h/h
1	73	m39	2	i/m	e/e	h/h
1	73	m40	2	d/b	x/x	h/h
1	37	M1	1	n/h	k/a	h/g
1	37	M2	1	n/h	g/g	h/h
1	37	M3	1	k/n	k/a	g/g
1	37	M4	1	f/h	k/g	h/g
1	37	M5	1	f/h	k/a	h/g
1	37	M6	1	n/h	g/a	h/g
1	37	M7	1	f/h	g/g	x/x
1	37	M8	1	f/h	g/a	h/g
1	37	M9	1	k/n	k/g	g/g
1	37	M10	1	l/h	k/a	g/g
1	37	M11	1	f/h	g/a	g/g
1	37	M12	1	l/h	k/g	h/g
1	37	M13	1	n/h	g/a	h/g
1	37	m1	2	n/h	g/g	h/g
1	37	m2	2	l/n	k/g	h/g
1	37	m3	2	x/x	x/x	x/x
1	37	m4	2	f/h	g/g	g/g
1	37	m5	2	f/h	g/g	h/h
1	37	m6	2	f/h	g/g	g/g
1	37	m7	2	f/h	g/a	g/g
1	42	M1	1	o/m	g/e	f/f
1	42	M2	1	m/c	x/x	x/x
1	42	M3	1	o/m	g/e	f/f

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
1	42	M4	1	m/c	x/x	f/f
1	42	M5	1	m/c	g/e	f/f
1	42	M6	1	o/m	x/x	x/x
1	42	M7	1	o/m	g/e	f/f
1	42	M8	1	m/c	x/x	x/x
1	42	m11	2	m/h	g/g	f/f
1	42	m12	2	m/h	g/e	f/f
1	42	m1	2	m/c	x/x	x/x
1	42	m2	2	m/f	x/x	x/x
1	42	m3	2	o/m	x/x	f/f
1	42	m4	2	m/c	x/x	f/f
1	42	m5	2	m/h	g/e	f/f
1	42	m6	2	o/m	g/e	f/f
1	42	m7	2	m/h	x/x	x/x
1	42	m8	2	o/m	g/g	f/f
1	42	m9	2	o/m	g/e	f/f
1	42	m10	2	m/h	g/e	f/f
2	53	M1	1	h/h	c/c	j/g
2	53	M2	1	h/b	c/b	h/c
2	53	M3	1	x/x	x/x	j/h
2	53	M4	1	m/b	c/b	j/h
2	53	M5	1	h/h	c/c	g/c
2	53	M6	1	x/x	x/x	j/h
2	53	M7	1	h/h	c/c	g/c
2	53	M8	1	x/x	x/x	j/h
2	53	M9	1	m/h	c/b	j/f
2	53	M10	1	h/b	c/b	j/h
2	53	M11	1	m/h	x/x	g/c
2	53	M12	1	h/b	x/x	h/c
2	53	M13	1	m/b	c/b	h/c
2	53	M14	1	l/h	c/a	h/c
2	53	M15	1	h/b	c/b	j/h
2	53	M16	1	l/m	c/a	j/h
2	53	M17	1	l/h	c/a	h/c
2	53	m2	2	m/h	c/b	f/c
2	53	m3	2	h/h	c/b	j/f
2	53	m4	2	m/l	c/a	j/h

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
2	53	m5	2	e/h	c/c	j/g
2	53	m6	2	m/b	x/x	h/c
2	53	m7	2	m/h	c/c	g/c
2	53	m8	2	h/h	x/x	j/g
2	53	m9	2	x/x	c/c	g/c
2	53	m10	2	x/x	c/a	h/c
2	53	m11	2	f/h	c/d	g/c
2	53	m12	2	m/b	c/b	j/h
2	53	m13	2	m/l	c/a	j/h
2	53	m21	2	m/b	c/b	h/c
2	53	m22	2	h/b	c/b	j/h
2	53	m23	2	h/m	c/d	j/g
2	53	m24	2	h/f	c/d	g/c
2	53	m25	2	f/h	c/b	d/c
2	53	m26	2	m/b	c/b	j/h
2	53	m27	2	h/b	c/b	j/h
2	53	m28	2	m/l	c/a	h/c
2	53	m29	2	l/h	c/a	h/c
2	53	m30	2	l/h	c/a	h/c
2	55	M1	1	n/m	e/a	k/i
2	55	M2	1	n/m	d/b	a/i
2	55	M3	1	n/m	d/b	a/i
2	55	M4	1	m/d	e/d	k/i
2	55	M5	1	n/m	e/a	h/k
2	55	M6	1	m/d	e/a	k/i
2	55	M7	1	n/m	x/x	h/h
2	55	M8	1	n/m	b/a	h/a
2	55	M9	1	n/m	e/a	h/k
2	55	M10	1	m/d	d/b	h/a
2	55	M11	1	m/d	d/b	h/a
2	55	M12	1	m/n	d/e	h/h
2	55	M13	1	m/d	d/f	i/i
2	55	M14	1	m/d	d/j	h/b
2	55	M15	1	o/d	d/d	h/k
2	55	M17	1	n/m	e/a	k/i
2	55	M18	1	m/d	b/a	i/a
2	55	M19	1	m/n	f/d	i/h

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
2	55	M20	1	m/n	d/d	k/h
2	55	M21	1	m/n	f/a	h/a
2	55	M22	1	m/d	j/d	i/h
2	55	m1	2	m/d	b/a	i/a
2	55	m2	2	m/d	d/e	h/h
2	55	m3	2	o/d	c/d	h/b
2	55	m4	2	n/m	e/a	i/i
2	55	m5	2	m/d	d/b	h/a
2	55	m6	2	n/m	e/a	i/k
2	55	m7	2	m/d	a/a	i/i
2	55	m8	2	n/m	b/a	i/a
2	55	m9	2	f/d	e/a	i/i
2	55	m10	2	n/m	d/b	i/a
2	55	m11	2	m/d	f/a	h/h
2	55	m12	2	o/d	g/a	h/b
2	55	m13	2	f/d	d/d	h/i
2	55	m14	2	o/d	e/a	h/k
2	55	m15	2	n/m	d/b	i/a
2	55	m16	2	n/m	b/a	i/a
2	55	m17	2	o/d	c/a	i/b
2	55	m18	2	n/m	e/a	k/h
2	56	M1	1	h/h	m/e	b/b
2	56	M2	1	e/j	m/j	h/g
2	56	M3	1	e/h	m/f	h/g
2	56	M4	1	i/e	m/e	g/b
2	56	M5	1	k/h	j/e	h/b
2	56	M6	1	e/h	f/e	g/b
2	56	M7	1	i/h	e/e	h/g
2	56	M8	1	i/e	e/e	h/g
2	56	m11	2	e/j	m/j	g/b
2	56	m12	2	e/h	m/e	j/b
2	56	m1	2	e/e	m/m	h/h
2	56	m2	2	i/e	f/e	h/g
2	56	m3	2	e/h	e/e	h/g
2	56	m5	2	i/h	j/e	j/b
2	56	m6	2	e/j	j/e	g/b
2	56	m7	2	h/j	j/m	j/g

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
2	56	m8	2	i/h	e/e	h/b
2	56	m9	2	e/h	n/m	b/b
2	56	m10	2	e/j	j/e	g/b
2	56	m21	2	e/h	f/e	j/g
2	56	m22	2	e/h	n/m	j/b
2	56	m23	2	e/j	j/e	g/b
2	56	m24	2	h/j	j/m	g/b
2	56	m25	2	e/j	j/e	g/b
2	56	m26	2	h/j	j/e	g/b
2	56	m27	2	h/g	m/k	g/b
2	56	m28	2	h/d	j/e	j/b
2	56	m29	2	h/j	j/e	g/b
2	56	m30	2	h/j	e/m	j/g
2	56	m31	2	h/e	m/j	h/b
2	56	m32	2	h/j	m/j	g/b
2	56	m33	2	h/j	m/k	j/g
2	56	m34	2	e/g	e/e	g/b
2	56	m35	2	e/i	m/e	j/g
2	56	m36	2	f/h	m/n	b/b
2	56	m37	2	m/e	j/e	h/b
2	56	m38	2	e/j	l/e	j/g
2	56	m39	2	f/h	m/f	j/b
2	56	m40	2	i/e	f/e	h/b
2	51	M1	1	e/g	x/x	x/x
2	51	M2	1	e/g	e/c	h/c
2	51	M3	1	e/g	x/x	x/x
2	51	M4	1	e/g	e/e	g/c
2	51	M5	1	c/g	e/e	f/f
2	51	M6	1	e/g	e/e	k/f
2	51	M7	1	c/g	e/e	g/c
2	51	M8	1	e/c	f/c	h/c
2	51	M9	1	c/g	f/m	h/f
2	51	M10	1	c/g	e/e	f/f
2	51	M11	1	c/g	f/e	f/f
2	51	M12	1	e/g	f/e	k/c
2	51	M13	1	i/e	g/e	x/x
2	51	M14	1	h/c	f/c	f/f

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
2	51	M15	1	e/g	f/e	f/c
2	51	M16	1	e/g	f/e	f/c
2	51	M17	1	e/p	f/l	g/c
2	51	M18	1	e/g	f/d	k/f
2	51	M19	1	e/g	f/e	f/c
2	51	M20	1	e/f	f/c	g/c
2	51	M21	1	c/g	f/d	x/x
2	51	M22	1	e/h	e/c	x/x
2	51	M23	1	e/g	e/m	j/c
2	51	m1	2	e/g	e/e	k/f
2	51	m2	2	e/g	f/f	k/c
2	51	m3	2	e/c	f/f	f/b
2	51	m4	2	x/x	e/e	k/f
2	51	m5	2	x/x	e/e	k/c
2	51	m6	2	i/e	f/l	f/c
2	51	m7	2	h/c	f/c	f/f
2	51	m8	2	c/g	f/m	h/f
2	51	m9	2	e/g	f/m	x/x
2	51	m10	2	e/g	f/f	g/c
2	51	m11	2	e/g	f/m	j/f
2	51	m12	2	h/c	f/e	j/c
2	51	m13	2	e/h	f/c	j/c
2	51	m14	2	e/i	e/l	c/c
2	51	m15	2	e/p	e/c	x/x
2	51	m16	2	e/h	e/c	j/f
2	51	m17	2	e/g	f/m	j/f
2	52	M1	1	b/g	g/a	j/g
2	52	M2	1	b/g	g/a	k/j
2	52	M3	1	f/b	f/c	k/b
2	52	M4	1	f/b	f/c	k/g
2	52	M5	1	b/d	f/f	k/h
2	52	M6	1	f/b	f/c	g/b
2	52	m11	2	e/b	f/c	j/g
2	52	m1	2	e/b	f/c	j/g
2	52	m2	2	c/b	f/c	j/g
2	52	m3	2	e/f	f/d	g/b
2	52	m4	2	b/g	g/a	k/j

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
2	52	m5	2	e/f	f/d	k/b
2	52	m6	2	e/f	f/c	g/g
2	52	m7	2	e/f	f/d	k/b
2	52	m8	2	b/d	f/f	k/h
2	52	m9	2	e/g	g/a	k/j
2	52	m10	2	f/b	f/d	g/b
3	63	M1	1	l/b	e/e	k/f
3	63	M2	1	l/b	j/j	b/b
3	63	M3	1	l/b	j/j	b/b
3	63	M4	1	x/x	j/j	e/b
3	63	M5	1	m/l	x/x	e/b
3	63	M6	1	m/l	x/x	b/b
3	63	M7	1	h/c	j/e	f/b
3	63	M8	1	m/l	x/x	e/b
3	63	M9	1	d/c	x/x	k/b
3	63	M10	1	m/l	e/e	f/e
3	63	m1	2	d/c	j/e	x/x
3	63	m2	2	m/l	x/x	e/b
3	63	m3	2	m/l	j/f	e/b
3	63	m4	2	d/c	j/j	g/b
3	63	m5	2	h/c	j/j	f/b
3	63	m6	2	m/l	j/e	l/f
3	63	m7	2	m/l	e/e	l/b
3	63	m8	2	m/c	j/j	f/b
3	63	m9	2	m/l	x/x	e/b
3	65	M1	1	x/x	x/x	l/g
3	65	M2	1	h/c	b/d	l/b
3	65	M3	1	m/f	x/x	g/g
3	65	M4	1	f/h	b/d	g/b
3	65	M5	1	m/f	b/d	g/b
3	65	M6	1	m/c	c/d	c/b
3	65	M7	1	m/c	b/d	l/g
3	65	M8	1	m/c	c/d	c/b
3	65	M9	1	f/h	d/d	i/b
3	65	M10	1	m/c	d/c	c/b
3	65	M11	1	h/c	b/d	l/b
3	65	M12	1	h/f	b/d	g/g

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
3	65	M13	1	m/k	d/d	g/a
3	65	M14	1	c/m	b/d	l/b
3	65	M15	1	h/f	b/d	g/g
3	65	m1	2	m/p	d/d	g/b
3	65	m2	2	m/h	x/x	g/a
3	65	m3	2	m/f	d/b	g/b
3	65	m4	2	f/h	x/x	b/b
3	65	m5	2	f/h	d/b	g/b
3	65	m6	2	m/c	d/c	g/c
3	65	m7	2	m/f	d/b	g/g
3	65	m8	2	m/f	d/b	g/b
3	65	m9	2	f/h	x/x	g/g
3	65	m10	2	k/m	d/d	g/i
3	65	m11	2	l/h	d/f	g/b
3	65	m12	2	f/m	d/c	g/b
3	65	m13	2	x/x	x/x	g/b
3	65	m14	2	f/h	d/c	b/b
3	65	m15	2	f/h	d/b	g/b
3	65	m16	2	m/k	x/x	b/b
3	65	m17	2	o/h	d/d	g/a
3	65	m18	2	m/c	d/c	g/b
3	65	m19	2	o/h	d/d	b/b
3	65	m20	2	m/k	d/d	b/b
3	65	m21	2	f/h	d/c	g/b
3	65	m22	2	f/m	d/b	g/b
3	65	m23	2	m/m	d/e	i/b
3	65	m24	2	o/h	d/d	b/b
3	65	m25	2	o/h	d/d	g/a
2	54	M1	1	d/g	k/f	k/j
2	54	M2	1	j/g	k/e	j/j
2	54	M3	1	c/m	f/e	g/d
2	54	M4	1	h/m	f/k	k/j
2	54	M5	1	j/m	e/e	g/d
2	54	m11	2	b/m	e/e	h/d
2	54	m12	2	f/g	e/k	j/j
2	54	m13	2	j/g	e/e	j/d
2	54	m14	2	j/g	e/e	j/d

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
2	54	m15	2	j/m	f/e	g/d
2	54	m1	2	l/g	x/x	j/j
2	54	m2	2	f/g	e/e	j/j
2	54	m3	2	m/h	f/e	j/d
2	54	m4	2	m/b	f/e	h/d
2	54	m5	2	m/f	f/k	j/d
2	54	m6	2	g/p	e/e	j/j
2	54	m7	2	m/j	f/k	j/j
2	54	m8	2	d/g	f/k	k/d
2	54	m9	2	c/g	x/x	x/x
2	54	m10	2	j/g	k/e	j/j
3	62	M1	1	x/x	x/x	c/b
3	62	M2	1	x/x	j/f	g/d
3	62	M3	1	x/x	j/f	d/b
3	62	M4	1	x/x	j/j	d/d
3	62	M5	1	x/x	x/x	d/b
3	62	M6	1	x/x	k/f	l/d
3	62	M7	1	x/x	x/x	c/b
3	62	M8	1	x/x	x/x	l/d
3	62	M9	1	x/x	k/h	c/b
3	62	M10	1	x/x	j/j	d/d
3	62	m1	2	x/x	j/j	c/b
3	62	m2	2	x/x	k/f	g/d
3	62	m3	2	x/x	x/x	c/b
3	62	m5	2	x/x	x/x	l/d
3	62	m6	2	x/x	k/h	l/d
3	62	m7	2	x/x	j/f	x/x
3	62	m8	2	x/x	j/f	l/d
3	62	m9	2	x/x	j/j	c/b
3	62	m10	2	x/x	j/f	x/x
3	69	M1	1	p/b	e/e	i/g
3	69	M2	1	h/b	e/e	i/b
3	69	M3	1	b/b	h/e	i/d
3	69	M4	1	p/d	I/e	d/b
3	69	M5	1	p/b	I/e	d/b
3	69	M6	1	h/b	h/i	i/i
3	69	m11	2	p/d	e/e	i/b

Location	Colony label	Individual label	Caste	PR2	Pb5	Pb6
3	69	m12	2	b/b	I/m	i/b
3	69	m13	2	h/b	f/e	d/d
3	69	m14	2	h/b	e/i	d/b
3	69	m1	2	p/b	I/m	i/b
3	69	m2	2	b/b	x/x	x/x
3	69	m3	2	x/x	e/i	i/b
3	69	m4	2	b/b	e/i	i/g
3	69	m5	2	b/b	x/x	g/g
3	69	m6	2	h/b	e/i	i/g
3	69	m7	2	b/b	e/e	i/g
3	69	m8	2	h/b	x/x	i/i
3	69	m9	2	h/b	e/i	i/g
3	69	m10	2	p/b	x/x	i/g

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5. A Genetic Component in the Determination of Worker Polymorphism in the Florida Harvester ant *Pogonomyrmex badius*

Published in: Insectes Sociaux 52 (2005): 1-6

Authors: Frank E. Rheindt, Christoph-P. Strehl, Jürgen Gadau

5.1 Summary

The interplay of genetic and environmental factors in the determination of social insect castes has long intrigued biologists. Though an overwhelming majority of studies establish that factors such as nutrition, pheromones and temperature determine the developmental fate of worker larvae, genetic components have recently been shown to play a role in the determination of morphological worker castes in leaf-cutting ants. Here we demonstrate that the determination of worker castes in the strongly polyandrous Florida harvester ant, *Pogonomyrmex badius,* has a genetic component. The overall distribution of caste members among patrilines in our study colonies is significantly different from the intracolonial caste ratio. Though this effect was not apparent in all colonies, our results suggest that workers of different patrilines in *P. badius* differ significantly in their propensities to develop into a certain worker caste. This genetic basis of worker polymorphism may go unnoticed in many social hymenopterans because of their low intracolonial genetic diversity due to monogamous colony structure. The worker polymorphism of *P. badius* is a taxonomic isolate and presumably a young trait in the genus. Therefore, a common genetic component of the determination of morphological and behavioral worker castes in social insects might be far-ranging taxonomically and may even be based on a genetic machinery inherent to all hymenopterans, but dormant in most.

5.2 Introduction

Unraveling the mechanisms of caste determination in social insects lies at the heart of elucidating the evolution of polyphenism (Evans and Wheeler, 2001). Most biologists have identified nutrition or other environmental cues as the key trigger responsible for inducing a developmental pathway that eventually leads to a certain caste (Wilson, 1971; Michener, 1974; Nijhout and Wheeler, 1982; Hölldobler and Wilson, 1990;

Wheeler, 1991). Hormones mediate the effects of these factors by acting at particular thresholds to initiate differential gene expression (Evans and Wheeler, 2001).

However, doubts about the universality of this explanation were voiced early on (Wheeler, 1937). Beginning with observations by Kerr (1950), it has been confirmed repeatedly that caste ratios and size proportions in many social insects are stable through environmental fluctuations (Johnston and Wilson, 1985; Passera et al., 1996; Fraser et al., 2000; Billick, 2002). This constancy may point to some genetic factor that regulates caste recruitability.

Non-monogamous species are the most likely candidates in which genetic effects on caste determination may be detectable and may have some selective advantage. Among them, polyandrous species, in which maternal effects can be ruled out as a confounding factor, would be the study object of choice.

A suite of studies established genetic queen-worker caste determination as a preponderant feature in hybridizing populations of *Pogonomyrmex* harvester ants and in three ant species that display queen polymorphism (summarized in Hughes et al., 2003). However, Hughes et al. (2003) remarked that all these cases of genetic caste determination may be exceptions to the rule of environmentally mediated caste determination, because they are associated with unusual reproductive characteristics involving queen castes or hybridization. Noting that none of these confirmed genetic elements pertained to the determination of worker castes, they set out to analyze caste determination in *Acromyrmex*, which is characterized by a pronounced worker caste system. Their major finding was a link between patriline affiliation and a worker’s propensity to develop into a certain caste. Hughes et al. (2003) concluded that worker caste determination appears to be the product of genetic predisposition and environmental plasticity, and that the latter may have helped mask the former in previous research. However, to demonstrate a universal role of genetics in worker caste determination, this result should be confirmed in independent taxonomic groups.

We investigated if worker caste determination has a genetic component in the strongly polyandrous (Rheindt et al., 2004) Florida harvester ant, *Pogonomyrmex badius*. This species belongs to a different tribe of Myrmicinae than *Acromyrmex* and is one of only two species in the genus that exhibit a distinct worker dimorphism (Taber, 1998). Members of the major seed-milling caste are the largest individuals that can be found among harvester ants (Johnson, 2000), and there is very limited morphological overlap with the minor caste (Tschinkel, 1998). In Florida, where our samples were collected, majors comprise on average 7% of the worker force and seem to be produced right from the beginning of a colony's existence (Tschinkel, 1998). *P. badius* is geographically isolated from all congeners, ranging from Louisiana to the Carolinas. Although its distribution is parapatric with *P. barbatus* along the western boundary, there are no known cases of hybridization or syntopy (Johnson, 2000).

5.3 Methods

We investigated workers from 18 colonies that had been collected across four different localities in Florida in August/September 2001 and in February 2003 (Table 1). Specimens were immediately put in 95% ethanol upon collection. We used the same extraction protocol as Gadau et al. (1996). Patrilines were determined using three microsatellite loci (for details see Rheindt et al., 2004, and Table 1).

Caste affiliation was determined by measuring headwidths to the nearest 0.01mm. Tschinkel (1998) characterized the headwidth frequency distribution of workers in *P. badius* as consisting of two contiguous normal distributions with very limited overlap. He separated minors from majors in each of his colonies by assessing the range of headwidths at which these two distributions intersect. In our analysis, all majors had a headwidth greater than 2.7mm, while all minors had a headwidth of less than 1.9mm. Both these cut-off points are beyond Tschinkel's (1998) intermediate range of headwidths in which both majors and minors could be found.

Patriline identity of workers was determined by the inferred haplotype of the queen's mates using three microsatellite loci (for details see Rheindt et al., 2004 and references therein). Determining the patrilines based on female offspring is made easier in hymenopterans due to the haplo-diploid sex determination mechanism, in which males are haploid. Worker genotypes were interpreted by making the assumption of monogyny; polygyny has never been reported in the genus and/or in the species despite extensive fieldwork (Taber, 1998; Tschinkel, 1998; Cole and Wiernasz, 2000; Gadau et al., 2003). Patrilines that were represented by less than three workers are not suitable for the statistical detection of caste skew because of their inherently low sample size and were therefore omitted from analysis. G tests for heterogeneity (Sokal and Rohlf, 1995) were used to assess whether patrilines were distributed across both castes according to the actual ratio of major/minor workers. One computational difficulty of this test is its utilization of ratios, which bars all data points equaling zero from analysis. However, a considerable proportion (23.88%) of our caste frequency ratios involved instances in which a patriline was entirely absent in one of the castes. To render possible the inclusion of these valuable data, frequencies of zero had to be replaced with 1. Note that this conservative practice significantly reduced our chances of finding an association between patriline and caste.

5.4 Results

We successfully genotyped 248 majors and 320 minors across 18 colonies (Table 1). In three colonies, one to two primers did not amplify (Table 1). Colony 15-O was omitted from further analysis because no patrilines were represented by more than two workers (Table 1).

There were significant differences of the within-patriline ratio of majors/minors from their actual intracolonial ratio in three out of 17 colonies (17.65%; see Table 1 for p-values). This significance did not hold when applying a sequential Bonferroni correction (Sokal and Rohlf, 1995). However, when all patrilines were viewed as a single dataset, the distribution of castes among patrilines departed significantly from their representation across each colony ($G = 90.314$, $df = 67$; $p = 0.030$; Table 1).

Table 2 shows the frequencies of both castes across all patrilines and colonies as well as the respective statistics. Only five out of 67 patrilines (7.46%) analyzed were characterized by a significantly skewed caste ratio (see Table 2). However, most patrilines in consideration (67.16%) had a representation range of between three and five workers, which is too low to warrant detectability of caste skew in many of the colonies. Only 10.45% of all patrilines analyzed comprised more than 10 workers. This small average patriline size in our dataset therefore explains the relatively rare incidence of significant within-patriline caste skew (as compared with the colony-level and study-wide analyses).

5.5 Discussion

In our study, the overall distribution of castes among patrilines departed significantly from their across-colony representation. Colony-specific analyses showed that almost a fifth of our study colonies displayed a significant within-patriline caste skew. Possibly half the patrilines in our dataset were too small for statistic detection of caste skew, and many more might have been suitable for the detection of substantial skew only. Still one thirteenth of all patrilines in consideration were significantly caste-skewed when given separate scrutiny. Even the smaller and more under-represented patrilines seemed to carry considerable signal and were therefore not deleted from analysis, as can be seen by the fact that the study-wide analysis and some of the colony-level ones yielded significant caste skew.

Our results are probably conservative, and the genetic component of the determination of worker polymorphism in *P. badius* is possibly more pronounced than we have demonstrated. Several factors may be accountable: While sampling more colonies than Hughes et al. (2003), our average number of workers per colony was admittedly low, mainly because the low ratio of majors in *P. badius* (7% per colony; Tschinkel, 1998) makes it exceedingly hard to procure more than 20 unequivocal majors during any single excavation (the size of the sampled colonies ranged from *ca.* 200 through 2000 workers, but large colonies are harder to find). Consequently, many of our smaller patrilines that seemed to display a fairly skewed distribution of caste members were beyond statistic detectability. In addition, some of the above mentioned peculiarities inherent to our dataset (e.g. the high incidence of patrilines that were absent in one caste) called for methodological measures that increased the chance of committing type 2 errors, i.e. to erroneously reject caste skew in patrilines that do exhibit it (see Methods).

Our results seem to be in line with those of Hughes et al. (2003) by demonstrating that workers belonging to different patrilines in *P. badius* show a significant level of variability in their propensities to develop into a certain morphological caste. *P. badius* would therefore constitute the second species in which a genetic component to the determination of morphological worker castes has been shown. Like Hughes et al. (2003), we find it likely that patriline/genotype is not solely responsible for caste destiny, since most of our patrilines (and with a higher sampling effort probably all of them) were actually represented in both castes. Rather, it appears that genotype specifies an individual’s threshold to environmental cues – presumably nutrition, pheromones and temperature – that govern developmental destiny (Nijhout and Wheeler, 1982; Hölldobler and Wilson, 1990; Wheeler, 1991). A similar mechanism has been confirmed in honey bee workers of morphologically identical “behavioral castes”, in which members of some patrilines have a higher predisposition to carry out certain tasks than others (Robinson and Page, 1988; Frumhoff and Baker, 1988; Page et al., 1989; Robinson, 1992; Oldroyd et al., 1994).

Worker dimorphism in *P. badius* is a relatively young autapomorphy. The only other congener that displays worker caste dimorphism is the geographically and taxonomically distant *P. coarctatus* (Taber, 1998). All other *Pogonomyrmex* workers are monomorphic. It is now clear that the genetic basis of worker caste determination is not restricted to the genus *Acromyrmex*, but occurs more widely across ants. The evolutionary youth and taxonomic isolation of the worker caste system in *P. badius* suggest that the genetic basis for caste determination may have been present in the ancestor, lying dormant until evolutionary events favored the adoption of worker castes. It is conceivable that this genetic machinery is common to a wide array of ants and maybe even homologous to the mechanism by which behavioral bee castes are genetically determined. If so, the genes for worker caste determination can apparently be switched on and off with the rise and fall of worker caste systems over space and time. We recommend that future research concentrate on the detection of a genetic basis for worker caste determination in other groups of ants and social insects and on the identification of genes that may be involved in the mechanism of caste determination.

5.6 Acknowledgments

We are indebted to Walter Tschinkel and all members of his laboratory for insight into the sociobiology of *P. badius* and for assistance in the field. Annett Endler helped to collect specimens and deserves our warmest thanks. This work was supported by a grant to JG from the DFG (SFB-C-5). The treatment of ants complied with German and U.S. laws.

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5.8 Tables

Table 1 (following page):

Colonies, number of successfully amplified loci, number of successfully genotyped majors and minors, total number of patrilines and number of patrilines that were represented by three or more workers (which is simultaneously the number of degrees of freedom in the G test); G test statistics and their p values are given for the hypothesis that the distribution of caste members across patriline does not differ from their intracolonial ratio; significant p values ($p<0.05$) are marked with an asterisk; colony labels refer to the following sites: Ar – Archbold Biological Station (N 27° 11’, W 81° 20’), W – Withlacoochee State Forest (N 28° 48’, W 82° 29’), O – Ocala National Forest (N 29° 16’, W 81° 49’), Ap – Apalachicola National Forest (N 30° 22’, W 84°29’).

Colony (incl. site label)	Number of successfully amplified loci	Number of workers successfully genotyped (majors, minors)	Total number of patrilines	Number of patrilines represented by >2 workers	G statistic of test for heterogeneity	P value of test for heterogeneity
1-Ar	3	9, 10	5	3	3.532	0.317
2-W	3	23, 17	18	6	9.693	0.138
3-Ar	3	9, 10	8	2	1.378	0.502
4-W	3	6, 10	7	2	2.843	0.241
5-O	3	10, 10	10	2	0.541	0.762
6-Ar	3	13, 6	7	3	0.963	0.810
7-Ar	3	7, 32	12	5	11.810	0.037 *
8-W	3	21, 18	16	3	1.727	0.631
9-W	3	17, 23	7	4	0.666	0.955
10-W	3	8, 30	27	2	6.125	0.047 *
11-O	3	16, 23	14	4	12.496	0.014 *
12-O	3	6, 13	9	3	2.727	0.436
13-Ar	3	8, 12	4	3	5.784	0.123
14-W	3	5, 15	12	1	0.104	0.747
15-O	2	7, 7	9	0	-	-
16-Ap	2	24, 24	20	10	17.087	0.145
17-Ap	3	31, 33	28	7	9.312	0.349
18-Ap	1	28, 27	11	7	8.803	0.383
Overall	-	248, 320	225	67	90.314	0.030 *

Table 2:

All patrilines and their colony affiliation, number of majors and minors, colony wide ratio of majors / (total workers) and G statistics testing the hypothesis that caste ratio within patrilines equals their colony-wide ratio

Colony label	Number of Majors	Number of minors	Colony-wide Major/(minor+Major)	G statistic (* p<0.05)
1 – Ar	4	1	0,474	2,257
1 – Ar	1	2	0,474	0,243
1 – Ar	2	5	0,474	1,032
2 – W	3	0	0,590	2,753
2 – W	1	2	0,590	0,801
2 – W	6	0	0,590	4,535*
2 – W	2	1	0,590	0,075
2 – W	2	3	0,590	0,728
2 – W	1	2	0,590	0,801
3 – Ar	2	3	0,474	0,110
3 – Ar	3	1	0,474	1,268
4 – W	2	2	0,375	0,258
4 – W	0	3	0,375	2,584
5 – O	1	2	0,500	0,340
5 – O	3	2	0,500	0,201
6 – Ar	3	3	0,684	0,875
6 – Ar	3	1	0,684	0,084
6 – Ar	2	1	0,684	0,004
7 – Ar	1	10	0,179	0,690
7 – Ar	1	6	0,179	0,068
7 – Ar	3	1	0,179	6,203*
7 – Ar	0	3	0,179	2,425
7 – Ar	0	3	0,179	2,425
8 – W	6	2	0,538	1,524
8 – W	2	1	0,538	0,203
8 – W	7	6	0,538	0,000
9 – W	4	5	0,425	0,014
9 – W	1	2	0,425	0,106
9 – W	6	6	0,425	0,273
9 – W	6	6	0,425	0,273
10 – W	2	1	0,211	2,886
10 – W	0	10	0,211	3,239
11 – O	0	5	0,410	3,844*
11 – O	3	1	0,410	1,903
11 – O	5	0	0,410	6,747*
11 – O	5	7	0,410	0,002
12 – O	1	2	0,316	0,004
12 – O	1	4	0,316	0,337
12 – O	0	3	0,316	2,385
13 – Ar	4	2	0,400	1,735

13 – Ar	0	5	0,400	3,722
13 – Ar	4	4	0,400	0,327
14 – W	1	2	0,250	0,104
16– Ap	1	2	0,500	0,340
16 – Ap	1	2	0,500	0,340
16 – Ap	4	2	0,500	0,680
16 – Ap	0	3	0,500	3,348
16 – Ap	5	1	0,500	2,911
16 – Ap	2	2	0,500	0,000
16 – Ap	2	2	0,500	0,000
16 – Ap	0	3	0,500	3,348
16 – Ap	2	1	0,500	0,340
16 – Ap	3	0	0,500	3,348
17 – Ap	2	3	0,516	0,269
17 – Ap	1	2	0,516	0,405
17 – Ap	2	1	0,516	0,280
17 – Ap	1	2	0,516	0,405
17 – Ap	3	0	0,516	3,227
17 – Ap	2	2	0,516	0,004
17 – Ap	3	0	0,516	3,227
18 – Ap	10	6	0,491	1,161
18 – Ap	5	3	0,491	0,581
18 – Ap	0	4	0,491	4,051*
18 – Ap	1	3	0,491	0,975
18 – Ap	1	2	0,491	0,304
18 – Ap	5	5	0,491	0,003
18 – Ap	2	1	0,491	0,377
18 – Ap	5	3	0,491	0,581
18 – Ap	0	4	0,491	4,051*
18 – Ap	1	3	0,491	0,975
18 – Ap	1	2	0,491	0,304
18 – Ap	5	5	0,491	0,003
18 – Ap	2	1	0,491	0,377
18 – Ap	5	3	0,491	0,581
18 – Ap	0	4	0,491	4,051*
18 – Ap	1	3	0,491	0,975
18 – Ap	1	2	0,491	0,304
18 – Ap	5	5	0,491	0,003
18 – Ap	2	1	0,491	0,377
18 – Ap	5	3	0,491	0,581
18 – Ap	0	4	0,491	4,051*
18 – Ap	1	3	0,491	0,975
18 – Ap	1	2	0,491	0,304
18 – Ap	5	5	0,491	0,003
18 – Ap	2	1	0,491	0,377
18 - Ap	5	3	0,491	0,581
18 - Ap	0	4	0,491	4,051*
18 - Ap	1	3	0,491	0,975
18 - Ap	1	2	0,491	0,304
18 - Ap	5	5	0,491	0,003
18 - Ap	2	1	0,491	0,377

6. Cladistic Analysis of Paleo-Island Populations of the Florida Harvester Ant (Hymenoptera: Formicidae) Based Upon Divergence of Mitochondrial DNA Sequences

Published in: *Florida Entomologist* 87(4) December 2004, pp. 576-581

Authors: Christoph-Peter Strehl, Jürgen Gadau

6.1 Abstract

To examine the relationships of geographically isolated paleo-island populations of *Pogonomyrmex badius* (Latreille 1802) in Florida we generated a phylogeographic hypothesis based on mitochondrial DNA (mtDNA) sequences. We found at least three distinct mtDNA lineages and a positive correlation between genetic and geographic distances. The relationships between nowadays isolated *P. badius* populations might resemble a long lasting separation due to either restricted gene flow caused by inbreeding, paleo-climatic events or the impact of novel invasive species. The current depletion of the only representative of the ant genus *Pogonomyrmex* in the south-eastern USA makes a more fine-scaled mapping of the remaining, small *P. badius* populations necessary to identify evolutionary distinct units for conservation purposes.

6.2 Resumen

Para examinar las relaciones de poblaciones de *Pogonomyrmex badius* (Latreille 1802) de paleo-islas geograficamente aisladas en Florida nosotros generamos una hipótesis filogeografica basada sobre las secuencias de ADN mitocondrial (mtDNA). Nosotros encontramos por lo menos tres linajes distintos de mtDNA y una correlación positive entre las distancias genéticas y geográficas. Las relaciones entre las poblaciones de *P. badius* aisladas de hoy dia puede representar una separación de largo plazo debido al flujo de genes restringidos causado por la reproducción entre individuos de la misma familia, los eventos paleo-climáticos o el impacto de nuevas especies invasoras. La reducción actual de la unica hormiga representativa del género *Pogonomyrmex* en el sureste de los Estados Unidos hace necesario que se traze un mapa de una escala mas

precisa para las poblaciones pequenasde *P. badius* restantes para identificar las distintas unidades evolucionarias para propósitos de conservación.

6.3 Introduction

The paleogeographic history of Florida and its islands during Pleistocene with regular flooding of major parts of the Florida peninsula is well documented (Faught & Carter 1998; Froede 2002; Cunningham et al. 2003; Portell et al. 2003). The general pattern is that animals expanded their range from their refuges with the end of the last glaciation period. This historical isolation of different populations of the same species could have created distinct strains (Ribera & Vogler 2004) with their own history and genetic composition. The genetic distinctiveness of those “paleo-island” populations might be further increased if the population structure is highly viscose.

Demographic responses to climate change and resulting range changes usually result in genetic manifestation, making them genetically traceable with adaptively neutral genetic markers (Hewitt 2000; Lessa et al. 2003). Therefore, the genetic analysis of current Florida paleo-island populations might provide us with insights into historical events. In between numerous exotic ant species inhabiting the Florida peninsula, *Pogonomyrmex badius* (Latreille 1802) is considered “the closest approach to an endemic genus” (Deyrup & Trager 1986; Deyrup et al. 1988). *Pogonomyrmex badius* was isolated in the xeric uplands of central Florida during Pleistocene (Deyrup & Trager 1986). These isolated populations came again into contact after climatic changes at the end of the Pleistocene. Recently, colonization of Florida by aggressive, invasive species like *Solenopsis invicta* (Whitcomb et al. 1972; Deyrup et al. 2000; Cherry 2001) and abundant agricultural land use resulted again in a restriction of *P. badius* to more or less isolated island populations in remnant sand hill and scrub habitats. Therefore, the current populations of *P. badius* are the remains of formerly larger populations with a very restricted gene flow between them.

Retained ancestral polymorphisms can yield distinct phylogenetic relationships (Bulgin et al. 2003). Therefore, assuming restricted gene flow between isolated “island” populations of *P. badius* even since Pleistocene, a DNA based cladogram should resemble the historical events of the withdrawal of *P. badius* into ice-age refuges before and after introduction of invasive species. Genetic distances should increase with geographic distances if the phylogenetic pattern between island populations is based on ancient colonization events rather than splitting of a wide population zone and subsequently random, but incoherent mutation events. To examine the relationships of geographically isolated populations of *Pogonomyrmex badius* in Florida, we analyzed population samples phylogenetically using mitochondrial DNA sequences.

6.4 Materials and Methods

Collection of Specimens

Specimens of *Pogonomyrmex* (*sensu stricto*) *badius* (Table 1) were collected from six populations throughout Florida (USA) and preserved in 70100% Ethanol for later DNA analyses. Similarly, *Pogonomyrmex* (*Ephebomyrmex*) *imberbiculus* and four additional *Pogonomyrmex* (*sensu stricto*) species (Table 1) were collected as outgroup-spec-imens for later phylogenetic analyses. Subgenusclassification followed Bolton (1995), whereas determination of species followed the keys of Taber (1998) and Cole (1968), and was confirmed by independent researchers where possible (Table 1).

Nestmates from the analyzed workers are deposited as pinned voucher specimens in the collections of Harvard University (Cambridge, MA, USA) and collections of P.S. Ward at University of California in Davis (USA) and preserved in 100% Ethanol (stored at -70°C) at Museum Koenig (Bonn, Germany).

DNA-Isolation

DNA was extracted from workers with their gasters removed by phenol/chloroform extraction (Gadau et al. 1996), with the DNeasy Kit (Quiagen; following manufacturers tissue-protocol A for insects), or the Puregene Kit (Biozym/Gentra Systems, following the protocol of Gadau et al. 2003). The latter two methods worked well for specimens conserved in 70% Ethanol, which was problematic for the phenol/chloroform extraction method. Genomic and mitochondrial DNA was not separated by this method. DNA was dissolved in low TE-buffer and the success of DNA-isolation was tested on agarose gels. Good samples were diluted 1:10 with HPLC-water to a final concentration of approximately 5-10 µM.

PCR

We amplified fragments of the Cytochrome c Oxidase I (COI, 1054 bp) and Cytochrome b (CytB, 439 bp) mitochondrial genes (Table 2). PCR reactions were performed on a Biometra thermocycler (heating rate 5°C/s) with the following primer pairs (degenerate positions of primersequences are placed within brackets; numbers in brackets following the 3’ end of each primer refer to the next nucleotide positions relative to the sequence of the *Apis mellifera* mitochondrial genome published by Crozier & Crozier 1993, GeneBank-accession number NC_001566.1): LCO (sense) 5’-GGTCAACAAATCATAAAGATATTGG-3’ [1835] and HCO (anti-sense) 5’-TAAACTTC-AGGGTGACCAAAAAATCA-3’ [2492] (Folmer et al. 1994), Jerry (sense) 5’-CAACATTTATTTTGA-TTTTTT-3’ [2502] (modified bee-primer Ca-J-2183 of Simon et al. 1994) and Ben3R (anti-sense) 5’-GC(AT)AC(AT)AC(AG)TAATA(GT)GTATCATG-3’ [2888] (Brady et al. 2000) for CoxI; CB1 (sense) 5’-TATGTACTACCATGAGGACAAATATC-3’ [11426] and CB2 (anti-sense) 5’-ATTACACCTC-CTAATTTATTAGGAAT-3’ [11858] (bee-primers CP-J-10933 and CB-N-11367 of Simon et al. 1994) for CytB. The reaction volume was 25 µl, containing 2 µl of 1:10 diluted DNA-extraction, 2.5 µl of 10× PCR Buffer (750 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$, 0.01% Tween 20), 0.2 mM of each dNTP, 2.0 mM $MgCl_2$, 0.52 µM of each primer, and 1.0U Taq DNA polymerase (MBI Fermentas, Lithuania). Cycling parameters were 3 min at 95°C for initial denaturation, followed by 33 cycles of denaturing 30 sec at 95°C, annealing 60 sec at 45°C, and elongation 30 sec at 72°C; two final steps of elongation 90 sec at 72°C and cooling down to 4°C were added. Amplicons were purified by ammonium acetate-precipitation (Sambrook et al. 1989) or with the Quiaquick purification kit (Quiagen). Sequencing reactions were performed by SeqLab (Göttingen, Germany).

Table 1:

Specimens of *Pogonomyrmex* used for mtDNA sequence analyses (Cytochrome c Oxidase I; Cytochrome Oxidase b); Acc.-No. = Accession Numbers (Numbers indicate collectors of specimen / determinators of species names: 1 = Annett Endler, 2 = Alexander Mikheyev; 3 = Christoph-P. Strehl (CPS); 4 = Juergen Gadau (JG); 5 = Juergen Liebig; 6 = Phil S. Ward; 7 = Robert A. Johnson (RAJ); 8 = Susanne Hoyer; 9 = Stefan P. Cover; 10 = Z. Punsak); Location = Place of collection, connected to GPS-data; COI/CytB = GeneBank accession numbers for Cytochrome c Oxidase I sequences / Cytochrome b sequences corresponding to samples.

Species	Acc.-No.	Location	Sequence Name [COI/CytB]
P. badius	CPS125 $^{10/7}$	Titusville, FL (N 28 32' W 80 50')	TIV [AY510637/-]
P. badius	CPS199 $^{3,5/3,5}$	Lake Placid, FL (N 27° 11' 37.7'' W 81° 20' 42.1'')	ABS [AY510636/AY538614]
P. badius	CPS200 $^{1,3/1,3}$	Withlacoochee, FL (N 28° 48' 42.4'' W 82° 29' 6.3'')	WIT [AY510633/AY538616]
P. badius	CPS201 $^{1,3/1,3}$	Ocala Ntl. Park, FL (N 29° 16' 26.5'' W 81° 49' 5.4'')	OCA [AY510635/AY538619]
P. badius	CPS203 $^{3/3}$	Fort Pierce, FL (N 27° 28' 26.5'' W 80° 17' 32.0'')	FTP [AY510638/AY538621]
P. badius	CPS204 $^{1,3/1,3}$	Lake Placid, FL(N 27° 13' 11.8'' W 81° 22' 48.5'')	LKP [AY510634/AY538615]
P. badius	CPS230 $^{2/2}$	Tallahassee, FL (N 30 27' W 83 20')	TA1 [AY510631/AY538618]
P. badius	CPS234 $^{2/2}$	Tallahassee, FL (N 30 27' W 83 20')	TA2 [AY510632/AY538617]
P. barbatus	CPS56-30 $^{3,4,8/3,7}$	Phoenix, AZ (N 33° 32' 40.7'' W 111° 38' 3.6'')	BRB [AY510639/AY538620]
P. californicus	RAJ2269 $^{7/6}$	La Chocera, Mexico (N 30° 30.96' W 116° 2.46')	CAL [AY510649/AY538625]
P. huachucanus	CPS123 $^{4,3/6,9}$	Portal, AZ (N 31° 55' 56.1'' W 109° 12' 26.2'')	HUA [AY510657/AY538623]
P. occidentalis	JG27 $^{3,4/3,5}$	Seligman, AZ (N 35 19' 37.5" W 112 52' 35.7")	OCC [AY510667/AY538622]
P. (Ephebomyrmex) imberbiculus	CPS268 $^{3/3}$	Portal, AZ (N 31° 55' 49.7'' W 109° 7' 59.4'')	IMB [AY510614/AY538624]

Table 2:

Pairwise distance between each sequence (Sequ.), calculated using PAUP 4.0b10 (Swofford 1998); Below diagonal: Total character differences; Above diagonal: p-distance matrix; Sequence names: compare Table 1.

Sequ.	IMB	TA1	TA2	WIT	LKP	OCA	ABS	FTP	BRB	CAL	HUA	OCC	TIV
IMB	-	0.18201	0.18137	0.18880	0.18953	0.18620	0.18942	0.18823	0.18510	0.19351	0.19411	0.20116	0.15907
TA1	252	-	0.00000	0.02617	0.04885	0.02602	0.05106	0.04973	0.10452	0.09134	0.14300	0.11041	0.03139
TA2	253	0	-	0.02601	0.04792	0.02585	0.04989	0.04893	0.10242	0.09122	0.14113	0.10949	0.03136
WIT	268	37	37	-	0.04096	0.00480	0.04457	0.04048	0.10688	0.09232	0.14897	0.11422	0.01766
LKP	267	69	68	60	-	0.04108	0.00140	0.00348	0.10624	0.09520	0.14732	0.11279	0.00314
OCA	258	36	36	7	59	-	0.04396	0.04120	0.10786	0.09608	0.15098	0.11011	0.01555
ABS	260	70	69	64	2	63	-	0.00285	0.10552	0.09749	0.14736	0.11312	0.00311
FTP	260	69	68	58	5	58	4	-	0.10587	0.09886	0.14342	0.11299	0.00314
BRB	258	145	143	155	153	154	150	150	-	0.11654	0.14747	0.12224	0.09953
CAL	254	121	121	126	130	129	130	135	158	-	0.16228	0.11672	0.09293
HUA	275	201	200	219	215	217	210	205	213	221	-	0.16021	0.13272
OCC	273	150	149	161	159	154	157	158	171	156	225	-	0.10316
TIV	50	10	10	7	1	6	1	1	34	32	46	36	-

Sequence Analysis

Obtained sequences were analyzed on Personal Computers. Proof reading was accomplished by comparing the forward and reverse amplicons and aligning them in a text-program with subsequent use of ClustalX (Thompson et al. 1997). Statistical analysis was performed with the programs PAUP 4.0b10 (Swofford 1998) and Mega 2.1 (Kumar et al. 2001). All sequences are deposited in GenBank (Table 1). For comparing population pairings we analyzed both types of sequences (COI, CytB) together or separately. Gene-trees were constructed in PAUP 4.0b10 using the Neighbor-Joining method (uncorr. *p*-distance; Kimura 2-parameter; HKY85; 100,000 bootstrap replicates), or Maximum Parsimony method (branch and bound search, 1000 bootstrap replicates).

We tested for a correlation of genetic distances with geographic distances between the analyzed populations. Geographic distances between samples were calculated with GPS data of Table 1 transformed into UTM-data (metric) and plotted against the genetic distances (uncorrected "*p*") calculated in PAUP 4.0b10 with the set of sequences used for constructing the gene-trees (Table 2). Between the Tallahassee samples, in which no detailed GPS-data were available, a geographic distance of 4 m was assumed.

6.5 Results

Sequencing of both gene fragments resulted in general in 1493 base pairs used as characters in the subsequent phylogenetic analysis. Among the variable characters, 213 were parsimony-uninfor-mative and 249 were parsimony-informative. Table 2 shows the absolute and p-distance between each sequence. Among the Pogonomyrmex badius samples there were 14 variable amino acids found among a total of 497.

Population pairings were identical with both sequences (COI, CytB) separately or together and either Neighbor-Joining (Fig. 1) or Maximum Parsimony analysis. We therefore show a tree based on both genes (Fig. 1). The populations of Fort Pierce and Lake Placid grouped together, as did those from Withlacoochee and Ocala. The separation of the Fort Pierce/Lake Placid populations from the Titusville population (CPS125) was not supported in the MP analysis, and we considered them as one mitochondrial lineage. Moreover, the characters used for the Titusville population are based on the shortest of all sequences, as only one of the primer pairs yielded a sequence out of the single worker available. We justify the inclusion of this sample into the data because omitting it did not change the pattern shown in Fig. 1, and it provided additional information about the putative range of the southern mtDNA-lineage. The grouping of the Withlacoochee/Ocala population lineage together with the Tallahassee population was well supported by high bootstrap values. Because our sampling was very limited, however, the dotted line in Fig. 1 should be seen as preliminary.

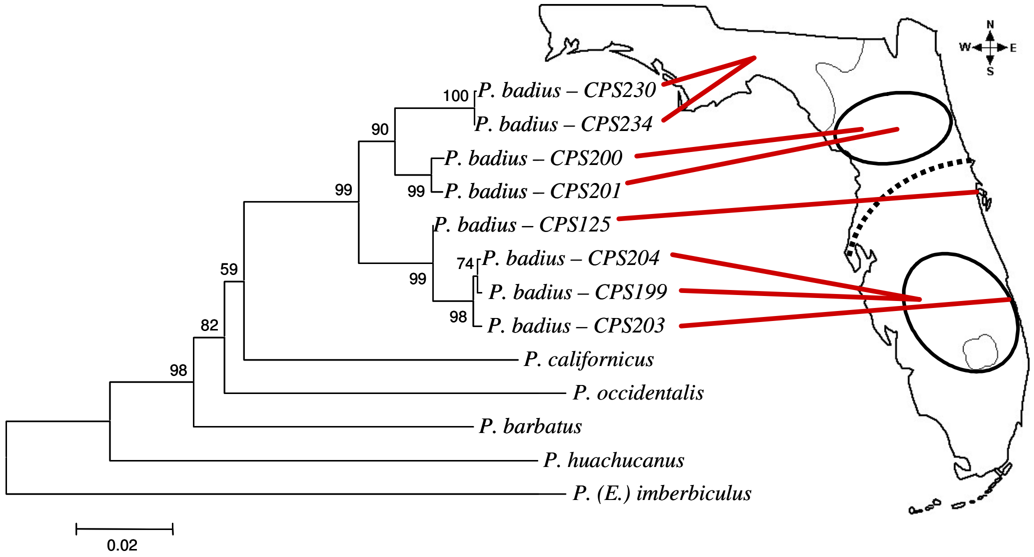

Figure 1:

Unrooted neighbor-joining tree of 1493-bp sequences (Cytochrome c Oxidase I and Cytochrome Oxidase b) of *Pogonomyrmex* spec., created with MEGA 2.1 [Distance method: Nucleotide: Kimura 2-parameter (Pairwise distance); Gaps/Missing Data: pairwise deletion; No. of bootstrap Reps: 100,000; SBL = 0.52206366] connected to the collection places of *Pogonomyrmex badius* on a contour of Florida, with circles/bows indicating hypothetical boundaries of genetically separated lineages (see text); numbers at branches indicate bootstrap replicates over 50%.

Genetic distance showed a positive linear correlation with geographic distances between all population samples (Fig. 2; $n = 28$, $R^2 = 0.259$, $t = 3.011$, $P = 0.00573$). By excluding the Titusville population because of their limited genetic information, this correlation became even stronger ($n = 21$, $R^2 = 0.498$, $t = 4.341$, $P = 0.00035$). To prevent a bias of those populations where two samples were available (Tallahassee, Lake Placid) compared to those with only one, we included only one of them (CPS204 and CPS234) and reanalyzed the data. This procedure did not increase the significance of correlation, but increased the R^2 value ($n = 10$, $R^2 = 0.793$, $t = 5.540$, $P = 0.000547$). This effect is mainly due to an exponential increase in genetic variability with distances over 100 km (62.14 mi).

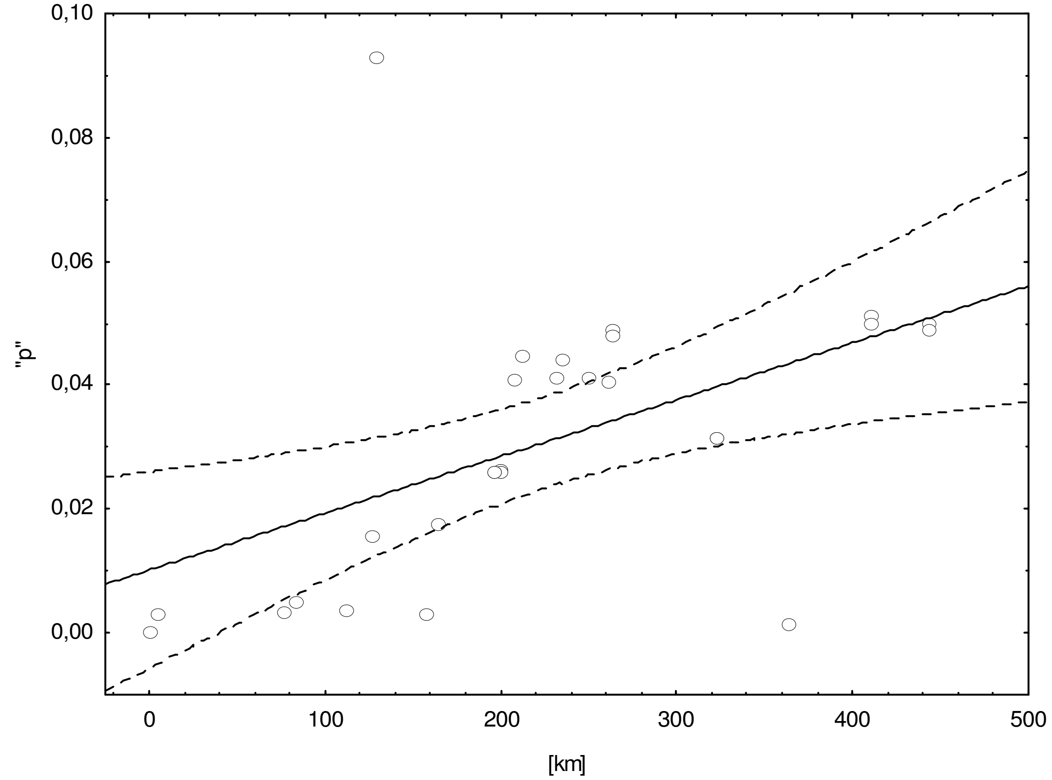

Fig. 2.

Plot showing the positive correlation for calculated geographic [km] and genetic (”p”) distances between Pogonomyrmex badius population samples sequenced for this study (P < 0.006, see text). The linear regression line is defined as “p” = 0.0102 +

9.176-5 [km], and accompanied by the corresponding 95% confidence interval (dotted line).

6.6 Discussion

Our analysis of mitochondrial DNA of *Pogonomyrmex badius* populations in Florida yielded three distinct lineages: (1) a southern lineage including samples from Lake Placid to Titusville, (2) a middle lineage including the Withlacoochee and Ocala populations, and (3) the northern lineage of the Tallahassee populations. The Withlacoochee and Ocala populations probably are more closely related to the Tallahassee populations. This view is further supported by a positive correlation of genetic with geographic distances.

The gene-tree and significant correlation of genetic and geographic distances suggest an ancient North to South movement of *P. badius* during its colonization of Florida with either very limited gene-flow back north, or less likely, higher mutation/fixation rates at the border of the ex-panding populations (Edmonds et al. 2004). Over all, the genetic relationships between the different population samples of *P. badius* might reflect past paleo-climatic events with a long lasting separation of populations withdrawn to protected “island” areas, like those found in the Lake Wales Ridge (Deyrup & Trager 1986). After the Pleistocene these populations might have come into secondary contact. This contact, however, did not lead to a sufficient flow and intermixing of the maternally inherited mitochondrial DNA before the *P. badius* populations were once again separated by anthropogenic devastation of their habitats, which was re-enforced by new and more competitive invasive species (Whitcomb et al. 1972; Deyrup et al. 2000; Cherry 2001).

This might be explained by the unique mating behavior of *P. badius* sexuals, and therefore dispersal of the gene carrying ‘units’ of this species. Mating behavior of *P. badius* is highly promiscuous (Page 1986; Crozier & Pamilo 1996; Rheindt et al. 2004) and makes gene flow between populations probable. However, there is also some indication for inbreeding, as females were reported to mate on their native nests, probably even with their brothers (Van Pelt 1953; M. Deyrup, Archbold Biological Field Station, Lake Placid/FL, pers. comm.). Additionally, no data are available on the dispersal of *P. badius* females, e.g., no reports on huge mating swarms similar to other *Pogonomyrmex* species (Hölldobler 1976). Rheindt (2003) showed that differences exist in allele frequencies of microsatellites (nuclear DNA) between three of the *P. badius* populations analyzed in this study (Withlacoochee, Ocala, Lake Placid). This was a first indication of either restricted gene flow between populations or rapid diversification in these distinct populations. As *P. badius* populations seem to show significant inbreeding (Rheindt 2003), restricted gene flow is likely between them. This is further corroborated by our analysis of mitochondrial DNA variations obtained from six different localities in Florida. Our analyses show clearly separated lineages and an increase of genetic distances with geographic distance.

A more fine-scaled mapping of *P. badius* populations will be needed to separate exactly the geographic range of the different mtDNA-lineages. However, we found at least three lineages which showed inter-population sequence variations that are normally found between *Pogonomyrmex* species (Strehl, unpublished data). Such phylogenetically distinct lineages, which are restricted in their geographical distributions might be characterized as Evolutionary Stable Units (ESUs), warranting protection because they may contain significant components of the evolutionary history of a species (Moritz 1994; Bulgin et al. 2003). This is of special interest because *Pogonomyrmex badius* is considered to represent an endemic ant genus of Florida (Deyrup & Trager 1986). It is also the only representative of the genus *Pogonomyrmex* east of Mississippi and the only North American *Pogonomyrmex* species with a substantial worker polymorphism (Taber 1990). Therefore, to protect the genetic diversity of the probably endangered ant species *P. badius* it is important to clarify the population structure and determine the range and extent of ESUs in Florida.

6.7 Acknowledgments

C.-P.S. thanks Mark Deyrup for kindly providing him with information about the substructuring of *P. badius* populations in Florida, which started the idea for conducting this study. We thank Juergen Liebig and especially Annett Endler for help during a sudorific collecting trip in 2001, and for helpful discussions. We thank Alexander Mikheyev and Robert Johnson for providing additional specimens, Phil Ward and Stefan Cover for species determinations, and two anonymous reviewers for comments on an earlier version of this manuscript. C.-P. S. thanks the Archbold Biological Field Station in Lake Placid for use of facilities during a convenient stay at the station in

August 2001. This study was supported by grants of the German Science Foundation (Deutsche Forschunsgemeinschaft/DFG) GA 661/1-2 and SFB 554-TP C-5.

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7. Colony structure and morphometrics in the queen dimorphic harvester ant, *Pogonomyrmex pima*

Publication status: submitted to Insectes Sociaux

Authors: Robert A. Johnson, Christoph-P. Strehl, Jürgen Gadau

7.1 Summary.

The North American seed-harvester ant *Pogonomyrmex pima* displays a dimorphism that consists of winged (alate) and wingless (intermorph) queens; both types of queens are fully reproductive. Surveys at our field site in southcentral Arizona, USA, demonstrated that most colonies contain multiple queens, but that only one type of queen (intermorph or dealate) occurred in each nest. Type of reproductives produced in a nest corresponded to the type of queen in that nest; colonies that contained dealate queens produced males and alate queens, whereas colonies that contained intermorph queens produced only males. Together, these data suggest that queen phenotype in P. pima is based on a genetically based polyphenism.

A morphometric analysis demonstrated that intermorph queens were intermediate in size to that of workers and alate queens, but that intermorph queens have retained all of the specialized anatomical features of alate queens (except for wings). Some colonies had queens that foraged and performed nest maintenance activities, and these queens sometimes accounted for a significant portion of colony foraging trips. Dissections revealed that these queens were uninseminated, but a portion of these queens retained reproductive capability and were able to produce males. We also overview mating behavior and colony reproduction for the both types of queens.

7.2 Introduction

Dispersal polymorphisms have played a significant role in our understanding of population dynamics, dispersal dynamics, life history, and the physiological basis of adaptation (Zera and Denno, 1997). Such polymorphisms are common among insects, and typically consist of one form that is fully capable of flight and a second form that is flightless. At the proximate level, these dimorphisms result from variation in genotype, environment, or from a combination of both; at the ultimate level, flight ability is associated with habitat persistence. Flight allows organisms to disperse from heterogeneous or temporary habitats, while flightlessness promotes survival in homogeneous or persistent habitats (Heinze and Tsuji, 1995; Roff, 1986, 1990; Zera and Denno, 1997).

In ants, reproductive females (queens) exhibit two types of dispersal polymorphisms, body size polymorphisms and wing polymorphisms (Heinze and Keller, 2000; Peeters and Ito, 2001; Rüppell and Heinze, 1999). Body size polymorphisms consist of two distinct size classes of queens (often referred to as macrogynes and microgynes), while wing polymorphisms consist of winged and wingless queens. In both cases, the two queen forms result in a dispersal polymorphism; macrogynes and winged queens typically disperse from the nest to mate and found colonies independently, whereas their small-bodied (microgyne) or wingless (ergatoid or intermorph) counterparts have limited ability to disperse, and employ dependent colony founding via readoption into established nests (McInnes and Tschinkel, 1995; Rüppell et al., 2001a). Studies on body size dimorphisms document differences in physiology of macrogynes and microgynes and in social structure of colonies with each of the two types of queens (Lachaud et al., 1999; McInnes and Tschinkel, 1995; Rüppell and Heinze, 1999; Rüppell et al., 2001a, b), but few such studies have been conducted on wing polymorphic species (Buschinger and Schreiber, 2002; Fersch et al., 2000; Murakami et al., 2000; Yamauchi et al., 1991).

This study was motivated by observations that both winged (alate, or dealate after the wings are shed) and wingless (intermorph) queens occur in nests of the seed-harvester ant *Pogonomyrmex* (*Ephebomyrmex*) *pima* Wheeler (subfamily Myrmicinae) (see also Heinze et al. 1992). Both dealate and intermorph queens also occur in the closely related *Pogonomyrmex* (*Ephebomyrmex*) *imberbiculus*, but information on queen dimorphism in this species is limited to populations that contain only intermorph queens (dealate queens were extremely rare) (Heinze et al., 1992). *Pogonomyrmex imberbiculus* was also inferred to be polygynous because colonies contained multiple inseminated, egg-laying intermorph queens. Heinze et al. (1992) also measured several morphological characters across the three female castes of *P. imberbiculus*, but a thorough morphometric analysis was hindered by lack of alate/dealate queens. Further understanding the dynamics of queen dimorphism within and across nests requires examining populations that contain both queen phenotypes.

This study examines colony structure of *P. pima* in a population that contains both dealate queens and intermorph queens. Our objectives were to: (1) determine if dealate queens and intermorph queens coexist within nests, (2) assess field colonies for a correlation between type of reproductive queen and type of sexual brood produced in that nest, and (3) conduct a morphometric analysis of workers, intermorph queens, and alate queens. Additionally, established colonies sometimes have queens that forage and participate in nest maintenance activities. We also assess this behavior, and overview mating behavior and colony reproduction for the two queen forms.

7.3 Methods

Study site

We studied *P. pima* at a site approximately 2 km south of the Sacaton Mountains, Pinal County, Arizona (32^{o}56'N, 111^{o}42'W; elevation 430 m). Habitat at the site was typical Sonoran Desert and consisted of a creosote bush (*Larrea tridentata*) association with scattered ironwoods (*Olneya tesota*). Substrate consisted of moderately coarse sand (see Johnson, 1992). Other common ants at the site included *Messor pergandei*, *Pheidole tucsonica*, *Dorymyrmex insanus*, and *Solenopsis xyloni*.

Colony excavations

We excavated nests of *P. pima* to determine number of workers and the number and type of queens. Excavation of most colonies involved digging a 3-4 cm deep depression that was about 15 cm in diameter and centered on the nest entrance. We then dripped approximately 1-1.5 liters of water onto the nest, which facilitated movement of individuals to upper portions of the nest; the colony was excavated 24-48 h later. A minority of colonies was excavated without adding water to the nest. Rarely did either method result in a complete colony excavation on the first day. Thus, we revisited nests on subsequent days, and excavated colonies around the area in which workers had reopened tunnels to the surface; excavation of each nest continued until workers or evidence of their activities had not been observed for several days. Several additional colonies were excavated during August and early September (when adult reproductive brood occur in nests) for the purpose of correlating the type of reproductive queen with the type of sexuals produced in that nest; these colonies were only excavated over one day. In all cases, we collected all individuals from each excavation, returned them to the laboratory, and scored each individual as worker, dealate queen, or intermorph queen under a binocular microscope.

Morphometric comparison of the three female castes

We measured 10 morphological characters (eight external, two internal) for workers, intermorph queens, and alate queens. External characters were head width, head length, scape length, maximum eye diameter, petiole width, post-petiole width, pronotal width, and diameter of the anterior ocellus; internal characters were number of ovarioles and presence/absence of a spermatheca (see also Heinze et al., 1992). The eight external characters were measured by projecting a 40x image from a binocular microscope to a video monitor; the image on the monitor was measured to 0.01 mm using NIH Image (available at http://rsb.info.nih.gov/nih-image/). The two internal characters were determined via dissections under a binocular microscope. Characters were measured on a maximum of two individuals per caste per colony.

We performed a morphometric analysis of the three female castes using multivariate analysis-of-variance (MANOVA). The data set included the seven continuous external characters; ocelli diameter was excluded from this analysis because workers lack ocelli. An *a posteriori* univariate F test was used to determine which variables contributed to overall differences among the castes (SPSS, 1990). Variables were transformed, as necessary, to meet the assumptions of MANOVA. We then assessed degree of overlap among the three castes by performing a discriminant analysis using the same seven characters. The discriminant analysis (SPSS, 1990) developed predictive discriminant functions for each caste, which were then applied to all individuals during the same execution of the model. The seven characters were entered into the model simultaneously using caste as the grouping variable. The model used *a priori* classification and equal prior probabilities.

The three discrete characters (diameter of the anterior ocellus, ovariole number, and presence/absence of a spermatheca) were compared across the three castes using a Kruskal-Wallis test (Siegel and Castellan, 1988).

Foraging by queens in established nests

We observed queen foraging on several occasions, and in each case the queen returned to an established colony. Such queen foraging is unusual among ants, and little is known about this behavior when it does occur. Consequently, we collected data on two aspects of this behavior: (1) the proportion of colony foraging trips made by queens, and (2) reproductive status (mated or unmated) of foraging queens. Relative foraging effort was quantified by observing two colonies known to have foraging queens. For each colony, we recorded status (worker or queen) of 25-30 successive foragers returning to the nest on each of two days.

We determined reproductive status of foraging queens by dissecting out the reproductive tract of each individual ($n = 20$) under a binocular microscope. Color of the spermatheca was used to assess mating status (inseminated or uninseminated): queens were scored as unmated if their spermatheca was clear and translucent, whereas those with an opaque or whitish spermatheca were scored as mated (see Alloway et al., 1982; Keller and Passera, 1993). We verified this visual assessment of mating status by examining the spermatheca of virgin alate queens and mated dealate foundresses. The ovarioles were also examined for developing oocytes or for evidence that they had become expanded for reproduction.

7.4 Results

Morphometric comparison of the three female castes

Size of the three female castes of *P. pima* differed significantly (Wilks' $\lambda = 0.105$, $F_{14,102} = 15.2$, $P << 0.0001$); all seven continuous characters varied among the three castes (univariate F tests within MANOVA, $P < 0.001$), as did all three discrete characters (Table 1). The pattern of variation differed by character; measures for intermorph queens were intermediate to those of workers and alate queens for five characters, similar to those of alate queens for four characters, and similar to those of workers for one character (Duncan's multiple range test for continuous characters, Kruskal-Wallis test for discrete characters, $P < 0.05$; Table 1).

Discriminant analysis correctly classified 91.7% (55 of 60) of all individuals (Table 2). As might be expected from the MANOVA, intermorph queens occupied a multivariate space intermediate to that of workers and alate queens. This intermediacy was also demonstrated by group centroids, with the centroid for intermorph queens being nearly equidistant to those of workers and alate queens (Fig. 1). Misclassification of individuals involved only workers and intermorph queens; a minority of workers were classified as intermorph queens, and several intermorph queens were classified as workers or alate queens (Table 2, Fig. 1). The standardized coefficients of the canonical functions indicated that head width and pronotal width were the primary contributors to discriminant function 1, which accounted for 82.1% of the variance; width of the petiole and post-petiole were the primary contributors to discriminant function 2, which accounted for the remaining 17.9% of the variance (Table 3).

Intermorph queens, though intermediate in size to workers and alate queens, were identical to alate queens in regard to reproductive structures and specialized queen anatomy. Both intermorph and alate queens possessed a spermatheca (which all workers lacked) (Table 1) and a similar number of ovarioles (n = 7-8 for intermorph and alate queens; n = 2 for workers) (Table 1), and both intermorph and dealate queens laid eggs that developed into workers. Additional anatomical structures common to both types of queens included ocelli (which workers lacked), and an enlarged and dorsally flattened mesosoma with a distinct suture between the sclerites; the mesosoma of workers was reduced and rounded with complete fusion of the sclerites (Fig. 2). Intermorph and alate queens also have coarse longitudinal rugae on the dorsum of the mesosoma, in comparison to the coarse rugo-reticulose sculpturing on the mesosoma of workers. Note, however, that intermorph queens varied in their degree of expression of some queen-like structures. For example, the site where the wing would be inserted was marked by a small, darkly pigmented spot on some intermorphs, while this pigment was lacking on other intermorphs (see also Heinze et al., 1992).

Colony structure and size

Colonies of *P. pima* are highly polygynous, as multiple queens occurred in 27 of the 34 (79.4%) nests in which we found queens (mean = 8.0 ± 2.0 queens per nest). Occurrence of dealate and intermorph queens was highly non-random because 26 of the 27 (96.3%) polygynous nests contained only one type of queen; the other nest contained five intermorph queens and one dealate queen. At the population level, the number of nests containing only dealate (*n* = 13) or intermorph queens (*n* = 20) was similar (χ^2 = 1.5, 1df, *P* > 0.10). Number of queens per nest was also similar for both types of queens (dealate queens; mean = 7.6 ± 2.5, intermorph queens; mean = 8.4 ± 3.0: t-test, t = 0.2, 31 df, *P* > 0.50).

The type of queens produced by a colony corresponded to that of its reproductive queens. Seven nests headed by intermorph queens contained males (mean = 51.9 males, range 11 – 119), but lacked alate queens. We also excavated seven nests headed by dealate queens: four of these nests contained both alate females and males, one nest contained only alate females, and two nests contained only males (males; mean = 35.1, range 0-65: alate females; mean = 29.0, range = 0-89). In addition, one laboratory colony headed by a dealate queen produced several alate females. Moreover, both dealate and intermorph queens produce males, but only dealate queens produce alate females.

Colony size (number of workers) ranged from 52-326 for the four colonies that we judged to have completely excavated. Partial excavation of other nests indicated that colonies typically contain >100 workers. The maximum number of workers was similar for colonies containing each type of queen (intermorph queen; N_{max} = 309 workers, dealate queen; N_{max} = 326 workers). Similarly, worker number was not correlated with queen number; the two largest colonies, with 309 and 326 workers, contained six intermorph queens and one dealate queen, respectively, while the colony with 52 workers contained 24 dealate queens.

Queen foraging

Foraging queens accounted for a variable but sometimes significant proportion of the foraging trips for colonies in which they occurred (mean = 21.7 ± 6.5%, range = 3.3 – 33.3%, n = 116 foraging trips collected over two observation periods for each of two colonies). Examination of the spermatheca indicated that all 20 of our foraging queens (2 intermorph, 18 dealate) were unmated. Surprisingly, 8 of the 20 queens contained one or more ovarioles that had become expanded and contained a large white body that appeared to be an oocyte; ovarioles in the other 12 queens were translucent and undeveloped, similar to those of alate queens. Presence of expanded ovarioles suggested that these unmated queen may retain reproductive capability. We investigated this possibility by collecting several dealate queen foragers plus workers from one colony, which were placed in a plaster-lined nest box inside a darkened incubator at 35^{o} C. The plaster was moistened periodically and queens were provided dead fruit flies every 1-3 days. These queens laid numerous eggs, and the several adults that emerged were all males, supporting the dissection data that these foraging queens are unmated but can retain reproductive capability. The low proportion of eggs that developed into adults suggests that these queens also produced trophic eggs.

7.5 Discussion

Evolution of queen dimorphism

Dispersal polymorphisms result from the ability of organisms to produce multiple phenotypes from single genotypes, i.e., polyphenisms. Polyphenisms occur in a wide variety of insects, and they are particularly well known in crickets, aphids, and ants (Zera, 2004). Alternate phenotypes can be triggered by genetic factors, environmental factors, or a combination of both types of factors (Nijhout, 1999; Zera, 2004). Four lines of evidence suggest that queen dimorphism in *P. pima* is a genetically mediated polyphenism. First, only one queen phenotype occurred within colonies that contained multiple queens. Second, colonies that contained dealate queens produced alate females and males, whereas those that contained intermorph queens produced only males. Intermorph queen nests appeared to also produce intermorph queens, given large numbers intermorph queens in some nests, but direct assessment was hindered by inability to distinguish between older and recently eclosed intermorph queens. Third, it is doubtful that environmental variation affected differential production of the two queen phenotypes given that both types of colonies occurred sympatrically throughout our 1-2 ha site. Lastly, the two queen phenotypes displayed an intermixed mtDNA phylogeny, indicating that both forms are part of the same gene pool, i.e., the two forms do not consist of separate lineages (Strehl et al., in prep.). This genetically mediated polyphenism contrasts with other ant species in which the queen phenotype is environmentally mediated (Yamauchi et al., 1991), or those in which intermorph and dealate queens can produce both queen phenotypes (Buschinger and Schreiber, 2002).

Intraspecific queen dimorphism in the form of winged and wingless morphs is uncommon in ants, with examples scattered across genera in several subfamilies of Formicidae (Heinze and Tsuji, 1995). In the genus *Pogonomyrmex*, intermorph queens occur in all three subgenera (*Pogonomyrmex, Ephebomyrmex, Forelomyrmex*). Both intermorph queens and alate queens occur in two North American sister species of *Pogonomyrmex, P. imberbiculus* and *P. pima* (this study, Heinze et al., 1992), suggesting that this is an ancestral polyphenism that both species retained following their divergence. Such queen dimorphism might also occur in *P. laevinodis*, the third member of this clade, but queens of this species are unknown. Intermorph queens (termed ergatoids in the original papers) also occur in at least three South American congeners, *P. mayri* (subgenus *Forelomyrmex*) (Kugler, 1978; Kugler and Hincapie, 1983), *P. cunicularius* (subgenus *Pogonomyrmex*) (Kusnezov, 1951), and *P. laticeps* (subgenus *Pogonomyrmex*) (Kusnezov, 1951); alate/dealate queens are thusfar unknown from these three species, and the functional significance of their ergatoid queens is unknown. Intermorph/ergatoid queens probably occur in additional South American species of *Pogonomyrmex* given our poor knowledge of this group. Overall, the widespread taxonomic occurrence of intermorph queens suggests that this phenotype has evolved several times within *Pogonomyrmex*, most likely as a secondary modification in response to local ecological conditions (Heinze and Tsuji, 1995; Zera and Denno, 1997).

Morphometrics

Intermorph queens constitute an integral part of the life history of a species that are considered to be a permanent evolutionary modification of the ancestral queen morphology (Peeters, 1991). In *P. pima*, intermorph queens have reproductive ability similar to that of alate queens, and they retain all anatomical and reproductive characters of alate queens, except for their loss of wings. This study provides one of the first multivariate studies of intermorph queens (see also Murakami et al., 2002; Visicchio et al., 2003), and shows that the primary difference of intermorph queens is their intermediate size relative to that of workers and alate queens (Fig. 1), which is a pattern that occurs for intermorph queens of other species (Heinze et al., 1992; Murakami et al., 2002). Interestingly, size of individual characters varied for intermorph and alate queens. Head size (length and width) was similar for intermorph and alate queens, but the mesosoma (pronotal width), petiole, and post-petiole were consistently smaller in intermorph queens. The smaller mesosoma of intermorph queens likely reflects loss of wings and their associated musculature.

Mating behavior

Occurrence of two queen dispersal morphs has profound consequences on life history of a species. In ants, queen dimorphism often results in multiple mating systems (male-aggregation and female-calling) combined with queens that employ both independent and dependent colony founding (Hölldobler and Bartz, 1985; Peeters and Ito, 2001). Alate females typically fly from the nest to mate at aggregations formed by males, then tear off their wings and found a colony independently, usually with one queen. In contrast, wingless queens invariably use female-calling to attract males, and then mate in or near their natal nest. After mating, these wingless queens return to their natal nest (resulting in secondary polygyny), and the colony later reproduces by budding (Peeters and Ito, 2001; Peeters, 1991).

Mating behavior of *P. pima* is poorly known, but it likely involves both male-aggregation and female-calling. Male-aggregation occurs via mating flights that occur on mornings following summer rains (see Johnson, 2000; R.A. Johnson, pers. obs.). Males form numerous small mating aggregations (usually <200 individuals) near the top of bushes (e.g., creosote, ca. 1-1.5 m above ground), and trees (e.g., ironwoods, ca. 3-4 m above ground); in the latter case, a second aggregation often forms near the base of the tree. Females fly to the aggregation, find a mate, and pairs fall to the ground to mate. Newly mated dealate queens found nests haplometrotically (R.A. Johnson, unpublished data). Moreover, it seems unlikely that nests readopt dealate queens, as this would result in co-occurrence of intermorph and dealate queens. The only exception to such queen readoption would be if an alate queen mates at the nest entrance, then returns to her natal nest.

In contrast, intermorph queens likely use female-calling to attract males; they then mate and probably return to their natal nest, resulting in secondary polygyny. Lack of dispersal by solitary intermorph queens is corroborated by finding dealate queens (n = 104), but not intermorph queens, on the ground searching for nest sites following a large mating flight in September 2004 (R.A. Johnson, pers. obs.). Colony budding is typical for polygynous wingless species of ants, such as these colonies headed by intermorph queens. We lack direct evidence of colony budding in *P. pima*, but field observations suggest that budding occurs in *P. imberbiculus* (S.P. Cover, pers. comm.). Intermorph queens sometimes also occur on the ground beneath mating aggregations where they mate with males (R.A. Johnson, pers. obs.). The extent of this behavior is unknown, but it is probably restricted to intermorph queens that occur in colonies located directly below aggregation sites.

Queen foraging

Several ant species have winged queens that sometimes forego mating and later perform tasks such as foraging and nest maintenance (Brown, 1999; Forder and Marsh, 1986; Peeters, 1997). Both dealate queens and intermorph queens of *P. pima* also perform such tasks, and queens that engage in such behaviors are invariably unmated. Few data exist on species in which unmated queens opt to forage, but observations suggests that foraging queens are short-lived in both *Messor andrei* and *Acromyrmex versicolor* (Brown, 1999; J. Weser, pers. comm.). In contrast, foraging queens of *P. pima* appear to be longer-lived given that we observed these queens in several colonies during May, about eight months after the sexual brood is normally produced. Foraging queens can represent a variable but sometimes significant portion of the foraging trips made by colonies, with 10 or more foraging queens being observed in a couple of colonies.

The mechanism that causes queens to forego mating and to perform worker-like tasks is unknown. One possibility may relate to occurrence of the rains that trigger their mating flights. Mating flights for both *P. pima* and *A. versicolor* appear to depend on very late season monsoon rains, which do not occur in some years. In such years, queens might dealate themselves to make the best of a bad situation, then perform worker-like behaviors that could still benefit the colony. Regardless of the mechanism causing this behavior, some of these foraging queens of *P. pima* remain fertile and can produce males, and their ability to lay trophic eggs provides an additional nutritional benefit to the colony.

Overall, this study documents within nest structure for a population of *P. pima* that exhibits intraspecific queen dimorphism, and this species appears to be one of very few queen dimorphic species in which queens produce only same-phenotype queens. Our data suggest that *P. pima* is ideal for examining both proximate and ultimate causes of caste determination. At the proximate level, this system provides opportunities to examine mechanisms that trigger caste determination as well as those that determine body size and wing development. In contrast, determining the phylogenetic occurrence of intermorph/ergatoid queens in South American congeners, where this phenotype probably evolved, would provide insight into the evolution of alternative queen phenotypes. This system can also provide insight into alternative mating strategies given that *P. pima* uses the male aggregation syndrome for males and alate queens, combined with the female calling syndrome (and probably some intranidal mating) for intermorph queens and possibly also for some alate queens.

7.6 Acknowledgments

This paper benefited from discussions with Stefan Cover and Ehab Abouheif. We also thank Jim Elser for allowing us to use his microscope to take photographs that we used to measure morphological characters, and Brian Fisher for providing the photographs in Figure 2.

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7.8 Table

Table 1:

Morphological measures (mean + 1 SE) for the three female castes of the seed-harvester ant *Pogonomyrmex pima* (in mm). N = 20 individuals per caste (< 2 individuals per colony). Significant differences among castes are given by the letters a-c: a > b > c. Groupings are based on univariate F tests within MANOVA followed by a Duncan's multiple range test (see text). Characters in bold font were used in the discriminant analysis.

		Caste	
Character*	**Worker**	**Intermorph queen**	**Alate queen**
Head			
Head width (HW)	1.05 ± 0.01^{b}	1.11 ± 0.01^{a}	1.10 ± 0.01^{a}
Head length (HL)	1.09 ± 0.01^{b}	1.14 ± 0.01^{a}	1.15 ± 0.01^{a}
Maximum ocular diameter (MOD)	0.24 ± 0.00^{c}	0.26 ± 0.00^{b}	0.28 ± 0.00^{a}
Scape length (SL)	0.73 ± 0.01^{b}	0.75 ± 0.01^{b}	0.78 ± 0.01^{a}
Ocellus diameter (OD)	Absent^{c}	0.03 ± 0.00^{b}	0.06 ± 0.00^{a}
Mesosoma			
Pronotal width (PW1)	0.72 ± 0.01^{c}	0.80 ± 0.01^{b}	0.85 ± 0.01^{a}
Petiole width (PW2)	0.29 ± 0.00^{c}	0.32 ± 0.01^{b}	$0.35 + 0.00^{a}$
Post-petiole width (PW3)	0.44 ± 0.01^{c}	0.52 ± 0.01^{b}	0.54 ± 0.01^{a}
Gaster			
Number of ovarioles	2.00 ± 0.00^{b}	7.86 ± 0.14^{a}	7.55 ± 0.17^{a}
Spermatheca present	No	Yes	Yes

* HW – maximum width of the head, positioned in perfect full face view, at a level above the upper eye margin; HL - Maximum length of the head, positioned in perfect full face view, from the midpoint of the anterior clypeal margin to the midpoint of the occipital margin; MOD – maximum diameter of the eye as measured with the head in full lateral aspect; SL – maximum length of the scape, excluding the basal condyle; OD – maximum diameter of the anterior ocellus; PW1-3 – maximum width of pronotum, petiole, and post-petiole, respectively, as seen from above, at right angles to the longitudinal axis of the mesosoma.

Table 2:

Summary classification of predicted group membership for workers, intermorph queens, and alate queens of *Pogonomyrmex pima* using discriminant analysis. The seven morphological characters used in the analysis are given in Table 3. Values are the number of individuals that discriminant analysis classified into each of the three female castes.

	Predicted group membership			
Caste	Worker	Intermorph queen	Alate queen	Total correct
Worker	18	2	0	18/20 (90.0)
Intermorph queen	2	17	1	17/20 (85.0)
Alate queen	0	0	20	20/20 (100.0)
Total	20	19	21	55/60 (91.7)

Table 3:

Standardized coefficients for canonical discriminant functions for the three female castes of *Pogonomyrmex pima*.

Predictor variable	**Function 1**	**Function 2**
Head width	-1.045	0.709
Head length	-0.101	0.444
Maximum ocular diameter	0.526	-0.365
Scape length	-0.200	0.110
Pronotal width	1.027	-0.624
Petiole width	0.244	-1.107
Post-petiole width	0.165	1.502

7.9 Figures

Figure 1:

Discriminant scores for the three female castes of the seed-harvester ant *Pogonomyrmex pima*. Scores of all individuals (*n* = 20 per caste) are projected onto the two-dimensional space defined by discriminant functions one and two. The larger symbols for each caste are the unstandardized canonical discriminant functions evaluated at the group means (centroids).

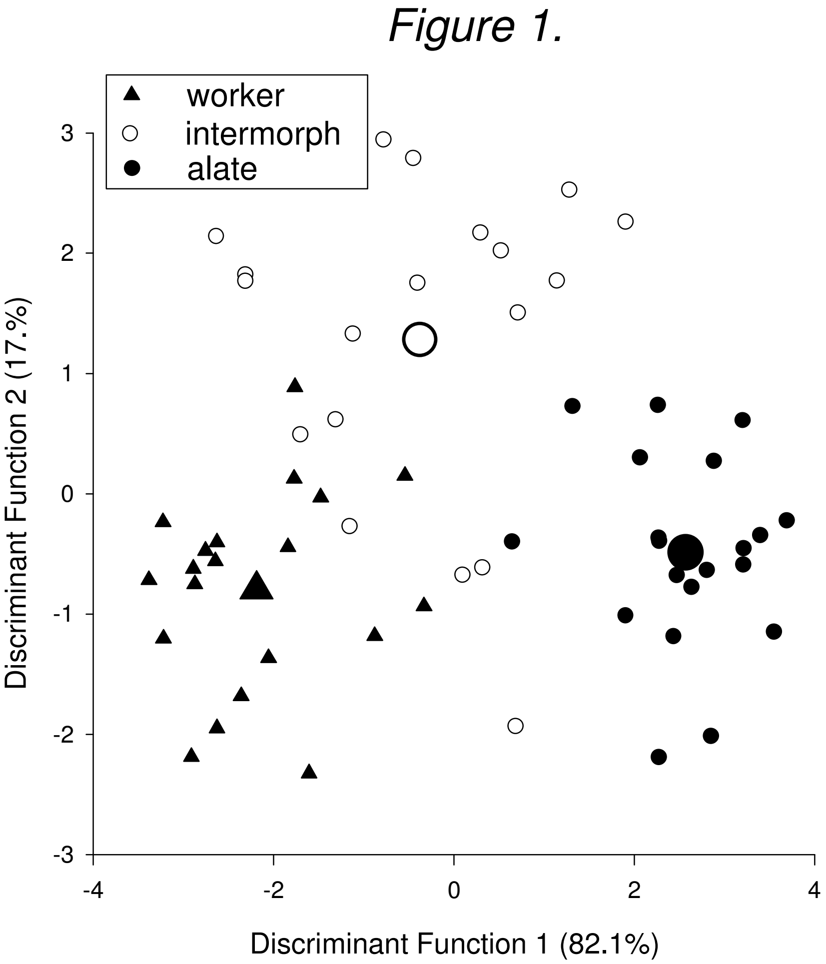


Figure 2.

Profile (left) and dorsal (right) view of the mesosoma for a worker (top), intermorph queen (center), and alate queen (bottom) of the seed-harvester ant *Pogonomyrmex pima*.

8. Colony Structure and Sociogenetics of the Queen Dimorphic Harvester Ant, *Pogonomyrmex* (*Ephebomyrmex*) *pima*

Publication status: in preparation

Authors: Christoph-P. Strehl, Robert A. Johnson, Jürgen Gadau

8.1 Abstract

We analyzed the structure of colonies of the queen dimorphic harvester ant *Pogonomyrmex* (*Ephebomyrmex*) *pima* sociogenetically. Based on microsatellite analyses, both, "intermorphic" and "dealate" queen morphs of *P. (E.) pima* had an effective mating frequency <2 (m_{eq}=1.03, m_{er}=1.36), indicating single mating. Therefore we reject as null hypothesis that this species has multiply mated queens as all other so far investigated *Pogonomyrmex* species. Among colony females (workers and virgin queens) the average relatedness was 0.63. The deviation from full sister relatedness in Hymenoptera (0.75) is mainly due to polygyny. Genotyping showed that both queen types produced female offspring and so both are fully functionally queens. Although dealate and intermorphic queens did no co-occur within nests, sequence analyses found no convincing genetic separation of mtDNA-lineages from both queen types in the studied population. This finding is discussed in the light of different dispersal strategies of both queen morphs. Colonies headed by intermorphic or dealate queens showed differences in their Sex Investment Ratios, with a lack of female-production in colonies headed by intermorphic queens. The population wide Sex Investment Ratio was highly male biased being 0.191. Additionally, we found Sex Investment Ratios of *P. (E.) pima* colonies to be positively correlated with within-colony relatedness, indicative of a possible worker control of sex ratio allocation.

8.2 Introduction

In only few cases it is that obvious to see morphology shaping the life history of its species than in the impressive diversity of forms found among sexually reproductive females of ants (Hymenoptera: Formicidae), the "queens". Despite inter- and even intra-specific size differences, a prominent character here is easily recognized: the presence or absence of wings. Wingless "apterous" morphs with a complete loss of flight ability occur in many species of ants (Wilson 1953; Hölldobler and Wilson 1990). In some ant species wingless “ergatoid” morphs are the exclusive form of female reproductives (Peeters 1991), whereas in others the incidence of winged or smaller, wingless “intermorphic” queens regularly occurs together (Heinze 1998).

To have wings or not is of importance for the fate of ant queens. Apterous and winged morphs typically follow different dispersal strategies (e.g.: Wilson 1971; Wilson 1975; Hölldobler and Wilson 1990; Doums et al. 2002). For apterous queens dispersal is restricted by morphology to their native nesting area, whereas winged queens ensure long-range dispersal. Additionally, both types of dispersal strategies often are also associated to nest founding strategies dependent or independent of the help from other females. Due to their different nest founding strategies, different queen morphs can affect colony structures by causing polygyny, i.e. having multiple queens per nest. For instance, limited dispersal might favor ant queens capable in founding together or being adopted into established nests, often found in parallel with social parasitic behavior. By this ant colonies founded by apterous queens often are characterized as having a polygynous structure.

Among the family of ants the Myrmicine ant subgenus *Ephebomyrmex* (Wheeler 1902) is an interesting object for studying the progression of apterous queen forms. For this subgenus polygyny and wingless, apterous queen morphs were reported several times (e.g.: Kusnezov 1951; Kugler 1978; Hölldobler and Wilson 1990; Heinze et al. 1992) *Ephebomyrmex* is embedded in the ant genus *Pogonomyrmex* (Mayr 1868), whose species are known as “harvesting ants”. Polygyny seems to be an exception or ancestral state within its genus, as in harvester ants of the derived subgenus *Pogonomyrmex sensu stricto* mostly single, morphologically invariable queens are heading a colony (Cole and Wiernasz 2000; Gadau et al. 2003).

Pogonomyrmex species often are ecologically dominant in the arid and semi-arid environments of the deserts in the Southwestern United States and Northern Mexico. Having high nest densities, and resource- and nest site-limitation via territoriality in an unstable environment *Pogonomyrmex* spp. show features favoring the evolution of polygynous nest founding strategies and of socially parasitic forms (Foitzik and Heinze 1999; Hoffmann and Merilä 1999). However, social parasites were rarely detected (*P. colei*, *P. snellingi*; all from the subgenus *P. sensu stricto*), and polygyny is reported for the North American subgenus *sensu stricto* for only one species, *P. huachucanus*, and one isolated population of *P. californicus* (Johnson 2002; Johnson 2004). In contrast, multiple queens per nest were observed in two of the three known North-American *Ephebomyrmex* species: *P.* (*E.*) *imberbiculus* (Heinze et al. 1992) and *P.* (*E.*) *pima* (Heinze & Schilder, cited as "unpublished report" by Heinze 1998; Johnson et al. submitted). Moreover, two queen types are characteristic for both species: "intermorphic" wingless queens and "dealate" queens, who had dropped their wings after mating for nest founding (Heinze et al. 1992; Johnson et al. submitted). Up to now no statements were made if colonies of these species are functionally polygynous or only one queen is the dominant egg-layer (functional monogyny). To detect functional polygyny and/or possible multiple mating in North-American *Ephebomyrmex* species, we representatively analyzed *P.* (*E.*) *pima* colonies sociogenetically. Additionally, we wanted to clarify if in *P.* (*E.*) *pima* queen dimorphism is correlated with specific colony structures and thus dissected and censured field colonies in more detail.

As only a single mitochondrial lineage is inherited in nests from their mother queen, mitochondrial strains might be separated by reduced gene flow between lineages with long-range or short-distance dispersal, e.g. winged queens, wingless queens, or queens with a social parasitic nest founding tactic. The evolution of inquilines via queen dimorphism for example is favored by dependent founding tactics/parasitic behavior, promoted by facultative polygyny (Stille 1996; Heinze and Keller 2000; Rüppell et al. 2001a; Rüppell et al. 2001b; Rüppell et al. 2001c). If in *P.* (*E.*) *pima* different queen morphs with separating founding tactics (Johnson et al. submitted) are more likely triggered by inherited genetic components rather than environmental cues, then different queen morphs should have common phylogenetical origins and should be distinguishable as dispersal lineages. By examining the occurrence of intermorphic and dealate queens we therefore also emphasized on the past genetic history of intermorphic queens and analyzed if intermorphic queens correspond to an own type of dispersal strategy, represented by genetically separated mitochondrial lineages.

Pogonomyrmex species analyzed sociogenetically so far all belonged to the subgenus *sensu stricto* and have all been identified to be strictly monogynous with the colonies queen being highly multiple mated (Cole and Wiernasz 1999a; Cole and Wiernasz 1999b; Cole and Wiernasz 2000; Helms-Cahan et al. 2002; Volny and Gordon 2002b; Gadau et al. 2003; Rheindt et al. 2004; Wiernasz et al. 2004). Sociogenetic analyses focusing on those species considered to represent the more ancient status of the *Pogonomyrmex* harvesting ants in North America (*P. huachucanus*, *P. imberbiculus*, *P.*

laevinodis, *P. pima;* (Parker and Rissing 2002; Gadau et al. 2003) were lacking so far. Preliminary sociogenetic results give hints to multiple mating in *P.* (*s.str.*) *huachucanus* (J. Gadau and C.-P. Strehl, *unpublished*). We therefore hypothesize *P.* (*E.*) *pima* to be multiple mated, like their congeners.

However, as predicted by the genetic variability hypotheses, polyandry is less common among polygynous than monogynous species (Keller and Reeve 1994). Therefore for *P.* (*E.*) *pima*, showing regularly polygyny (Johnson et al. submitted), alternatively a low number of matings per queen might exist (assuming an influence of the number of queens per colony on the mating frequency of females). Moreover, with 50-350 workers per colony (Johnson 2000; Johnson et al. submitted) *P.* (*E.*) *pima* has smaller-sized colonies than its P. sensu stricto congeners (600~12000 workers; Hölldobler and Wilson 1990). The 'sperm-need' hypothesis (Cole 1983) stated that especially those species with smaller colonies should show less often multiple mating. Therefore, we use the genetic variability and sperm-need hypotheses as foundation for our alternative hypothesis of a low effective mating frequency to be expected in *P.* (*E.*) *pima*.

8.3 Materials and Methods

Nest excavation and Censuring of field-colonies

Samples were collected in September 2002, before mating flights took place (for collection of sexuals), and from March – May 2003 at "McCartney Rd." (Pinal Co., Arizona/USA; 32°56'N, 11°42'W). Nests normally were located by following foraging workers back to their nests. Up to several liters of water were then poured on each nest, which was excavated the following day. With this procedure usually the whole colony was collected, as brood and sexuals moved upward during night. Colonies were censured in laboratory and distinctions between dealate queens, intermorphic queens and workers were made under binocular microscopes. Intermorphic queens were defined as individuals, having no indications for formerly wearing wings, but opposite to workers having queen-like structures (ocelli or visible thoracic segmentation). Several colonies also produced males and these were collected to more easily ascertain queen genotypes and for Sex Ratio determination.

Sex Ratio calculation

Sex Ratios were calculated as the ratio of the number of females to males found within colonies. Particularly, for calculating the Sex Investment Ratios (SIR) the numbers of females were multiplied with an additional factor representing the energetic-cost ratio (*C*) between the castes, calculated by exponentiating the mean female to male dry-weight ratio (*D*) with the approximated coefficient 0.7 (Boomsma 1989). *D* was calculated by using mean dry weights over colony-measurements of a total of 110 alate female (four colonies) and 167 males (nine colonies) each. Additionally 186 workers (seven colonies) were dried and weighted to compare their investment with the alate queen caste. All Individuals were dried for one week in an incubator at 57°C and weighted, pooled for colonies on a Mettler AE 200 scale (Mettler-Toledo, Switzerland/USA). From the obtained data we calculated mean intracolonial dry weights, which were then tested for normal distribution using a Shapiro-Wilk test. We assumed normal distribution of dry weights within colonies as no individual measurements were performed, and tested within each caste for deviations in the mean dry weights of single colonies from others by using a one-way ANOVA. For differences in the mean dry weights between pairs of castes was tested using T-tests.

Laboratory rearing

Multiple queens sometimes occurred in colonies, with the majority of these likely uninseminated queens that had not yet dispersed (Johnson et al. submitted). Laboratory rearing helped to examine the females mating frequencies (see below) by disentangling impacts of polygyny and possible polyandry on the relatedness structure of offspring. Therefore, when multiple queens were found in a nest, each queen and a group of workers were isolated and kept in laboratory at about 30-32°C using standard rearing conditions. Queens generally laid eggs within several days of initiating the colony. As the emerging individuals belonged genetically to one female, they are subsequently assigned to “families” (e.g. compare Fraser et al. 2000). To insure that samples resulted from these isolated queens, we collected for genotyping only brood (mostly larvae) from her families.

Analysis of colony sociogenetic structure

Material intended for genotyping was preserved in 95% Ethanol and stored at –20°C. After approx. 1 month of laboratory rearing, larvae and pupae were preserved and stored. This material was used for microsatellite analysis.

DNA was extracted from whole, Ethanol-preserved individuals following the modified version of the Puregene Kit protocol (Gentra Systems/Biozym, Germany) used by (Gadau et al. 2003). Prior to this isolation procedure, samples were washed in distilled water for 1 min to remove Ethanol. DNA was extracted from eight field colonies and six laboratory reared families and yielded successful amplifications in 396 individuals, the sum of (numbers for lab-families in brackets) 310 (99) females [217 (37) workers, 31 (0) alate females, 62 (62) larvae] and 86 (0) males.

We screened 24 microsatellites with primers developed for other ants within the subfamily Myrmicinae (Hamaguchi and Itô 1993; Bourke et al. 1997; Foitzik et al. 1997; Volny and Gordon 2002a; Gadau et al. 2003). Among 14 successfully amplified loci three microsatellites, Pb8 (Volny and Gordon 2002a), LxAGT1 (Bourke et al. 1997), and L18 (Foitzik et al. 1997), showed heterozygosity, and were useful for analyzing offspring of both, field colonies and laboratory reared families.

Polymerase chain reaction (PCR) amplifications were performed in total volumes of 25µl, containing 3-8 ng genomic DNA, 2.5µl of 10x PCR Buffer (750mM Tris-HCl, 200mM (NH4)2SO4, 0.01% Tween 20), 0.2mM of each dNTP, 2.0mM $MgCl_2$, 0.52µM of each primer, and 1.5U Taq DNA polymerase (MBI Fermentas, Lithuania).

For detailed genetic analyses loci were amplified at heating rates of 5°C/sec (thermocycler of Biometra, Germany) with 9 cycles of denaturing 40 sec at 94°C, annealing 40 sec at 56°C (L18: 55°C) -0.1°C (L18:-0.2°C) per cycle, and elongation 40 sec at 72°C, followed by a second set of 23 cycles of 30 sec at 94°C, 30 sec at 55°C (L18: 53°C), and 40 sec (plus 1 sec per cycle) at 72°C; an initial denaturation step of 3 min at 94°C and a final cooling step pausing at 4°C was added. For the microsatellite screening similar, but more unspecific touchdown parameters were used with decreasing steps of 2-5°C for the annealing temperatures, covering a total range of 48°C up to 56°C.

Screens to test for polymorphisms and genotyping of colony samples was performed on Spreadex EL 800 gels and using a LiCor 4300 sequencer. For the latter, primers were labeled (LiCor, USA) with IRDye700 (LxAGT1) and IRDye800 (Pb8 and L18).

Heterozygosities and inbreeding coefficients of loci were calculated using the program GDA 1.0 (Lewis and Zaykin 2001) for diploid female genotypes only. Relatedness of colonies was calculated with colonies weighted equally using the program Relatedness 5.0.8 (Queller and Goodnight 1989; Goodnight 2001). Mating frequencies were calculated for colonies where matrilines could genetically be separated: from the frequency of patrilines within colonies (m_{eq}), also corrected for sample size (m_{eqc}) (Starr 1984; Rheindt et al. 2004), and from obtained relatedness results (m_{er}) (Pamilo 1993; Gadau et al. 2003). The failure to obtain male or female genotypes was used to estimate the impact of null-alleles in the loci used. A Regression analysis was performed to test for a possible correlation of Sex Investment Ratios with the relatedness of females in colonies.

Marking-off mitotypes

To resolve genetic substructuring of maternally inherited material, we compared mitochondrial sequences obtained from different colonies. DNA, isolated from three intermorph headed colonies (“I”) and three colonies headed by dealate queens (“D”), was used to amplify a 657 bp region of the mitochondrial Cytochrome c Oxidase subunit 1 (CO1) gene using primers LCO and HCO (Folmer et al. 1994). Each amplification was performed with 2µl of 1:20 diluted DNA-extraction, in 25µl reaction volumes containing 2.5µl of 10x PCR Buffer (750mM Tris-HCl, 200mM $(NH_4)2SO_4$, 0.01% Tween 20), 0.2mM of each dNTP, 2.0mM $MgCl_2$, 0.52µM of each primer, and 1.0U Taq DNA polymerase (MBI Fermentas, Lithuania). Loci were amplified at heating rates of 5°C/sec (thermocycler of Eppendorf, Germany) after an initial denaturation step of 3 min at 95°C, with 33 cycles of denaturing 30 sec at 95°C, annealing 60 sec at 45°C, and elongation 30 sec at 72°C; two final steps for elongation 90 sec at 72°C and cooling down to 4°C were added. Amplicons were sequenced (SeqLab, Germany) using LCO as sequencing primer.

Obtained sequences were aligned by eye to 632 bp of CO1-sequence of the North-American species *Pogonomyrmex* (*Ephebomyrmex*) *imberbiculus* (GenBank Acc.-No.: AY510614), the phylogenetically closest relatives of *P.* (*E.*) *pima* (Parker and Rissing 2002; Strehl et al. in preparation). Aligned sequences were subsequently analyzed using

Mega 2.1 (Kumar et al. 2001). Two trees, a neighbor-joining tree (Kimura 2-parameter, pair wise distances) and a maximum-parsimony tree (standard parsimony; Min-Mini heuristic with search factor of 2) were constructed with 100,000 bootstrap repetitions each. This was done in combination with known sequences of *P.* (*E.*) *pima* provided in GenBank (Tab. 3; GenBank Acc.-No.: AY510622, AY510623, AY510659; (Strehl et al. in preparation)) to check for monophyly of the analyzed population samples. Where known, the type of colony ("I" or "D") was mapped onto the tree.

All sequences are deposited in GenBank (Tab. 1). Surplus nestmates from the analyzed workers are deposited as voucher specimens: pinned in the collections of Harvard University (Cambridge, Mass., USA) and of P. S. Ward at University of California in Davis (USA); preserved in 100% Ethanol (stored at –70°C) at Museum Koenig (Bonn, Germany).

Statistics

Statistics were performed using the program Statistica 6.1 (StatSoft, USA). For all analyses a Bonferroni-correction for multiple testing was applied.

8.4 Results

Composition of field colonies

The data on colony compositions of 24 field colonies are summarized in table 1. The mean colony size in *P.* (*E.*) *pima* was 57±38 SD (N=24) workers per colony, with a median of 45 workers per colony. These values agree very well with those of Johnson (2000) and of (Johnson et al. submitted). Note, that this is a minimum estimate because we may have missed parts of the colony. The colony size of field colonies did not differ between mono- and polygynous nests or nests containing intermorphic or dealate queens (one-way ANOVAs; p>0.5). Both queen types were found in polygynous (N=11) and monogynous nests (N=4). Therefore, monogyny made up 27% among those colonies analyzed, containing two colonies of both queen types each. Accordingly, the presence of intermorphic or dealate queens was not correlated with the presence or absence of more than one queen (polygyny vs. 'monogyny'; N=15; Phi^2=0.00162; Fishers exact p=0.6615).

Seven colonies contained intermorphic, eight colonies dealate queens, and in eight colonies no individual morphologically distinct from workers was collected. In our data set no mixing of intermorphic (N=19) and dealate queens (N=17) was found inside single queenright colonies. This is consistent with an additional data set from excavations of over 30 nests in this population, where a similar result was found for nearly all nests except for one, which contained a dealate queen together with 5 intermorphic queens (Johnson et al. submitted).

Colony Sex ratio and Production of Sexuals

In *P.* (*E.*) *pima* there seemed to be a weak trend of more sexuals produced in bigger colonies (Tab.1). However, colony size in *P.* (*E.*) *pima* was not correlated with sexual output ($p>0.05$).

The mean dry weight (± SD) was calculated for alate females being 1.088µg±0.225 SD, for males 0.662µg±0.080 SD, and for workers 0.601µg±0.040 SD. We did not measure weight of intermorphic queens. The mean dry weights of colonies showed normal distribution (Shapiro-Wilk test; $p>0.05$), and no differences in their variance within each caste was observed (one-way ANOVAs; $p>0.05$). The mean dry weights of colonies for both, workers (*w*) and males (*m*) were significantly different to the weight of alate females (*f*) ($t_{m/f}=-5.211$, $t_{w/f}=-5.690$; $FG_{m/f}=11$, $FG_{w/f}=9$; $p_{m/f}=0.0003$; $p_{w/f}=0.0003$), but not between males and workers ($t=-1.574$, $FG=4$, $p=0.138$). For *P.* (*E.*) *pima* an alate female to male dry-weight ratio (*D*) of 1.644 and an energetic-cost ratio (*C*) of 1.416 was calculated.

Included in the measurement of Sex Investment Ratios (SIR) are only alate females and males from colonies collected in 2002 (Tab.1). From the colonies' SIR (N=23) a highly male biased (Shapiro-Wilk W=0.64290, p=0.00000) population SIR of 0.191±0.328 SD was calculated, only marginally differing from the numerical Sex Ratio (without including *C*), which was 0.173. We observed no split in Sex Ratios between colonies, although some colonies specialized in total male production whereas other colonies yielded a more female biased ratio (Tab.1).

Sex Investment Ratios were positively correlated with the relatedness of females in colonies (Tab.1; Fig.1: $p<0.003$).

Sociogenetic analysis

In our study population of *P.* (*E.*) *pima* inbreeding coefficients for Pb8 and L18 were different from zero (Tab. 2; Pb8: t=3.55, DF=13, $p<0.005$; L18: t=6.70, DF=12, $p<0.0005$), but not for LxAGT1 (t=0.86, DF=13, $p>0.05$). The lowest allele variability – and consequently lowest H_o – was found for L18, maybe explaining its high F_{IT} value in *P.* (*E.*) *pima* (Tab.2).

As the loci used were not developed for *P.* (*E.*) *pima*, we analyzed the possibility of occurring null-alleles (Holm et al. 2001) by focusing on male haploid genotypes (Tab.2). Here we found only for Pb8 that the amplifications of male genotypes failed in a higher rate than in the female castes. This might explain the high inbreeding coefficient, in line with the higher expected than observed heterozygosity for the locus Pb8 in *P.* (*E.*) *pima* (Tab.2).

For three colonies, representing each colony type (Tab.1), a high number of putative matrilines was determined, irresolvable with the three loci used. Additionally, in these colonies male production by more than one female was detected by comparing the haploid genotypes of male sexuals. Because of uncertainties in these colonies for assigning patrilines and matrilines, they were not used for measuring mating frequencies.

The probability of not detecting a second patrilines was <1% ($P_{non\text{-}det}$=[1-0.810]*[1-0.945]*[1-0.647]=0.190*0.055*0.353=0.004), based on H_e (Tab.1). The average number of patrilines detected was 1.18, lying in between the effective mating frequencies calculated for 11 colonies (Tab.1), being 1.03 (m_{eq}) and 1.36 (m_{er}). The effective mating frequency based on m_{eq}, but corrected for sample size following Pamilo (1993), was even lower with m_{eqc}= 0.86. Effective mating frequencies seemed not to be different between colonies headed by dealate ("D") or intermorphic ("I") queens (Tab.1). However, more colonies will be needed for a specific comparison of mating frequencies of both queen types. Due to the present small sampling size of sociogenetically analyzed colonies where an additional calculation of mating frequencies was possible for "D"- and "I"-colonies (two vs. one) this comparison certainly will not be valid using the data at hand.

The mean intracolonial female relatedness was 0.603±0.069 (Tab.1) and not different from the overall relatedness for the study population being 0.632±0.069 SD. Male to male relatedness for the study population was 0.418±0.072 SD. In laboratory reared families relatedness did not differ from 0.75 (Tab.1), which can be explained by single mating of queens. Intracolonial female relatedness was lowest in those colonies having multiple matrilines (Tab.1).

Marking-off mitotypes

Sequences for the mtDNA analysis of different colony types can be found in GenBank, accession numbers AY515285-AY515291 (Tab.3). Nine sites were parsimony informative within sequences of *P.* (*E.*) *pima* (21 for all; Tab. 3). The phylogenetic analysis of these sequences of intermorphic ("I") and dealate ("D") queen headed colonies yielded the same topology by both neighbor joining (Fig.2; SBL=0.16943511) and maximum parsimony (CI=0.897, RI=0.656, RCI=0.589). One dealate queen sample was grouped in between a cluster of intermorphic queen-samples. This sequence of CPS225 had 4 bp difference to the sequence of "I" CPS219, two of them each being 2- and 4-fold degenerate sites (all at third codon positions), with only one site being parsimony informative within samples of *P.* (*E.*) *pima*.

8.5 Discussion

Pogonomyrmex (*Ephebomyrmex*) *pima* regularly shows polygyny as 3/4 of queenright colonies typically contain multiple queens (Johnson et al. submitted), which was also found in this study. Similarly, polygyny was not connected to either dealate or intermorphic queens. We found multiple matrilines within several field colonies of *P.* (*E.*) *pima* (Tab.1). Due to polygyny within-colony relatedness in *P.* (*E.*) *pima* was lowered noticeably: among colony females (workers and virgin queens) the average relatedness was 0.63. In one case (CPS207) intracolonial relatedness deviated from full sister relatedness of 0.75 by a drop down to a value of 0.20 (Tab.1): females in this colony then shared only 1/5 of their genes with nestmates. For both queen morphs we found a reproduction by more than one queen in some of the genetically analyzed field colonies. Moreover, the occurrence of several matrilines among males in 3 of 8 field colonies indicates functional polygyny at least during sexual reproduction. However, we do not explain this with fully functional polygyny as throughout the study population male to male relatedness in colonies was close to 0.50, which is in Hymenoptera the expected value for male-production by single females. Multiple matrilines among workers in field colonies might have been produced by their accumulation from foreign nests (brood raids, drifting workers), or due to nest fusion events with maintaining existing queens, or via a queen-turnover in reproduction during the colonies lifetime.

Overall, the data at hand point to a weak functional polygyny with minor repression of other reproducible *P.* (*E.*) *pima* females by one dominant egg-laying queen. Moreover, queen-turnover might be a frequent event in *P.* (*E.*) *pima*. Subsequent genetic analyses of whole colonies including egg-laying females should clear this point.

The occurrence of multiple queens is either achieved by pleometrosis during the founding stage of colonies, or by (re-)adopting mated females from nearby or the same nest (Heinze and Keller 2000). The latter is likely to occur in *P.* (*E.*) *pima* as here two ways of colony founding do exist (Johnson et al. submitted): 1) independent haplometrotic founding by alate queens, and 2) a mode of dependent founding of intermorphic queens, with readoption into their natal nests after mating. Therefore, limited dispersal of (intermorphic) queens could account for the inbreeding found for two of the three microsatellite loci used, although this might also be caused by null alleles in the locus Pb8 and by poor heterozygosity in L18.

Queen dimorphism in *P.* (*E.*) *pima* is found together with an almost complete lack of co-occurring intermorphic and dealate queens (Johnson et al. submitted). As dimorphism of queens can be seen as adaptation to the species dispersal strategies and founding tactic (Tschinkel 1996; Rüppell and Heinze 1999; Heinze and Keller 2000; Rüppell et al. 2001b; Rüppell et al. 2001a), different explanations for the lack of co-occurrence of intermorphic and dealate queens might hold true. One explanation might be that there is a reciprocal exclusion of both queen types due to the behavior of intermorphic queens, e.g. as social parasites, leading to fixed phylogenetic lineages. In some species queen size dimorphism became genetically fixed, which is well studied in several Myrmicine ant species (Heinze and Buschinger 1987; Fersch et al. 2000; Buschinger and Schreiber 2002). In *Temnothorax* (*Leptothorax*) *rugatulus* (Rüppell et al. 2001c) a high transmissibility from generation to generation of queen body size is found, additionally influenced by social (queen) influences. Determination of queen size in *T. rugatulus* seems to be an adaptive adjustment to alternative reproductive strategies (Rüppell et al. 2001c).

In *P.* (*E.*) *pima* the mtDNA based tree topology (Fig. 2) brought no convincing explanation for a genetically fixed dimorphism of intermorphic and dealate queen lineages (“I” and “D”). It can only be suggested that the intermorphic queen type arose from the dealate queen type, as the nodes within the study-population were only weakly supported in the NJ-tree (Fig. 2). A sequencing of longer stretches of DNA (mtDNA or nuclear DNA) might yield a better resolution of the population samples in this study, and should be compared with additional samples from other populations. However, the intermixing of “I” and “D” queen morphs found by the present analysis of mtDNA lineages (Fig.2) is most parsimonious explained with phenotypic variability of queens, all belonging to the same gene pool.

Colonies might be flexible in their behavior to rear dealate or intermorphic queens, responding to its external or internal environment. E.g., intermorphic queens might be produced during a form of ‘emergency rearing’ in case a colonies’ old queen died, or alate queens might be produced if climatic conditions limit resource availability. Similar to *Ectatomma ruidum* (Lachaud et al. 1999) higher energetic production costs might be assumed for winged female sexuals of *P.* (*E.*) *pima* as their dry weights generally were greater, at least compared to males or workers. This might be in parallel to clear morphological distinctions, with intermorphic queens being intermediate between workers and dealate queens (Johnson et al. submitted). Possibly in *P.* (*E.*) *pima* lowered resource consumption for the production of intermorphic compared to alate queens then might be assumed, although still being higher than for the production of workers. This should be tested by measuring the energetic investments into both queen morphs.

Resource availability is one of the causes for development of intermorphic females (Murakami et al. 2002). Resource limitation was considered to underlie wing reduction in congeners of *P.* (*E.*) *pima*, namely *Pogonomyrmex* (*Ephebomyrmex*) *imberbiculus*, *P.* (*sensu stricto*) *laticeps*, *P.* (*s.str.*) *huachucanus*, *P.* (*s.str.*) *longibarbis andinus*, or *P.* (*Forelomyrmex*) *mayri* (Heinze et al. 1992). Especially in deserts resources are often only temporally available, mainly after monsoon rains, and patchily distributed, especially around bushes and trees. Living in a desert habitat, and depending on grass seeds and occasional collected insects, for *P.* (*E.*) *pima* resources are highly variable in time. This would additionally support the evolution of a dependent colony founding tactic with re-adoption of queens (Tinaut and Heinze 1992) like possibly the case for the intermorphic queens in *P.* (*E.*) *pima* (Johnson et al. submitted).

Ross *et al.* (2003) showed for *Solenopsis geminata* that its polygyne form originated via a founder event from a local monogyne population, leading to lower genetic diversity and different gene frequencies than the monogyne form. Further they stated that these polygnous *S. geminata* emerged due to a breakdown in discrimination abilities followed by the acceptance of multiple queens in colonies (Ross et al. 2003).

This could have happened also in the polygynous forms of *P.* (*E.*) *pima*. Only the well-fed, often older colonies might be able to shift into the production of female sexuals, whose dimorphism might be determined by a complex set of environmental factors. E.g. "self-determination" (Bourke and Ratnieks 1999) of caste by female larvae is thinkable, which is favored by small queen-worker dimorphism in addition to larval control over resource intake (Bourke and Ratnieks 1999). Maybe in polygynous colonies or orphaned colonies of *P.* (*E.*) *pima* a certain control mechanism, triggered by dealate (single) queens, disappears, resulting in increased proportions of female larvae developing into reproductives.

Caste control in *P.* (*E.*) *pima* colonies should be tested experimentally, especially as this will give important insights into their generic neighbors, for which the *P.* (*s.str.*) *rugosus* X *barbatus* hybrids (Gregg 1963; Cole 1968) recently became most prominent (Helms-Cahan et al. 2002; Julian et al. 2002; Parker and Rissing 2002; Volny and Gordon 2002b; Helms-Cahan and Keller 2003). Cross fostering experiments are needed to show if dealate queens produce intermorphic queens and vice versa. This would underline a morphological flexibility, or – if not found – proof the hypothesis of separated genetic lineages. For this a de-queening of dealate colonies could probably show if an emergency rearing of (intermorphic) queens in the orphaned colony does occur.

Beside polygyny, another aspect affecting the relatedness structure of *P.* (*E.*) *pima* field colonies could have been multiple mating by queens and subsequently an increase of patrilines. In our study we don't have a good comparison of mating frequencies and relatedness measurements between intermorphic and dealate queen colonies, as well between monogynous and polygynous colonies. However, in *P.* (*E.*) *pima* we found for both, intermorphic and dealate queen morphs an effective mating frequency far below two matings (m_{eq}=1.03, m_{er}=1.36). In laboratory reared families of *P.* (*E.*) *pima* relatedness did not differ from 0.75, corresponding to the expected condition of monogyny and monandry here taking place. Although sample size was low ($N_{colonies}$=11; $N_{indiv.}$=396), we believe that the matching results of both analyzes on field colonies, and on laboratory reared monogynous families are persuasive. Furthermore, the detection probability of additional patrilines was >99% and seemed not to be affected by inbreeding effects.

With our result of single mating we reject the hypothesis that *P.* (*E.*) *pima* has multiply mated queens as its congeners. *P.* (*E.*) *pima* has small colony sizes, being not different between mono- and polygynous nests, or nests containing intermorphic or dealate queens (Johnson et al. submitted). As queen mating frequencies in polygynous *P.* (*E.*) *pima* are markedly lower than in its bigger sized monogynous congeners this is in line with the expectations of the genetic variability and sperm-need hypotheses (Cole 1983; Keller and Reeve 1994). However, single mating in *P.* (*E.*) *pima* is more likely explained using the genetic variability hypothesis, as the sperm-need hypothesis could not yet be confirmed despite extensive reviews (Page 1986; Boomsma and Ratnieks 1996).

Like other species of the subgenus *Ephebomyrmex*, *P.* (*E.*) *pima* is phylogenetically basal to North-American *Pogonomyrmex sensu stricto* species (Kusnezov 1951; Snelling 1981; Strehl et al. in preparation). Therefore, the finding of single mating within this species supports the hypothesis of an early evolution of multiple mating in an ancestor of the North-American *Pogonomyrmex* (*sensu stricto*) species (Gadau et al. 2003; Rheindt et al. 2004). We conclude that other North-American *Ephebomyrmex* species than *P.* (*E.*) *pima* will show similar low female mating numbers, as they have similar life history traits.

Intracolonial relatedness in a single-mating species like *P.* (*E.*) *pima* is changed only via functional polygyny. Polygyny, in general, connected with dependent founding has important implications (Heinze and Keller 2000) for colony structure and sociogenetics of colonies previously founded by one single mated queen. Here lowered relatedness is leading to possible conflicts among nest mates over reproduction with shifts from female biased to more male biased sex allocation ratios, sometimes including worker reproduction (Hölldobler and Bartz 1985; Pamilo 1991; Evans 1995; Heinze and Keller 2000). However, in addition to the relatedness of nestmates, environmental influences like resource availability also play important roles in determining sex allocation ratios (Kim 1999; Napoleon and King 1999).

We calculated a highly male biased population wide Sex Investment Ratio of 0.191±0.328 SD for *P.* (*E.*) *pima.* This certainly is due to the fact that 22 of the 23 sexually reproducing colonies in our study (96%) produced males (Tab.1). The male-biased investment ratio is likely to be explained (Pamilo 1990) with restricted queen dispersal in *P.* (*E.*) *pima*. Noticeably female producing colonies of *P.* (*E.*) *pima* normally produced a certain number of males, too (Tab.1). This result in *P.* (*E.*) *pima* probably matches that found for *Temnothorax* (*Leptothorax*) *nylanderi*, where many colonies investing highly in females, are still producing a certain number of males (Foitzik 1998), consistent with the constant male hypothesis (Frank 1987).

In nests of *P.* (*E.*) *pima* containing intermorphic queens we found in no single case female sexuals (Tab.1). We doubt that virgin queens were confused with the counted intermorphic queens, as their numbers and frequencies were not different from those numbers found in dealate headed colonies (Tab.1). However, the occurring dimorphism of female reproductives should be taken into consideration for future studies. The possible absence of queen-production in intermorphic colonies is surprising as intermorphic queens have the capacity to mate and to produce female worker-offspring (Johnson et al. submitted). Therefore, in *P.* (*E.*) *pima* queen physiology might lead to a specialization in the reproduction by one or two sexes, e.g. due to a form of “genetic sex ratio variation”, which has been found before in parasitoid Hymenoptera (Bourke and Franks 1995). Breeding experiments could resolve the question if intermorphic or dealate queen determination is connected to a genetic component, or if the fate of a fertilized egg is solely determined by environment.

If queens in *P.* (*E.*) *pima* do *not* vary in their tendency to produce queen-destined eggs, i.e. they produce the same ‘primary’ sex ratios, then the observed pattern of ‘secondary’ sex ratios are environmentally controlled. Some hints for such an explanation come from studies in closely related species. E.g., in *Myrmica brevispinosa* sex ratios can be shifted through nutritional influences on female caste determination (Bono and Herbers 2003). For other *Pogonomyrmex* species, *Pogonomyrmex* (*s.str.*) *montanus*, *Pogonomyrmex* (*s.str.*) *subnitidus* and *Pogonomyrmex* (*s.str.*) *rugosus*, it was shown that similar proportions of energy are invested between the production of males and females, but since females are larger, more males are produced (MacKay 1985). If *Pogonomyrmex* nests were provided with additional food, they invested more energy in the production of males, whereas control nests, nests which had the food removed and older nests invested equally in the production of the two sexes (MacKay 1985).

In *P.* (*E.*) *pima* the observed positive correlation of Sex Investment Ratios with relatedness (Fig. 1) points to a Sex Ratio Determination more via internal environmental factors (e.g. nest mates) than external environmental factors (e.g. climate). Unclear is the mechanism by which this occurs, if there is total queen control, an interference of the nestmates (workers) or a mixture of both. The most likely factor of the intracolonial environment to control sex ratios is the worker caste. Hints for a worker control of sex allocation determination were found in within-species studies, where high relatedness asymmetry was associated with mainly female production, and low relatedness asymmetry with male-biased production of sexuals (Chapuisat and Keller 1999). Similarly to multiple mating, polygyny is predicted to lead to workers policing each others' male production with the result that virtually all males are produced by the queen. By this the interests of a colony's queen converge with her daughters interests about the amount of males produced among the reproductive output (Boomsma and Grafen 1990; Tsuji 1996; Foster and Ratnieks 2001; Mehdiabadi et al. 2003). Therefore, the positive correlation of Sex Investment Ratios with intracolonial relatedness of females (Fig. 1) is indicating a possible worker control over sex allocation in *P.* (*E.*) *pima*.

8.6 Acknowledgements

We thank Bert Hölldobler and Heike Feldhaar for helpful comments and fruitful discussions throughout this study. Laboratory work was evidently supported by Karin Moeller. This study is supported by grants of the German Science Foundation (Deutsche Forschungsgemeinschaft / DFG) GA 661/1-2 and SFB 554-TP C-5.

8.7 Tables

Table 1 (following page):

Census and relatedness results (bold) of field colonies (upper part) containing dealate or intermorphic queens, or with unknown queen types, and relatedness results of laboratory reared families (lower part); asterisks indicate colonies sequenced for 632 bp of mtDNA (Fig.2). *Abbreviations*: genot. = number of individuals in left column genotyped; NSR = Numerical alate female to male Sex Ratio; SIR = alate female to male Sex Investment Ratio, including a calculated energetic-cost ratio (*C*; Boomsma 1989) of 1.416; M / P = number of matrilines / patrilines detected by genotyping; § = indicates field colonies genotyped as highly polygynous, with unreliable assignment of patrilines; m_{eq} = effective mating frequency calculated from P; intr. rel. / J/loci = intracolonial relatedness / Jackknife values calculated with Relatedness 5.0.8 from female genotypes; m_{er} = effective mating frequency calculated from Relatedness (Crozier and Pamilo 1996).

	colony:	dealates	intermophs	workers	genot.	female sexuals	genot.	males	genot.	NSR	SIR	M	P	m_{eq}	intr. rel.	± SD (J/loci)	m_{er}
dealate queens (field)	CPS #221	15	0	31		10		26		0,28	0,35						
	CPS #220*	6	0	146	46	105	9	10	8	**0,91**	**0,94**	1	1	1,00	0,759	± 0,077	0,982
	CPS#261	4	0	23		89		69		0,56	0,65						
	CPS #227	3	0	70		0		1		0,00	0,00						
	CPS #216	2	0	25		0		44		0,00	0,00						
	CPS #225*	2	0	16	7	24	6	0	-	**1,00**	**1,00**	1	2	1,17	0,599	± 0,034	1,433
	CPS #206	1	0	31		0		9		0,00	0,00						
	CPS #208*	1	0	93	32	0	-	52	16	**0,00**	**0,00**	§	-	-	0,371	± 0,077	
intermorphic queens (field)	**CPS #219***	0	11	104	20	0	-	119	20	**0,00**	**0,00**	§	-	-	0,305	± 0,054	
	CPS #210*	0	3	25	19	0	-	17	10	**0,00**	**0,00**	2	1	1,00	0,407	± 0,094	3,185
	CPS #218	0	3	103		0		0		-	-						
	CPS #226*	0	3	83		0		24		0,00	0,00						
	CPS #214	0	2	16		0		11		0,00	0,00						
	CPS #223	0	1	35		0		88		0,00	0,00						
	CPS #224	0	1	116		0		88		0,00	0,00						
unknown queen type (field)	**CPS #207**	0	0	98	27	0	-	76	16	**0,00**	**0,00**	§	-	-	0,196	± 0,089	
	CPS #209	0	0	54	24	28	8	34	8	**0,45**	**0,54**	1	1	1,00	0,516	± 0,044	1,880
	CPS#262	?	?	10	5	20	8	15	8	**0,57**	**0,65**	1	1	1,00	0,520	± 0,021	1,852
	CPS #211	0	0	75		0		93		0,00	0,00						
	CPS #212	0	0	60		17		65		0,21	0,27						
	CPS #213	0	0	65		0		57		0,00	0,00						
	CPS #215	0	0	35		0		3		0,00	0,00						
	CPS #217	0	0	32		0		10		0,00	0,00						
	CPS #222	0	0	18		0		19		0,00	0,00						
dealate queens	**CPS#263**	1	-	-	16	-	-	-	-	-	-	1	1	1,00	0,870	± 0,100	0,806
	EP/3-13, DQ1	1	-	-	18	-	-	-	-	-	-	1	1	1,00	0,677	± 0,052	1,171
intermorphic queens	**EP/Old, IQ2**	-	1	-	14	-	-	-	-	-	-	1	1	1,00	0,866	± 0,121	0,812
	EP/Old, IQ3	-	1	-	14	-	-	-	-	-	-	1	2	1,15	0,761	± 0,088	0,978
	EP/3-10, IQ4	-	1	-	28	-	-	-	-	-	-	1	1	1,00	0,849	± 0,117	0,835
	EP/3-10, IQ6	-	1	-	9	-	-	-	-	-	-	1	1	1,00	0,722	± 0,015	1,059

Table 2:

Characteristics of loci used for genotyping: He / Ho = expected / observed heterozygosity; FIT = inbreeding coefficient; He, Ho, and FIT were calculated for diploid females only, using the program GDA 1.0 (Lewis and Zaykin 2001); % m / % f = frequency of males / females in dataset without alleles (Nm = 86 / Nf = 310).

Locus	Allele-number	size range (bp)	H_e	H_o	F_{IT} ± SD	% m	% f
Pb8	11	273-291	0.810	0.743	0.127 ± 0.134	0.233	0.120
LxAGT1	29	211-307	0.945	0.960	0.008 ± 0.035	0.070	0.197
L18	4	132-139	0.647	0.500	0.264 ± 0.142	0.012	0.148

Table 3:

Variable sites (N=28) in the sequences of *P.* (*E.*) *pima* used for constructing a Cladogram (Fig. 3). Colony types “I” or “D” correspond to the presence of intermorphic or dealate queens in the colony samples. Positions refer to relative positions of base pairs differing in at least one of the used sequences. GenBank Accession Numbers are indicated for each sample in the rightmost column.

colony type	Acc.-No.	positions																												GenBank
		28	31	40	55	58	73	84	88	115	175	208	263	304	313	349	391	424	466	467	487	520	552	554	559	568	574	598	628	
"D"	CPS225	G	A	G	G	T	G	A	T	C	C	A	T	C	G	T	C	C	C	A	T	T	T	T	A	C	G	T	G	AY515291
"I"	CPS219	.	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	G	T	A	.	.	AY515290
"I"	CPS226	.	G	A	A	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	AY510622
"I"	CPS210	.	.	.	.	.	.	.	?	.	?	.	C	.	.	.	.	.	.	.	.	?	C	C	.	.	A	?	.	AY515289
"D"	CPS220/m6	.	G	.	.	C	.	.	G	.	.	G	C	.	.	.	.	.	.	.	.	C	.	.	.	.	A	?	.	AY515285
"D"	CPS220/f1	.	G	.	.	C	.	.	?	.	.	G	C	.	.	.	.	.	.	?	.	?	.	.	.	.	A	C	?	AY515286
	CPS222	.	G	.	?	C	A	G	G	.	.	.	C	.	.	.	.	.	.	?	.	C	.	C	.	.	A	C	T	AY510623
"D"	CPS208	A	.	A	R	C	.	.	A	Y	T	.	C	.	A	C	.	.	?	?	.	C	.	C	.	.	A	C	?	AY515288
	6258AZ	?	?	?	?	?	?	?	?	T	?	.	C	T	.	.	T	T	T	C	.	?	.	C	.	.	A	C	T	AY510659

8.8 Figures

Figure 1:

Scatterplot showing the correlation of the Sex Investment Ratios (SIR) of genotyped field colonies with their within-colony relatedness: R=0.8948, R²=0.8007, corr. R²=0.7675, F(1,6)=24,113, p<0.00268; Solid line = regression line (y=0.3188+0.3583 x); dashed lines = 95% confidence interval; numbers above dots indicate the CPS accession numbers and type of colony, where known (comp. Fig.2).

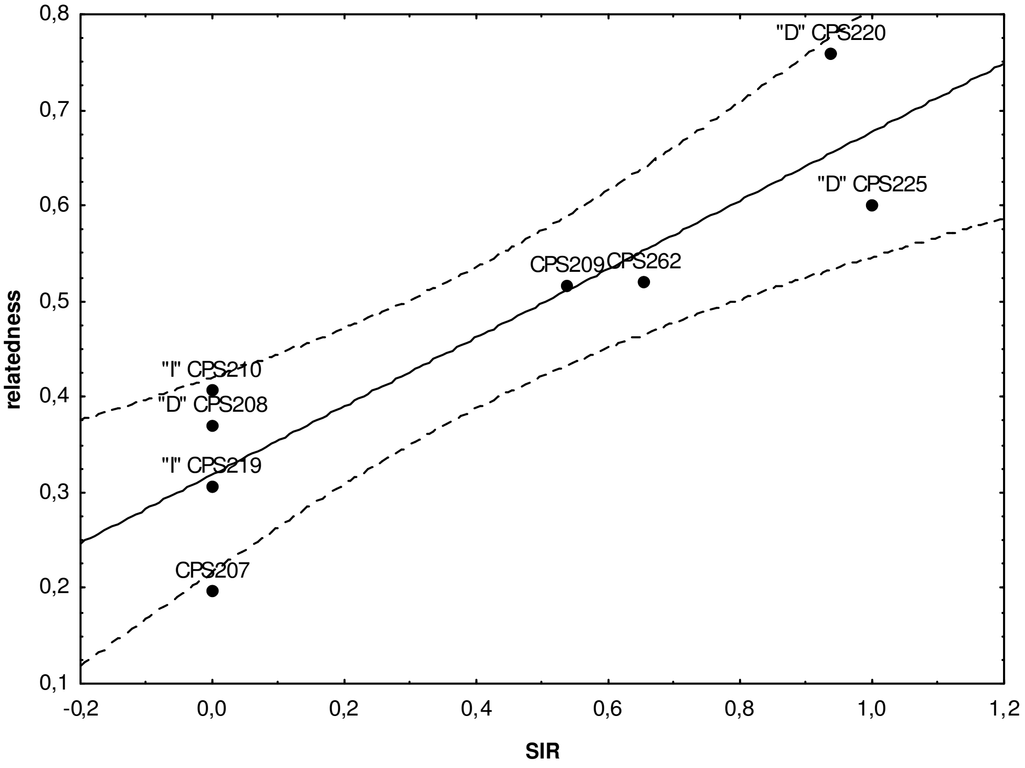
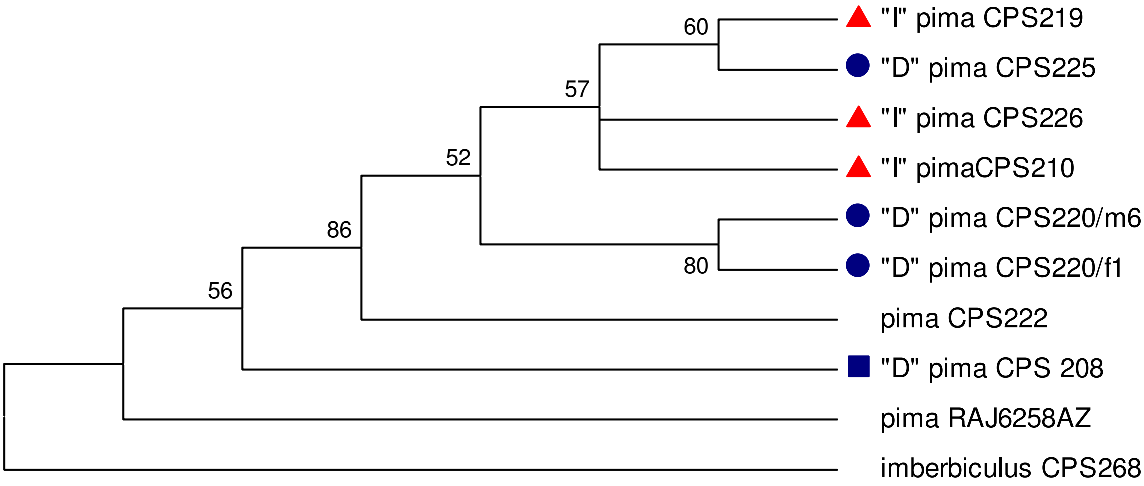


Figure 2

Unrooted neighbor-joining tree of 8 Cytochrome c Oxidase I sequences (632 bp) of *Pogonomyrmex* (*Ephebomyrmex*) *pima* samples from the study population at McCartney Rd., Pinal Co. (AZ), with *P.* (*E.*) *pima* from Yavapai Co. (AZ) and *P.* (*E.*) *imberbiculus* as outgroups; bootstrap-values (100.000 reps) are indicated at branches; triangle = Polygynous "I" colony with intermorphic queens (red), circle = Polygynous "D" colony with dealate queens (blue), square = monogynous "D" colony with dealate queen (blue).



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9. Phylogeny of the Harvester Ant Genus *Pogonomyrmex* (Hymenoptera: Formicidae) in North-America and its relation to South-American *Pogonomyrmex* species

Status of publication: in preparation

Authors: Christoph-P. Strehl, Heike Feldhaar, Jürgen Gadau, Robert A. Johnson, Berthold Hölldobler

9.1 Abstract

The harvester ant genus *Pogonomyrmex* has 61 described species. It is adapted to xeric and arid habitats and endemic to the New World. *Pogonomyrmex* shows an interesting biogeographic pattern with about half of the described species occurring in South America (n=30) and the other half in North America (n=27). Only one species occurs in Guatemala and two species are endemic to the Hispaniola island.

We sequenced parts of two mtDNA genes (CoxI = 1043 bp and CytB = 430 bp) and one nuclear gene (EF1a = 845 bp) from 39 species to create a representative molecular phylogeny of the whole genus. Nuclear gene trees indicated a basic split into two lines including all analyzed species of the subgenus *Ephebomyrmex* and the subgenus *sensu stricto* species, respectively. Hence, the subgenus *Ephebomyrmex* might be elevated to a separate genus as proposed by earlier authors.

9.2 Introduction

Among the currently known over 11,000 ant species (Antbase; Clarke 2002; Agosti 2004)), harvester ants form an important ecological clade, especially in areas of arid and semiarid environments (MacMahon et al. 2000). Half of the harvester ant species in the world belong to the genus *Pogonomyrmex*, which is exclusively found in North, Central, and South America and consists of 61 described species (Cole 1968; Hölldobler and Wilson 1990; Bolton 1995; Fernández C. and Palacio G. 1997; Taber 1998; MacMahon et al. 2000) and one undescribed species (*species B*: Taber, 1998). *Pogonomyrmex* harvester ants inhabit mostly dry areas, but some species also occur in humid environment (e.g. *P. sylvestris*; Lattke 1990).

Pogonomyrmex (Mayr 1868; Wheeler 1911) shows an interesting biogeographic pattern with about half of the described species occurring in South America (n=30) and the other half in North America (n=28). Only one species occurs in Guatemala (*P. guatemaltecus*) and two species are endemic to the Hispaniola island (*P. saucius*, *P. schmitti*). The evolution of the North-American *Pogonomyrmex* species has been speculated to have evolved from boreal North-American *Myrmica* species which then spread to South-America (Wheeler 1914). But more conceivable is recent theory (Taber 1998) that *Pogonomyrmex* originated like most North-American species in South-America and then spread over Middle-America into the area of the United States (compare *P. badius*: Deyrup and Trager 1986).

Taxonomic positions and the cladistic relation between the two big geographical lineages in North- and South-America or between possible subgenera are still not fully understood. According to Bolton (1994, 1995), three subgenera can be found: *Pogonomyrmex*, *Ephebomyrmex* and *Forelomyrmex*. However, the subgenus *Forelomyrmex* (= junior homonym of *Janetia*) consists of only one species, *P. (F.) mayri* (Forel 1899; Wheeler 1902; Wheeler 1913; Kugler 1978; Bolton 1994; Bolton 1995), which was recently classified to be part of the subgenus *Ephebomyrmex* (Taber 1998). The remaining two subgenera *Ephebomyrmex* and *Pogonomyrmex* therefore consist of 15 and 46 species, respectively (Cole 1968; Bolton 1995; Taber 1998). The status of *Ephebomyrmex* as a subgenus of *Pogonomyrmex* (Wheeler 1902; Creighton 1956; Cole 1968; Snelling 1981; Bolton 1995; Parker and Rissing 2002) was frequently shifted to generic status by several authors (Kusnezov 1959; Kempf 1972; Wheeler and Wheeler 1986; Fernández C. and Palacio G. 1997; Taber 1998), where differences in morphological and life-history traits (e.g.: Heinze et al. 1992; Taber 1998; Johnson 2000; Johnson 2001) seem to support this opinion.

Ephebomyrmex and *Pogonomyrmex* vary in their occurrence, biology and life history traits, which is best studied in their North-American representatives. In North America 25 species of *Pogonomyrmex sensu stricto* exist, which first Cole (1968) reviewed to belong to four morphological "complexes" (BADIUS, BARBATUS, MARICOPA, OCCIDENTALIS). The second half of *Pogonomyrmex sensu stricto* species (N=21) is living in South-America and was separated by Kusnezov (1951) into six "complexes" (BISPINOSUS, BREVIBARBIS, COARCTATUS, CUNICULARIUS, INERMIS, RASTRATUS). For *Pogonomyrmex (Ephebomyrmex)* species (N=15) two complexes have been named (ANGUSTUS, NAEGELII; Kusnezov 1951). For North-American

Pogonomyrmex sensu stricto species Parker and Rissing (2002) confirmed in their DNA-based phylogeny Cole's morphological complexes. Additionally, they confirmed cases of hypothesized hybridization within the BARBATUS species complex (Cole 1968; Ashe and Oldroyd 2002; Helms-Cahan et al. 2002; Julian et al. 2002). Hybridization even between species complexes has recently found for MARICOPA X OCCIDENTALIS (Cole 1968; Anderson 2002). These evidences for hybridization in, at least the North-American, *Pogonomyrmex sensu stricto* are emphasizing the flexibility and/or recent rise of species barriers.

Pogonomyrmex species play an important role in their environments as granivores, predictors of the food-web structure and converters of the soils composition and have few functional equivalents for their roles (Folgarait 1998; MacMahon et al. 2000). Therefore, a deeper understanding of their ecology is essential for management and conservation efforts, e.g. in the species-rich hotspot of the deserts in the Southwestern USA (Whitfield 2002). To resolve the uncertainties in phylogenetic relationships between *Pogonomyrmex* harvester ants a cladistic analysis was done by the use of mitochondrial and nuclear DNA sequences.

For reconstructing phylogenies we chose to use DNA-sequences from the mitochondrial genes of Cytochrome c Oxidase I (CoxI) and Cytochrome b (CytB) which promised to show useful resolution at generic and population level in a number of previous studies (e.g.: Chiotis et al. 2000; Leys et al. 2000; Hedin and Maddison 2001; Lopez-Vaamonde et al. 2001; Monteiro and Pierce 2001; Jordal et al. 2002; O'Grady and Kidwell 2002). Additionally, CytB was needed to compare the consistency of our gene-trees with the one published by Parker and Rissing (2002), which is based on N-American *Pogonomyrmex* samples of this gene. For resolving deeper splits we compared sequences obtained from a nuclear gene, Elongation Factor – 1 alpha (EF1a), which is promising for this purpose (e.g.: Danforth et al. 1999; Jordal et al. 2002).

A detailed knowledge of the cladistics in the genus *Pogonomyrmex* is needed to understand their species biogeography, but also the evolution of certain life history traits shaping the characteristics of single colonies, like their for ants unusual high mating frequency of *Pogonomyrmex* (*s.str.*) queens (e.g.: Rheindt et al. 2004; Wiernasz et al. 2004), and also the evolution of biological features like pheromones (Hölldobler et al. 2001) or venoms (Schmidt and Blum 1978; Schmidt et al. 1980; Schmidt et al. 1986; Leluk et al. 1989; Schmidt 1998). Therefore, first reflections founded on the received molecular phylogeny will be made on the evolution of female multiple mating in *Pogonomyrmex*.

9.3 Material and Methods

Most specimens were collected during a field trip in the United States during August and September 2000. Animals were killed and conserved in 95% Ethanol. Material from species hard to discover, or from which their distribution was not in the range of the collecting sites, were obtained by using already collected specimens or by donations of other people (compare Tab.1). Some of these donations contained species fixated in 75% Ethanol or even lower concentrations of Ethanol, which made a special treatment of these samples necessary (see below).

For this analysis we sequenced and used (1) a 1043 bp fragment of the mitochondrial gene Cytochrome c Oxidase I (CoxI) from 40 *Pogonomyrmex* species (61 individuals), (2) 430 bp of the mitochondrial gene Cytochrome b (CytB) from 33 *Pogonomyrmex* species (56 individuals), and (3) a 845 bp fragment of the Q-I region of the nuclear Elongation Factor one alpha (EF1a) from 24 *Pogonomyrmex* species (22 individuals). Similar to other aculeate Hymenoptera (e.g.: Danforth and Ji 1998) two copies of EF1a were distinguishable in *Pogonomyrmex*: one copy ("F2"), used in this study, was specifically amplified by the use of primers CHO6trs and U114 (Tab. 1), whereas a second copy of EF1a ("F1") was identified during a pre-study for its use in three *Pogonomyrmex* species (*P. comanche*, *P. rugosus*, *P. snellingi*) by specific amplification with primers TRS1R and U114 or Uant377 (Tab. 1; GenBank Acc.-Nos. AY553232-AY553234), and an EF1a-sequence paralogous to the honeybees "R-region" by the use of primers TRS2F and TRS10R (Tab. 1; GenBank Acc.-Nos. AY553229-AY553231).

The genus *Hylomyrma* is suggested to be the closest relative to *Pogonomyrmex* (Taber, 1998) and would have been the optimal outgroup for phylogenetic analyses, but was not accessible. For phylogenetic analyses at generic level we therefore used as outgroups sequences obtained from *Myrmica* (Myrmicinae) as the next close relative, and additionally from an *Odontomachus* (Ponerinae) species (Tab. 2).

Genomic DNA usually was isolated using a modified version of the Puregene Kit protocol (Gentra Systems/Biozym, Germany) published in Gadau *et al.* (2003). For material critical in yielding enough of DNA we used different treatments for extracting

DNA, usually a Phenol-Chloroform extraction method (Gadau et al. 1996)and in some cases the QuiaGen DnEasy Tissue Kit (with the protocol for insect tissues no. I; see also (Dobler and Müller 2000). For DNA-isolation single workers were grind up in liquid nitrogen. Gasters were cut off to prevent contamination with bacterial DNA or PCR-inhibiting gland secretions.

PCR amplifications were performed in 25µl reaction volumes at heating rates of 5°C/sec (thermocycler of Biometra, Germany). The reaction mix contained 2µl of usually 1:10 diluted genomic DNA (approximately 4ng DNA), 2.5µl of 10x PCR Buffer (750mM Tris-HCl, 200mM $(NH_4)_2SO_4$, 0.1% Tween 20), 0.2mM of each dNTP, 2.0mM $MgCl_2$, 1U Taq (MBI Fermentas, Lithuania) and 0.5µM of each primer (Tab. 1). CoxI and CytB fragments were amplified in 35 cycles using the following parameters: 40 sec at 94°C, 60 sec at 45°C, and 40 sec at 72°C; an initial denaturation step of 3 min at 94°C and a final elongation step of 90 sec at 72°C was added. Similar parameters were used for the amplification of EF1a fragments, but the annealing temperature was set to 50°C for 23 cycles, after a touchdown over 12 cycles with -0.5°C per cycle (starting at 56°C) to accumulate highly specific amplicons.

In some cases successful DNA amplifications had to be multiplied for obtaining a great enough amount of PCR products for the subsequent steps. PCR products were purified by standard Ethanol-precipitation or using ExoSAPit (USB Corporation, USA). Purified amplicons were sequenced either commercially at SeqLab laboratories (Göttingen, Germany) or in Wuerzburg (Department of Zoology 1) on an ABI sequencer using products of standard sequencing PCR reactions with Perkin Elmer premix (Perkin Elmer, USA) and purified amplicons as templates.

Sequences were checked and aligned on a Personal Computer using the programs Chromas 1.45 (McCarthy 1998), MEGA2 (Kumar et al. 2001), BioEdit 5.0.9 (Hall 1999), Textpad 4.5.0 (Helios Software Solutions), and ClustalX (Thompson et al. 1997). Gaps were excluded from the final alignment before phylogenetic analyses (Salemi and Vandamme 2003, p.65), and treated as missing data. All obtained sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/Genbank; Tab. 2). Nestmates from the analyzed workers are deposited as pinned voucher specimens in the collections of Harvard University (Cambridge, MA, USA) and collections of P.S. Ward at University of California in Davis (USA) and preserved in 100% Ethanol (stored at -70°C) at Museum Koenig (Bonn, Germany).

Phylogenetic analyses were performed using standard parameters in PAUP* 4.0b1 (Swofford 1998), MEGA2 (Kumar et al. 2001), and Mr. Bayes (Huelsenbeck and Ronquist 2001). Appropriate models for Maximum Likelihood analyses were found using Modeltest 3.06 (Posada and Crandall 1998).

Parsimony, Neighbor Joining (NJ), Bayesian, and, where manageable, Maximum Likelihood (ML) analyses were performed (1) separately for subsets of the mtDNA-gene fragments (CoxI, CytB) and the EF1a-gene fragment, (2) using a matrix having all species with complete sets of mtDNA-fragments combined (up to 4 sequences per species), (3) using all sequences of available South-American species plus representatives of the North-American species complexes, all having complete sets of mtDNA- and EF1a-fragments. Some samples with missing gene subsets e.g. due to sequencing failure had to be omitted from some of the combined data sets, as here phylogenetic information for binding it into the gene trees was missing. EF1a-sequences were mainly analyzed for a comparison of the North- and South-American species complexes and for validating the subgenus-hypothesis for *Ephebomyrmex* and *Pogonomyrmex s.str.* species.

Parsimony analyses were performed generally using 2,500 bootstrap replicates, except for the CoxI-subset where only 1,000 bootstrap replicates were obtained. The NJ-analyses were performed using 100,000 bootstrap replicates. Bayesian analyses were run for at least 1 Mio. generations, with collection of every 100th tree, and consensus trees were built after a sufficient burn-in phase as described in the programs manual. Maximum Likelihood (ML) analyses were performed for each subset using the GTR+I+G model, except for the the EF1a-gene subset, for which the appropriate model was TrN+G. ML-analyses were performed for the CoxI-subset using 25 bootstrap replicates, for the CytB-subset using 90 bootstrap replicates, and for the EF1a-subset using 100 bootstrap replicates. Generally, subsequent analyses of the obtained tree topologies were made on bootstrap 50% majority rule consensus trees.

9.4 Results

Successful sampling included 39 of the described 61 *Pogonomyrmex* species, specifically 26 of Middle- and North- American and 13 of South-American *Pogonomyrmex* species, comprising 53% (N=8) of all described *Pogonomyrmex* (*Ephebomyrmex*) species, and 67% (N=31) of *Pogonomyrmex sensu stricto* species. For North- and Middle-American species only four of 25 described *sensu stricto* species were missing (*P. bigbendensis*, *P. guatemaltecus*, *P. texanus*, *P. wheeleri*), and one of 5 *Ephebomyrmex* species respectively (*P. schmitti*). A representative sampling including species of all actually described complexes was ensured by addition of 13 South-American *Pogonomyrmex* species. Only the single representative of the former subgenus *Forelomyrmex* was not able to be included (*P. mayri*).

At all, 206 sequences were obtained and deposited in GenBank (Tab. 2; Accession numbers: AY510606-700, AY515285-291, and AY646357-365 for CoxI-sequences; AY538614-625, AY552475-523, and AY647220-222 for CytB-sequences; AY651887-917 for EF1a-sequences). Generally, sequences were obtained readily with the described methods. However, problems occurred with some, mainly *Ephebomyrmex* species in obtaining CytB-sequences (e.g. *P.* (*E.*) *odoratus*), probably due to primer mismatch. Additionally, not all of the obtained sequences were useful for phylogenetic analyses. The BEN/JERRY-fragment AY646360 (*P. inermis*) contained two insertions causing Stop-codons; this fragment was not repeatable and therefore the problematic region was treated as missing in the analyses. Additonally, sequences AY552491 (*P. "owyheei" / salinus*), AY510607 and AY510609 (both *P. angustus*) were not included in the final phylogenetic analyses as they might represent pseudogenic regions, always outgrouping in pre-run gene trees; the position of these sequences outside the mitochondrial genome should be verified in subsequent analyses including additional, suitable techniques.

Several gene trees have been created with matrices including either only the sequenced DNA fragments or combinations of them combined. If possible, the runs were hold as equal, only the number of bootstrap replicates was reduced when needed to reduce computing time.

General pattern of the obtained gene trees

Among the obtained gene trees three main groups were found, which are separated into several subgroups: 1) New Word *Ephebomyrmex* species group "EPHEBOMYRMEX", with North-American and South-American species separated, 2) North-American *sensu stricto* group "HUACHUCANUS" (PHA), and 3) South-American *sensu stricto* group "LOBATUS" (PLB). Additionally, South-American *Ephebomyrmex* species either formed own groups within the whole *Pogonomyrmex* ingroup, or clustered generally with the North-American *Ephebomyrmex* species. *Ephebomyrmex* species commonly did not group within the *sensu stricto* sub-groups PHA or PLB. Accordingly, both *sensu stricto* sub-groups generally clustered together. The analyses for resolving the deeper splits between *Ephebomyrmex* and *sensu stricto* using nuclear DNA always showed a clear separation of both groups in the obtained gene trees. However, in the Bayesian analyses of mtDNA (Fig. 4) the PHA-group and *Ephebomyrmex* species (except *P. odoratus*) formed one clade.

The following groups of species were found among the obtained gene-trees: (1) BARBATUS-complex (PBR; Cole 1968) = *tenuispina* + PBR-1 (*barbatus* + *rugosus*) + PBR-2 (*desertorum* + *colei* + *anergismus* + *barbatus* + *rugosus* var. *fuscatus*) + PBR-3 (*bicolor* + *apache*); (2) OCCIDENTALIS-complex (POC; Cole 1968) = *anzensis* + *subdentatus* + (*montanus* + (*brevispinosus*, *subdentatus*)) + (*occidentalis* + *salinus* / "*owyheei*"); (3) CALIFORNICUS-complex (PCL; Taber 1990) = *magnacanthus* + *badius* (=BADIUS-complex / PBD; Cole 1968) + MARICOPA-complex (PMR ; Cole 1968) (*maricopa* + *comanche* + *californicus*; loosened in the CytB-ML analysis); (4) HUACHUCANUS-group (PHA; new, named by the species at the base of this group, comprising all North-American *Pogonomyrmex sensu stricto* species) = *huachucanus* + PBR, POC, PCL; in the EF1a ML analysis PBR, POC and PCL species formed a single group, whereas *huachucanus* was grouped outside into the single polytomy; (5) RASTRATUS-complex (PRS; Kusnezov 1951) = *rastratus* / msp458 + *longibarbis* + *vermiculatus* + *brevibarbis* (representing the BREVIBARBIS-complex of Kusnezov 1951; grouped into *Ephebomyrmex* by Taber 1998); (6) LATICEPS-complex (PLT, new) = (*laticeps* + *pronotalis*) (both: formerly RASTRATUS-complex of Kusnezov 1951) + (*inermis* + *uruguayensis*) (representing the INERMIS-complex and the former BISPINOSUS-complex of Kusnezov 1951); (7) LOBATUS-group (PLB; new, comprising all South-American *Pogonomyrmex sensu stricto* species) = *lobatus* (representing the

COARCTATUS-complex of Kusnezov 1951) + *bispinosus* (formerly BISPINOSUS-complex of Kusnezov 1951) + PRS + PLT. Note: the PLB-group was not validated in the EF1a ML analysis; (8) LAEVINODIS-complex (ELE; new, comprising all North- and Middle-American *Pogonomyrmex Ephebomyrmex* species): (*laevinodis* + *saucius*) + (*pima* + *imberbiculus*); (9) NAEGELII-group (ENE, Kusnezov 1951): *naegelii* + *cunicularius* (formerly CUNICULARIUS-complex of Kusnezov 1951) + ELE. *P.* (*E.*) *odoratus* was the single representative of the ANGUSTUS-complex (Kusnezov 1951) and not placed within one of the above groups (from this species only the BEN/JERRY-fragment of the CoxI gene could be sequenced; see Tab. 2).

Gene trees representing the obtained pattern are provided for the Bayesian analyses (Fig. 1-5), and also for the ML-analyses of the subsets of CoxI (Fig. 6), CytB (Fig. 7) and EF1a (Fig. 8). Between the obtained gene-trees some species inconsistently grouped with others: (1) *P. (E.) cunicularius* mostly grouped into, or was associated to the ELE-complex (CoxI+CytB+EF1-a, CytB, EF-1a), but in two gene-trees (CoxI+CytB, CoxI) was found incorporated into PBR together with *P.* (*s.str.*) *bicolor* and *P.* (*s.str.*) *apache* (Fig. 1, 6), which in most analyses clustered together ("PBR-3"; except the ML analysis where *P. bicolor* was untied from the PBR-group in the CytB-ML analysis into the base of the North-American PHA-*sensu stricto*-group). The main cause for the inconsistent grouping of *P. cunicularius* might lie in long branch attraction of the CoxI-sequences. Therefore, the correct grouping of *P. cunicularius* was assumed to be within the other *Ephebomyrmex*-samples, as done first by Taber (1998). (2) The grouping of *P. (s.str.) snellingi* was ambiguous, as this species clustered either into group PHA (specifically PCL; CoxI, CoxI+CytB+EF1a; Fig. 1, 6, 5), or group ELE (EF1a; Fig. 3, 8); no CytB-gene sequence was obtained. Therefore, the results on the grouping of *snellingi* into either *Ephebomyrmex* or *sensu stricto* should be analyzed with additional genes. Morphologically, *P. snellingi* is classified as part of the subgenus *sensu stricto* (Taber 1998). (3) The North-American *Myrmica* outgroup species clustered for the CytB-gene-tree (Fig. 2, 7) into group PBR-1, whereas in all other analyses this species was grouped together with the *Myrmica rubra* samples from Europe, forming the outgroup to all other *Pogonomyrmex* samples. Unique sequence features might have led to this result, indicating problems in the sequencing of the CytB-fragment and therefore calling for caution in interpreting the obtained CytB gene-trees.

9.5 Discussion

For the gene-trees obtained in this analysis all complexes of Cole (1968) and some of Kusnezov (1951) could be validated, and additionally new groups / complexes were defined. Moreover, the morphological classification of *Pogonomyrmex* species into *sensu stricto* and *Ephebomyrmex* was secured in the gene trees, although a strict separation of these two (sub-) genera was not confirmed in all analyses. Solely *P.* (*E.*) *odoratus* as the single representative of the ANGUSTUS-complex (Kusnezov 1951) was not placed within one of the found groups (s. Results). This finding might indicate a basic split between at least some of the *Ephebomyrmex* species. However, for validation of the ANGUSTUS-complex also its other members, *P.* (*E.*) *angustus* and *P.* (*E.*) *laevigatus*, should be added into a subsequent genetic analysis.

The hypothesis of seeing *Ephebomyrmex* as a genus separate of *Pogonomyrmex* (Kusnezov 1959) was not disproved in our analysis. To finally resolve this problem a detailed and combined genetic and morphological analysis will be needed. In the phylogeny of Taber (1998, p.76) lineages throughout the genus *Pogonomyrmex* can be recognized explained by geographical isolation. The two subgenera *Ephebomyrmex* and *sensu stricto* were documented as being clearly distinct, like within the subgenus *sensu stricto* the North- and Middle-American members being separate from their South-American congeners. The same pattern could be recognized throughout the gene-trees obtained in the present analyses. Moreover, there were indications that this geographical separation also was found in the subgenus *Ephebomyrmex*. Therefore, we believe geographical separation is playing an important role in the evolution of the *Pogonomyrmex* harvester ants. The observed pattern is supporting an evolutionary scenario where geographical separation was following a morphological separation of *Ephebomyrmex* and *Pogonomyrmex sensu stricto*. Seemingly an early separation event was splitting up the ancestors of *Pogonomyrmex* into further separately evolving lineages. Consequently, the erection of a separate genus *Ephebomyrmex* seems to be justified and supported by our analyses.

The species groups found in our analyses were mostly comparable to the phylogenies of Taber (Taber 1990; Taber 1998), which are based on both, morphological and biological traits. Important differences were found in the groupings of certain species in our gene-trees: (1) *P. anzensis* was placed in the OCCIDENTALIS-complex, (2) *P. montanus* is closely related to *P. brevispinosus* and *P. subdentatus*, (3) *P. salinus* was consistently placed near *P. occidentalis* (see also Parker and Rissing, 2002), and (4) *P. snellingi* seemingly is not a close relative of *P. comanche* (Taber 1998, p.76), and only loosely associated to the CALIFORNICUS-complex (see also discussion above).

The second published *Pogonomyrmex*-phylogeny of Parker and Rissing (2002) was based on CytB-sequences and showed only minor deviations to our CytB-gene trees (Fig. 2, 7). Two differences were manifested by the gene trees implementing additional genes (1) in the grouping of *P. bicolor* into the BARBATUS-complex as close relative of *P. apache*, and (2) of *P. anzensis* into the OCCIDENTALIS-complex (Fig. 1).

An interesting result is that the sequences obtained from the specimen of *P. owyheei* and *P. salinus* showed differences in only 2 of the 1,473 analyzed base pairs, and no differences in the amino acid composition. Accordingly, both sequences always were clustered in the gene trees. This finding does agree with the statement that *P. owyheei* is a synonym of *P. salinus* (Shattuck 1987).

The most parsimonious explanations for the inconsistent grouping of *P. cunicularius*, *P. snellingi*, and *Myrmica sp.* between the obtained gene-trees might be unspecific primer-binding sites (possible for CoxI sequences; H. Feldhaar, *pers. obs.*) resulting in incorrect sequences, or specific sequencing of undetected pseudogenes / gene-duplicates. Future studies should address this problem in more detail.

An intermixing of *P. rugosus* and *P. barbatus* morphotypes was found across all gene trees, with at least two different strains among them. This result is similar to the gene-tree of Parker and Rissing (2002) and is explained by frequent hybridization between both species (Cole 1968; Ashe and Oldroyd 2002; Helms-Cahan et al. 2002; Julian et al. 2002; Parker and Rissing 2002; Volny and Gordon 2002; Helms-Cahan and Keller 2003; Parker 2004). Indications for possible hybridization between *P. maricopa* and *P. occidentalis* (Cole 1968; Anderson 2002) were not found in our gene trees.

This phylogenetic analysis was based on a successful sampling of 64% of the yet described species of the harvester ant genus *Pogonomyrmex*. Especially the sampling of the North- and Middle-American species was sufficient, as we here did include 84% of species. However, more specimens collected over a broad geographical sampling range will be needed for a more fine-scaled resolution of complexes, especially as hybridization might be a frequent event, and for finding possible geographical substructuring within species other than *P. badius* (Strehl and Gadau 2004). For these purposes the use of additional markers will be helpful, e.g. RFLP, RAPD, or microsatellites (e.g.: Julian et al. 2002; Volny and Gordon 2002; Gadau et al. 2003; Wiernasz et al. 2004).

The present phylogenetic reconstruction allows mapping certain life history and colony characteristics of *Pogonomyrmex* harvester ants to give possible explanations for the evolution of some of these traits. One issue is the high mating frequency of *Pogonomyrmex* (*s.str.*) queens: For ants unusual high numbers of mating of *Pogonomyrmex* ant queens might be a plesiomorphic attainment of the derived North-American *sensu stricto* complexes (Hölldobler 1976; Crozier and Pamilo 1996; Cole and Wiernasz 1999; Cole and Wiernasz 2000; Julian et al. 2002; Volny and Gordon 2002; Gadau et al. 2003; Rheindt et al. 2004; Wiernasz et al. 2004). Additionally, there are hints for multiple mating in South-American *sensu stricto* species (*P. pronotalis*: R. Pohl, *pers. comm.*) and for *P.* (*s.str.*) *huachucanus* (m_e=2, analyzed using microsatellites; J. Gadau and C.-P. Strehl, *unpublished*), a species basal to the North-American *sensu stricto* complex. The smaller effective mating number of queens in *P. huachucanus* compared to its *sensu stricto* relatives could mirror a change during the evolution of more advanced species. Possibly extreme high mating frequencies like those of *P. badius* (Rheindt et al. 2004) evolved in parallel with the invention of new colony and life-history characteristics, most impressive in the furious nest defense and pronounced territoriality in some species, often in line with high toxicity of their venoms (e.g. *P. maricopa*: Schmidt and Schmidt 1989).

As *Ephebomyrmex* species seem to be close relatives of the ancestors of all *sensu stricto* species, it finally is of importance to resolve the question if *Ephebomyrmex* representatives also show multiple mating of queens. First data on queen mating frequency in *Ephebomyrmex* for the North-American species *P.* (*E.*) *pima* disprove this idea as here single mating was found (m_e=1, analyzed using microsatellites; Strehl et al. in prep.). Therefore, multiple mating in *Pogonomyrmex* seems to be a trait invented during the evolution of the *sensu stricto* clade(s). This modern origin of queen multiple mating among *sensu stricto* branches (or the whole subgenus) will allow to test for proximate and ultimate causes triggering the evolution of multiple mating, e.g. male-male competition, female choice, parasite resistance, task allocation optimization, or lowering of the queen-worker kin conflict.

By adding additional species a detailed genus-wide phylogeny of *Pogonomyrmex* harvester ants could be made available, allowing two clear the (sub-)generic status of *Ephebomyrmex* and to map and discuss additional important features like within-colony relatedness e.g. (Rheindt et al. 2004; Wiernasz et al. 2004; Strehl et al. in prep.), pheromones e.g. (Ruano et al. 2000; Hölldobler et al. 2001; Liu and Liu 2002), or toxicity/venoms e.g. (Schmidt 1998).

9.6 Acknowledgments

For kindling providing us with specimen we would like to thank Alex Wild, Phil Ward, Kristina Ingram, Stefan Cover, Jerry L. Cook, Flavio Roces, and Joel Parker. Useful primers for sequencing were found by support of Ted R. Schultz, Miriam Brandt, Michaela Strätz and Riitta Savolainen. For technical support in the laboratory we thank K. Moeller. For support during our collecting trips we want to acknowledge Susanne Hoyer, Kirk and Belynda Anderson, Phil Ward, Jennifer Fewell, and the marvelous team at the Southwestern Research Station of the American Museum of Natural History. This study was supported by grants of the German Science Foundation (Deutsche Forschungsgemeinschaft / DFG), GA 661/1-2 and SFB 554-TP C-59.

9.7 Figures

The following figures are corresponding to gene-trees cited in the text (for sequence names: see table 2); numbers are corresponding to bootstrap values (ML-trees) or numbers of generations (Bayesian analyses).

Figure 1: Mr. Bayes-tree of CoxI-subset

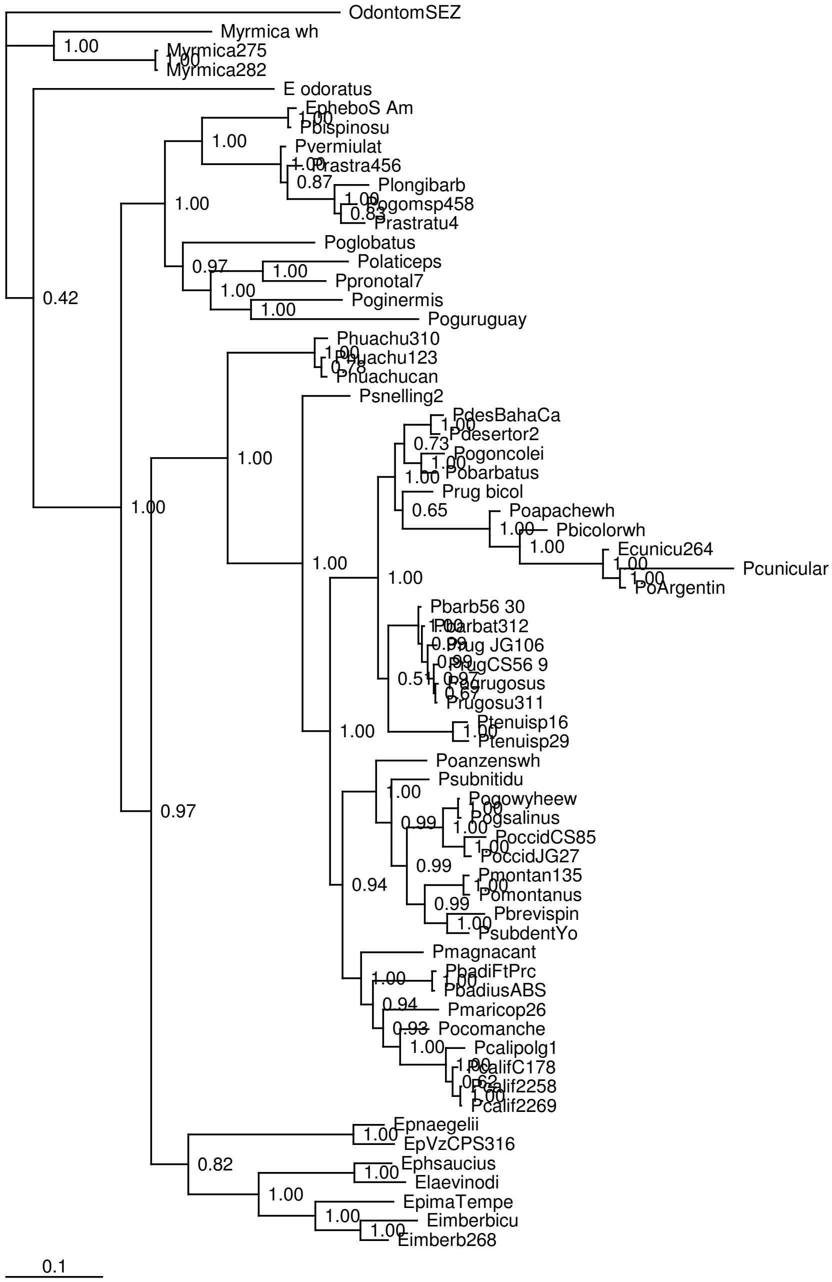

Figure 2:

Mr. Bayes-tree of CytB-subset

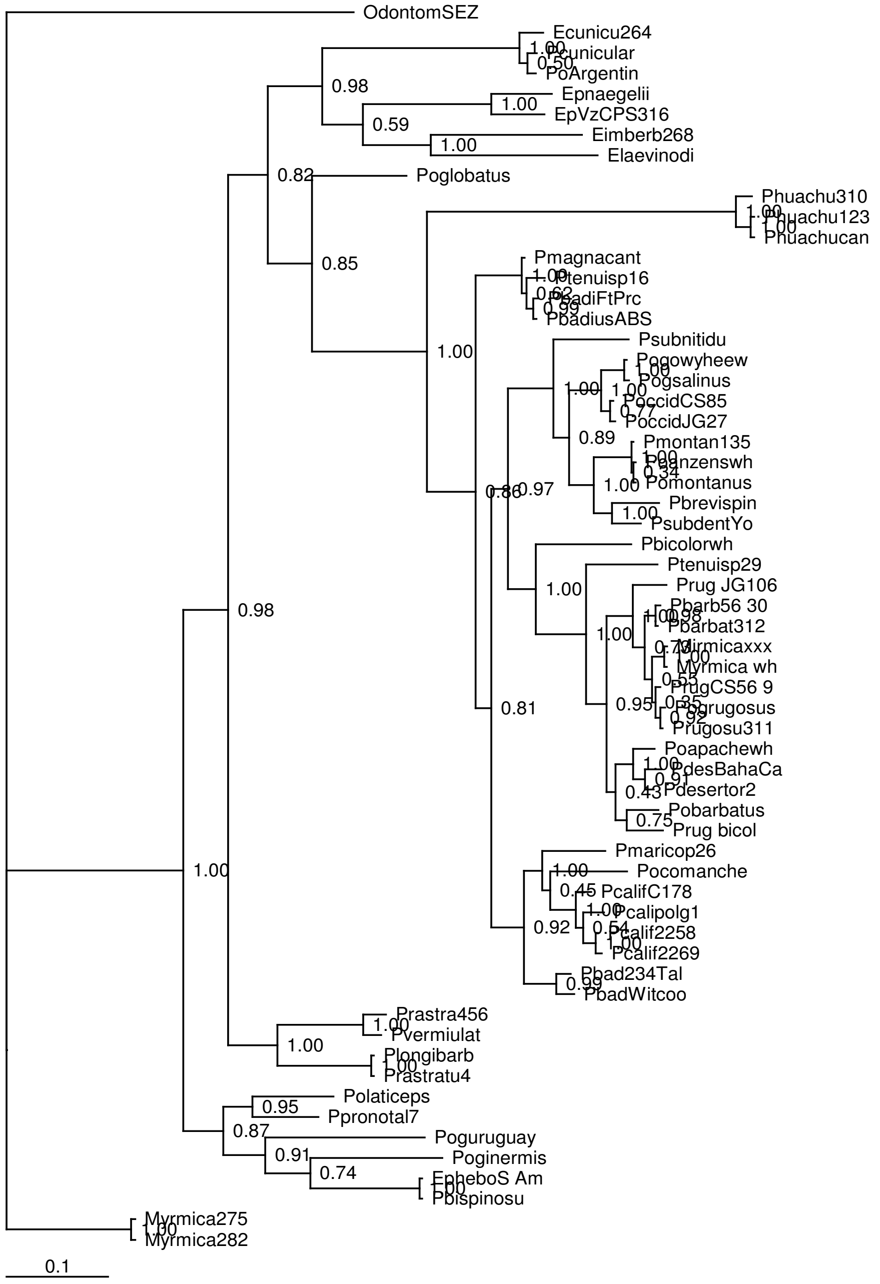

Figure 3:

Mr. Bayes-tree of EF1a-subset

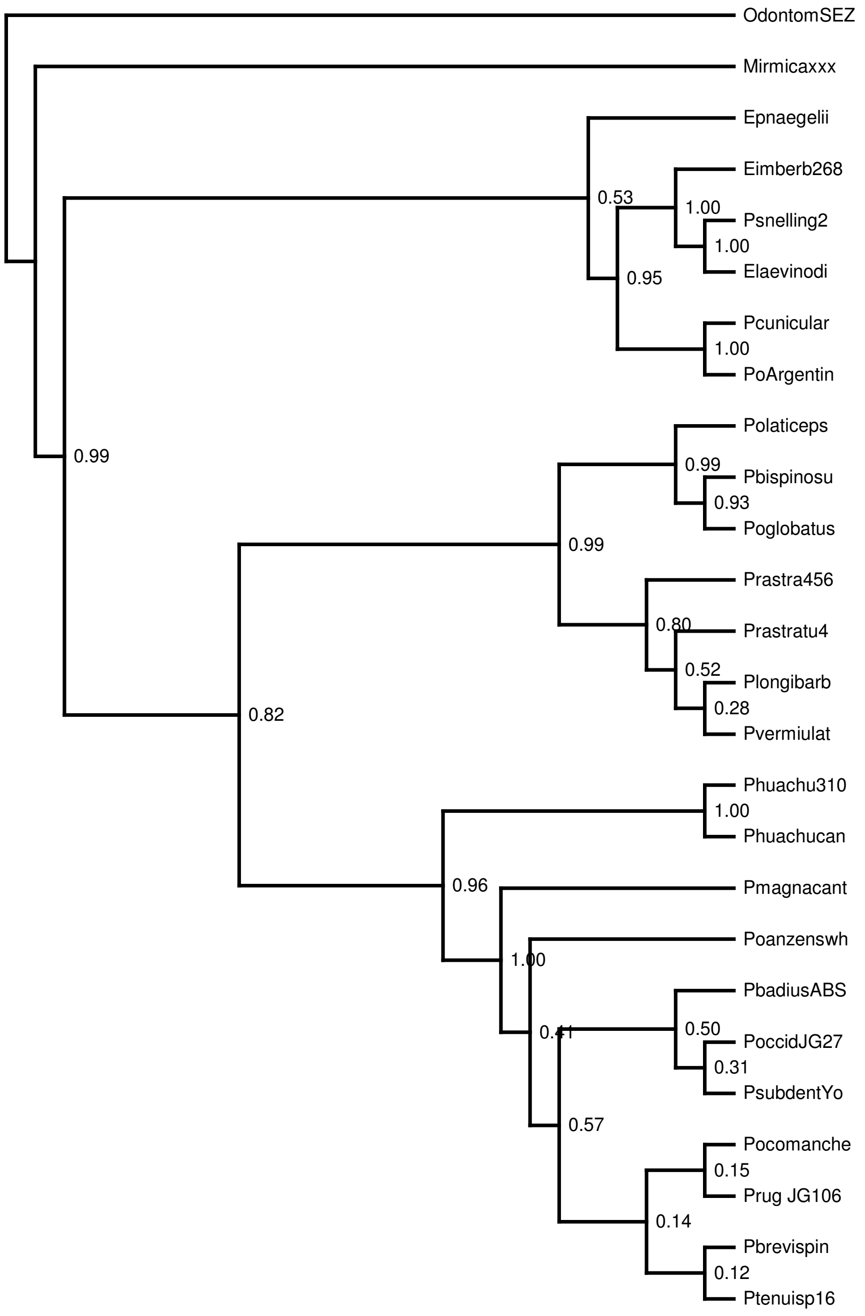

Figure 4

Mr. Bayes-tree of mtDNA-subset (CoxI plus CytB)

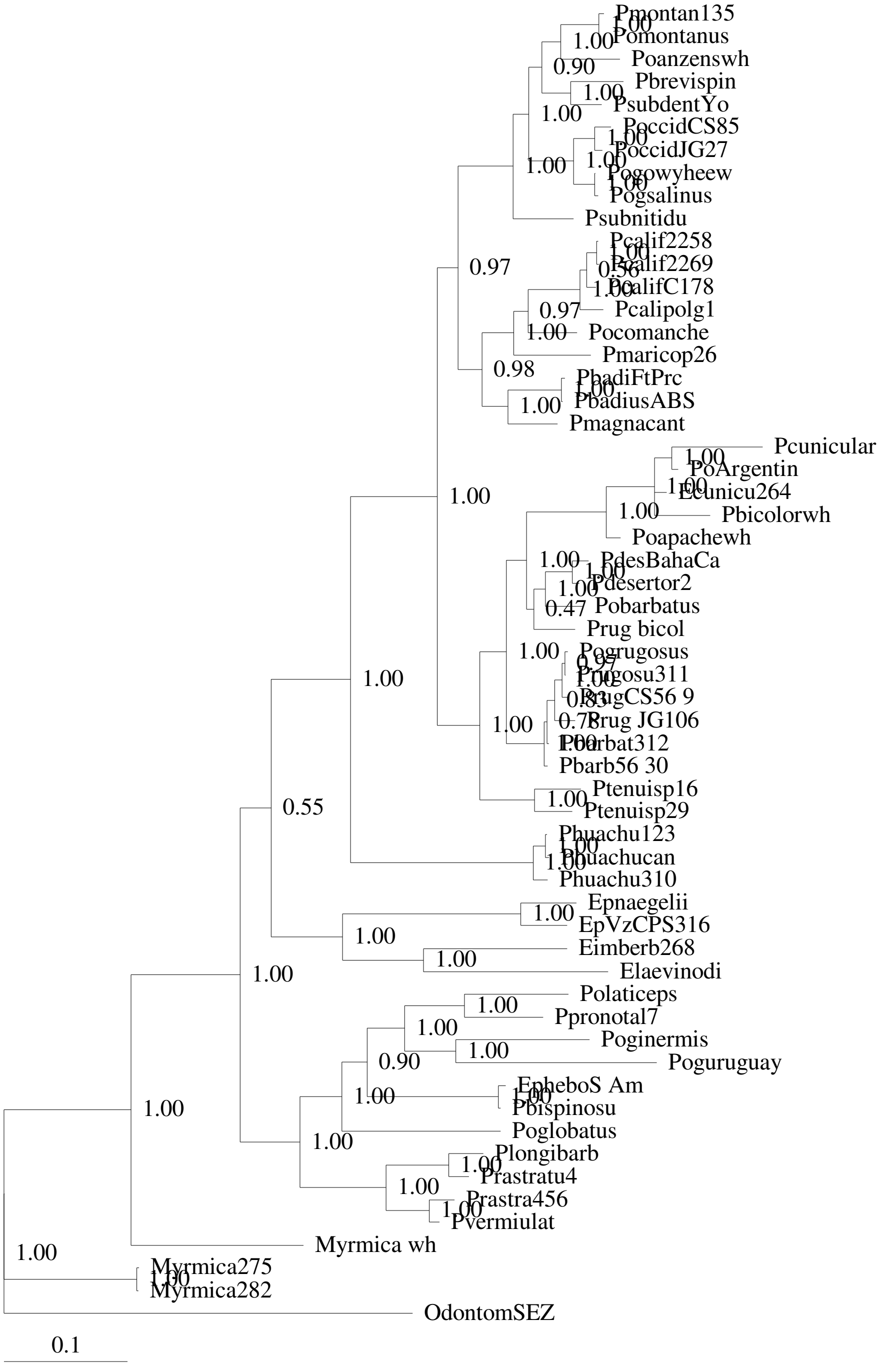

Figure 5

Mr. Bayes-tree for subset with all genes combined (CoxI plus CytB plus EF1a)

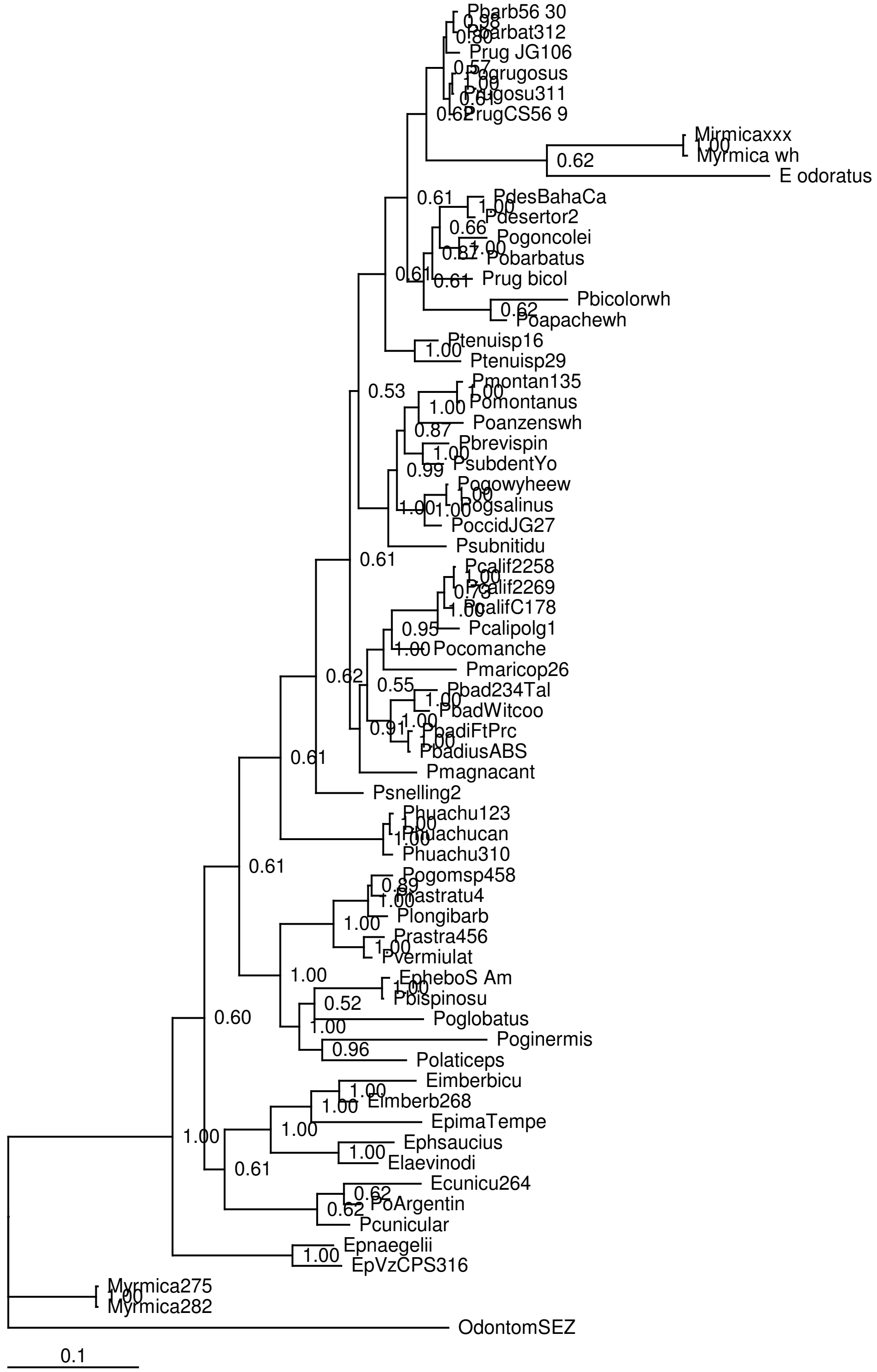

Figure 6 (following page): ML-tree for CoxI-subset

```
/-------------------------------------------------------------------------------------- OdontomSEZ
|                                                                              /------- Pogomsp458
|                                                                       /--56--+
|                                                                       |      \------- Prastratu4
|                                                               /--92---+
|                                                               |       \-------------- Plongibarb
|                                                        /--60--+
|                                                        |      \---------------------- Prastra456
|                                                 /-100--+
|                                                 |      \----------------------------- Pvermiulat
|                                          /--52--+
|                                          |      |                            /------- EpheboS Am
|                                          |      \------------100-------------+
|                                          |                                   \------- Pbispinosu
|                                          |
|                                          |                                   /------- Poginermis
|                                          |                            /--96--+
|             /-------------84-------------+                            |      \------- Poguruguay
|             |                            +-------------72-------------+
|             |                            |                            |      /------- Polaticeps
|             |                            |                            \--88--+
|             |                            |                                   \------- Ppronotal7
|             |                            |
|             |                            \------------------------------------------- Poglobatus
|             |
|             |              /--------------------------------------------------------- Psnelling2
|             |              |
|             |              |                                                 /------- Pogoncolei
|             |              |             /----------------88-----------------+
|             |              |             |                                   \------- Pobarbatus
|             |              |             |
|             |              |             |                            /-------------- Ecunicu264
|             |              |             |                            |
|             |              |             |                    /--76---+      /------- Pcunicular
|             |              |             |                    |       \--76--+
|             |              |             |             /--64--+              \------- PoArgentin
|             |              |             |             |      |
|             |              |             +-----64------+      \---------------------- Pbicolorwh
|             |              |             |             |
|             |              |             |             \----------------------------- Poapachewh
|             |              |             |
|             |              |             |      /------------------------------------ Pbarb56 30
|             |              |             |      |
|             |              |             |      |      /----------------------------- Pbarbat312
|             |              +-----60------+--96--+      |
|             |              |             |      |      |                     /------- Pogrugosus
|             |              |             |      |      |              /--72--+
|             |              |             |      \--60--+              |      \------- Prugosu311
|             |              |             |             |      /--83---+
|             |              |             |             |      |       \-------------- PrugCS56 9
|             |              |             |             \--83--+
|             |              |             |                    \---------------------- Prug JG106
|             |              |             |
|             |              |             |                                   /------- PdesBahaCa
|             |              |             +----------------100----------------+
|             |              |             |                                   \------- Pdesertor2
|             |              |             |
|             |              |             +------------------------------------------- Prug bicol
|             |              |             |
|             |              |             |                                   /------- Ptenuisp16
|             |              |             \----------------100----------------+
|             |      /--64---+                                                 \------- Ptenuisp29
|             |      |       |
|             |      |       |                                                 /------- PbadiFtPrc
|             |      |       |             /----------------100----------------+
|             |      |       |             |                                   \------- PbadiusABS
|             |      |       |             |
|             |      |       |             |                                   /------- Pcalif2258
|      /--96--+      |       |             |                            /-100--+
|      |      |      |       |             |                            |      \------- Pcalif2269
|      |      |      |       |      /--72--+                    /--54---+
|      |      |      |       |      |      |                    |       \-------------- PcalifC178
|      |      |      |       |      |      |             /-100--+
|      |      |      |       |      |      |             |      \---------------------- Pcalipolg1
|      |      |      |       |      |      |      /--72--+
|      |      |      |       +--68--+      |      |      \----------------------------- Pocomanche
|      |      |      |       |      |      \--69--+
|      |      |      |       |      |             \------------------------------------ Pmaricop26
|      |      |      |       |      |
|      |      |      |       |      \-------------------------------------------------- Pmagnacant
|      |      |      |       |
|      |      |      |       |                                                 /------- Pbrevispin
|      |      |      |       |                                          /--88--+
|      |      +-100--+       |                                          |      \------- PsubdentYo
|      |      |      |       |                                  /--88---+
|      |      |      |       |                                  |       |      /------- Pmontan135
|      |      |      |       |                                  |       \--96--+
|      |      |      |       |                                  |              \------- Pomontanus
|      |      |      |       |                           /--72--+
|      |      |      |       |                           |      |              /------- PoccidCS85
|      |      |      |       |                           |      |       /--96--+
|      |      |      |       |                           |      |       |      \------- PoccidJG27
|      |      |      |       |                           |      \--100--+
|      |      |      |       |                    /--80--+              |      /------- Pogowyheew
|      |      |      |       |                    |      |              \--88--+
+--64--+      |      |       |                    |      |                     \------- Pogsalinus
|      |      |      |       \---------84---------+      |
|      |      |      |                            |      \----------------------------- Psubnitidu
|      |      |      |                            |
|      |      |      |                            \------------------------------------ Poanzenswh
|      |      |      |
|      |      |      |                                                         /------- Phuachu123
|      |      |      |                                                  /--76--+
|      |      |      |                                                  |      \------- Phuachucan
|      |      |      \-----------------------100------------------------+
|      |      |                                                         \-------------- Phuachu310
|      |      |
|      |      |                                                                /------- Ephsaucius
|      |      |                                                 /-----100------+
|      |      |                                                 |              \------- Elaevinodi
|      |      |                                                 |
|      |      +-----------------------96------------------------+              /------- Eimberbicu
|      |      |                                                 |       /--64--+
|      |      |                                                 |       |      \------- Eimberb268
|      |      |                                                 \--68---+
|      |      |                                                         \-------------- EpimaTempe
|      |      |
|      |      |                                                                /------- Epnaegelii
|      |      \------------------------------100-------------------------------+
|      |                                                                       \------- EpVzCPS316
|      |
|      \------------------------------------------------------------------------------- E odoratus
|                                                                       /-------------- Myrmica wh
\----------------------------------86-----------------------------------+      /------- Myrmica275
                                                                        \-100--+
                                                                               \------- Myrmica282
```

Figure 7

ML-tree for CytB-subset

```
/-------------------------------------------------------------------------- OdontomSEZ
|
|                                                      /------------- Eimberb268
|                                          /-----88----+
|                                          |           \------------- Elaevinodi
|                      /---------66--------+
|                      |                   \-------------------------- Pcunicular
|                      |
|                      |                                    /------------- Myrmica wh
|                      |                                    |
|                      |                       /-----55----+------------- Pbarb56 30
|                      |                       |           |
|                      |           /-----86----+           \------------- Prugosu311
|                      |           |           |
|                      |           |           \---------------------- Prug JG106
|                      |           |
|                      |           |                       /------------- PdesBahaCa
|                      |           |           /-----65----+
|                      +-----87----+           |           \------------- Pdesertor2
|                      |           +-----72----+
|                      |           |           \---------------------- Poapachewh
|                      |           |
|                      |           +---------------------------------- Pobarbatus
|                      |           |
|                      |           \---------------------------------- Ptenuisp29
|                      |
|                      |                                   /------------- PbadiFtPrc
|                      |                       /-----77----+
|                      |                       |           \------------- PbadiusABS
|                      |           /-----53----+
|                      |           |           \---------------------- Pmagnacant
|                      +-----99----+
|                      |           \---------------------------------- Ptenuisp16
|                      |
|                      +---------------------------------------------- Pbicolorwh
|                      |
|           /----71----+                                   /------------- Pbrevispin
|           |          |                       /-----79----+
|           |          |                       |           \------------- PsubdentYo
|           |          |                       |
|           |          |           /-----63----+           /------------- Pmontan135
|           |          |           |           |           |
|           |          |           |           \-----99----+------------- Poanzenswh
|           |          |           |                       |
|           |          |           |                       \------------- Pomontanus
|           |          |           |
|           |          |           |                       /------------- PoccidCS85
|           |          +-----56----+           /-----73----+
|           |          |           |           |           \------------- PoccidJG27
|           |          |           +-----81----+
|           |          |           |           |           /------------- Pogowyheew
|           |          |           |           \-----85----+
+-----97----+          |           |                       \------------- Pogsalinus
|           |          |           |
|           |          |           \---------------------------------- Psubnitidu
|           |          |
|           |          +- -------------------------------------------- Pcalif2258
|           |          |
|           |          + -------------------------------------------- PcalifC178
|           |          |
|           |          |                               /------------- Phuachu123
|           |          +--------------100--------------+
|           |          |                               \------------- Phuachu310
|           |          |
|           |          +---------------------------------------------- Pmaricop26
|           |          |
|           |          \---------------------------------------------- Pocomanche
|           |
|           \--------------------------------------------------------- Pbispinosu
|
|                                                  /------------- Myrmica275
\------------------------100-----------------------+
                                                   \------------- Myrmica282
```

Figure 8:

ML-tree for EF1a-subset

```
/-------------------------------------------------------------------------------------- OdontomSEZ
|
|                                                               /---------------------- Psnelling2
|                                          /---------74---------+
|                                          |                    \---------------------- Elaevinodi
|                    /---------76----------+
|                    |                     \------------------------------------------- Eimberb268
+---------59---------+
|                    |                                          /---------------------- Pcunicular
|                    \--------------------96--------------------+
|                                                               \---------------------- PoArgentin
|
+-------------------------------------------------------------------------------------- Epnaegelii
|
+-------------------------------------------------------------------------------------- Mirmicaxxx
|
|                                                               /---------------------- PbadiusABS
|                                                               |
|                                                               +---------------------- Pbrevispin
|                                                               |
|                                                               +---------------------- Pmagnacant
|                                                               |
|                                                               +---------------------- Poanzenswh
|                                                               |
+------------------------------80-------------------------------+---------------------- PoccidJG27
|                                                               |
|                                                               +---------------------- Pocomanche
|                                                               |
|                                                               +---------------------- Prug JG106
|                                                               |
|                                                               +---------------------- PsubdentYo
|                                                               |
|                                                               \---------------------- Ptenuisp16
|
|                                                               /---------------------- Pbispinosu
|                                          /---------77---------+
|                                          |                    \---------------------- Poglobatus
+--------------------67--------------------+
|                                          \------------------------------------------- Polaticeps
|
|                                                               /---------------------- Phuachu310
+------------------------------100------------------------------+
|                                                               \---------------------- Phuachucan
|
|                                                               /---------------------- Plongibarb
|                                                               |
|                                                               +---------------------- Prastra456
\------------------------------63-------------------------------+
                                                                +---------------------- Prastratu4
                                                                |
                                                                \---------------------- Pvermiulat
```

9.8 Tables

(see following pages)

Gene	Primer name	sequence (5' to 3' direction)		Reference
CoxI	LCO (sense)	GGTCAACAAATCATAAAGATATTGG	[1835; NC_001566.1]	A
	HCO (anti-sense)	TAAACTTCAGGGTGACCAAAAAATCA	[2492; NC_001566.1]	A
	Jerry (sense)	CAACATTTATTTTGATTTTTT	[2502; NC_001566.1]	B
	Ben3R (anti-sense)	GC(AT)AC(AT)AC(AG)TAATA(GT)GTATCATG	[2888; NC_001566.1]	C
CytB	CB1 (sense)	TATGTACTACCATGAGGACAAATATC	[11426; NC_001566.1]	D
	CB2 (anti-sense)	ATTACACCTCCTAATTTATTAGGAAT	[11858; NC_001566.1]	E
EF-1a	U114 (sense)	AAGTACGCTTGGGTGTTGGT(CT)AA	[550; X52884] [547; AF015267]	F
	Uant377 (sense)	TGGGCGTGAAGCAGCTGAT	[811; X52884] [808; AF015267]	F
	CHO6trs (anti-sense)	GCTTC(AG)TGGTGCAG(CT)TC(AGCT)AC	[1456; X52884] [1474; AF015267]	G
	TRS1R (anti-sense)	ACCTGGTTTAAGAT(AG)CCGGT	[1168; X52884]	F
	TRS2F (sense)	ATTTTAAGGTATGTTGGTAACTT	[1422; X52884] [1440; AF015267]	F
	TRS10R (anti-sense)	ACGGC(GC)AC(GT)GTTTG(AT)C(GT)CATGTC	[1864; X52884] [2162; AF015267]	F

Table 1 (Sequencing-primers used in this study):

Numbers and GeneBank-accession numbers (Walldorf and Hovemann 1990; Crozier and Crozier 1993; Danforth and Ji 1998) following the 3' end of each primer refer to the next nucleotide positions relative to sequences of *Apis mellifera*. Degenerate positions of primer-sequences are placed within brackets.

References:

A	Folmer et al. 1994
B	Modified bee-primer Ca-J-2183 of (Simon et al. 1994)
C	Brady et al. 2000
D	Bee-primer CB-J-10933 of Simon *et al.*(1994)
E	Bee-primer CB-N-11367 of Simon *et al.*(1994)
F	Designed by Ted R. Schultz (*unpublished*)
G	Primer rcM52.6 of (Cho et al. 1995), modified by Bryan Danforth and Ted R. Schultz (unpublished)

Table 2 (following three pages):

Samples of *Pogonomyrmex* species used in this study, and of *Myrmica* and *Odontomachus* outgroup species respectively. All sequences submitted to GenBank are listed. Headings: "(Sub-)genus" = suspected classification of the species into one of the *Pogonomyrmex* subgenera *Ephebomyrmex* or *Pogonomyrmex* following Cole (1968), Taber (1998) and Kusnezov (1951), and of the outgroup genera *Myrmica* and *Odontomachus* respectively; "species" = suspected species name; "sequence" = name of the sequence used in the phylogenetic analyses; "gene" = abbreviations of those genes where successfully amplicons where obtained (CoxI = mitochondrial gene Cytochrome c Oxidase I; CytB = mitochondrial gene Cytochrome b; EF1a = nuclear gene Elongation Factor 1 alpha, F1-copy); "specimen" = names and accession numbers of the voucher specimen collected by ALW (Alex L. Wild), CPS (Christoph-P. Strehl), JDP (Joel D. Parker), JG (Juergen Gadau), JLC (Jerry L. Cook), KI (Kristina Ingram), PSW (Phil S. Ward), RAJ (Robert A. Johnson), and SPC (Stefan P. Cover); "locality" = area where the specimen was collected (if known); "GenBank acc. no." = number by which the listed sequence is accessible via internet at GenBank.

(sub-)genus	**species**	**sequence**	**gene**	**specimen**	**locality**	**GenBank acc. no.**
Pogonomyrmex	anergismus	Panergismu	CoxI	RAJ1546 / CPS289	n.a.	AY510629
Ephebomyrmex	angustus	Poangustus	CoxI	PSW12719	S-America	AY510607, AY510609
Pogonomyrmex	anzensis	Poanzensis	CoxI, CytB, EF1a	SPC4807	Anza (CA)	AY510665, AY552483, AY651891
Pogonomyrmex	apache	Papache267	CoxI, CytB	CPS267	Portal (AZ)	AY510630, AY552495
Pogonomyrmex	apache	Pogoapache	CoxI	RAJ#AZ1037	Portal (AZ)	AY510672
Pogonomyrmex	badius	PbadiusBEN	CoxI	CPS125 (from RAJ)	n.a.	AY510637
Pogonomyrmex	badius	PbadiusABS	CoxI, CytB, EF1a	CPS199	LkPlacid (FL)	AY510636, AY538614, AY651892
Pogonomyrmex	badius	PbadWitcoo	CoxI, CytB	CPS200/8	Withlacoochee (FL)	AY510633, AY538616
Pogonomyrmex	badius	PbadiOcala	CoxI, CytB	CPS201	OcalaNtlPk (FL)	AY510635, AY538619
Pogonomyrmex	badius	PbadiFtPrc	CoxI, CytB	CPS203	FtPierce (FL)	AY510638, AY538621
Pogonomyrmex	badius	PbadLkPlac	CoxI, CytB	CPS204	LkPlacid (FL)	AY510634, AY538615
Pogonomyrmex	badius	Pbad230Tal	CoxI, CytB	CPS230	Tallahassee (FL)	AY510631, AY538618
Pogonomyrmex	badius	Pbad234Tal	CoxI, CytB	CPS234	Tallahassee (FL)	AY510632, AY538617
Pogonomyrmex	barbatus	PbarbCS236	CoxI	CPS236	Portal (AZ)	AY510642
Pogonomyrmex	barbatus	Pbarbat312	CoxI, CytB	CPS312	Sierra Ancha (AZ)	AY510640, AY552497
Pogonomyrmex	barbatus	Pobarbatu2	CoxI	CPS5	Tombstone (AZ)	AY510643
Pogonomyrmex	barbatus	Pbarb56-30	CoxI, CytB	CPS56-30	Phoenix (AZ)	AY510639, AY538620
Pogonomyrmex	barbatus	Pobarbatus	CoxI, CytB	JDP233	Tarrant (TX)	AY510641, AY552496
Pogonomyrmex	bicolor	Pogbicolor	CoxI, CytB	RAJ#SON1025	Sonora/Mexico	AY510644, AY552498
Pogonomyrmex	bispinosus	Pbispinosu	CoxI, CytB, EF1a	CPS260	Laguna Verde/Chile	AY510645, AY552499, AY651893
Pogonomyrmex	brevibarbis	Pbrevib457	CoxI, EF1a	"P. brevibarbis 457" (from RAJ and JG)	Argentina	AY646359, AY651890
Pogonomyrmex	brevispinosus	Pbrevispin	CoxI, CytB, EF1a	JG154	Wasco (CA)	AY510646, AY552500, AY651894
Pogonomyrmex	californicus	Pcalif2258	CoxI, CytB	"2258" (from RAJ)	Baha Californica (MX)	AY510647, AY552475
Pogonomyrmex	californicus	Pcalif2269	CoxI, CytB	"2269" (from RAJ)	Baha Californica (MX)	AY510649, AY538625
Pogonomyrmex	californicus	Pcalipolg1	CoxI, CytB	CPS121 (from ASU)	California	AY510651, AY552477
Pogonomyrmex	californicus	PcalifC178	CoxI, CytB	CPS178 (from RAJ)	SaltRiver (AZ)	AY510648, AY552476
Pogonomyrmex	californicus	Pogoncalin	CoxI	PSW13114	SandSpringDunes (NV)	AY510674
Pogonomyrmex	californicus	Pcaliforni	CoxI	RAJ#AZ1719	LaPaz (AZ)	AY510650
Pogonomyrmex	colei	Pogoncolei	CoxI	RAJ1001 / CPS287	n.a.	AY510671
Pogonomyrmex	comanche	Pocomanche	CoxI, CytB, EF1a	CPS174 (from JLCook)	Texas	AY510668, AY552501, AY651895
Ephebomyrmex	cunicularius	Pcunicular	CoxI, CytB, EF1a	CPS154 (from KI)	S-America	AY510652, AY552502, AY651896
Ephebomyrmex	cunicularius	Ecunicu264	CoxI, CytB	CPS264 (from ALW)	Paraguay	AY510613, AY552503
Pogonomyrmex	desertorum	PdesBahaCa	CoxI, CytB	CPS337	Tempe (AZ)	AY510653, AY552505
Pogonomyrmex	desertorum	Pdesertoru	CoxI	CPS4	Portal (AZ)	AY510655
Pogonomyrmex	desertorum	Pdesertor2	CoxI, CytB	JG72	Portal (AZ)	AY510654, AY552504
Pogonomyrmex	huachucanus	Phuachu123	CoxI, CytB	CPS123	Portal (AZ)	AY510657, AY538623

Pogonomyrmex	huachucanus	Phuachucan	CoxI, CytB, EF1a	CPS3	Portal (AZ)	AY510658, AY552507, AY651897
Pogonomyrmex	huachucanus	Phuachu310	CoxI, CytB, EF1a	CPS310	Sierra Ancha (AZ)	AY510656, AY552506, AY651898
Ephebomyrmex	imberbiculus	Pimberbicu	CoxI	CPS2	Superior (AZ)	AY510617
Ephebomyrmex	imberbiculus	Eimberbicu	CoxI	CPS2	Superior (AZ)	AY510616
Ephebomyrmex	imberbiculus	Eimberb268	CoxI, CytB, EF1a	CPS268	Portal (AZ)	AY510614, AY538624, AY651899
Ephebomyrmex	imberbiculus	Eimberb313	CoxI, EF1a	CPS313	Sierra Ancha (AZ)	AY510615, AY651900
Pogonomyrmex	inermis	Poinermis1	CoxI, CytB	"P. inermis 1" (from RAJ and JG)	Argentina	AY646361, AY552522
Pogonomyrmex	inermis	Poinermis2	CoxI	"P. inermis 2" (from RAJ and JG)	Argentina	AY646360
Ephebomyrmex	laevinodis	Elaevinodi	CoxI, CytB, EF1a	RAJ2679	Baha Californica (MX)	AY510618, AY552508, AY651901
Ephebomyrmex	laevinodis	Elaevinowh	CoxI	RAJ2679-w.h.	Baha Californica (MX)	AY510619
Pogonomyrmex	laticeps	Polaticeps	CoxI, CytB, EF1a	"1227" (from RAJ)	Salta/Argentina	AY510660, AY552509, AY651902
Pogonomyrmex	laticeps	Polaticeps	CoxI	CPS155 (from KI)	S-America	AY510661
Pogonomyrmex	lobatus	Poglobatus	CoxI, CytB, EF1a	CPS266 (from ALW)	Paraguay	AY510670, AY552510, AY651903
Pogonomyrmex	longibarbis	Plongibarb	CoxI, CytB, EF1a	"1220" (from RAJ)	Salta/Argentina	AY510662, AY552511, AY651904
Pogonomyrmex	magnacanthus	Pmagnacant	CoxI, CytB, EF1a	RAJ#CAL1006	Riverside (CA)	AY510663, AY552487, AY651905
Pogonomyrmex	maricopa	Pmaricop26	CoxI, CytB	CPS26	St. Xavier (AZ)	AY510664, AY552512
Pogonomyrmex	maricopa	Pomaricopa	CoxI	RAJ#AZ902	Superior (AZ)	AY510678,
Pogonomyrmex	montanus	Pomontanus	CoxI, CytB	CPS135	SanBernardino (CA)	AY552488, AY510680
Pogonomyrmex	montanus	Pomontanus	CoxI, CytB	JG133	SanBernardino (CA)	AY510679, AY552489
Myrmica	rubra	Myrmica275	CoxI, CytB	CPS275	Nuremberg (GER)	AY510626, AY552481
Myrmica	rubra	Myrmica282	CoxI, CytB	CPS282	Neustadt/Aisch (GER)	AY510627, AY552480
Myrmica	sp.	Mirmicaxxx	CoxI, CytB, EF1a	RAJ2132	USA	AY510625, AY552486, AY651887
Myrmica	sp.	Myrmica_wh	CoxI	RAJ2132	USA	AY510628,
Ephebomyrmex	naegelii	Epnaegelii	CoxI, CytB, EF1a	"E. naegelii 401" (from RAJ and JG)	S-America	AY646357, AY647221, AY651915
Pogonomyrmex	occidentalis	PoccidCS85	CoxI, CytB	CPS85	Portal (AZ)	AY510608, AY552484
Pogonomyrmex	occidentalis	Pooccident	CoxI	JDP195	Boulder (CO)	AY510681
Pogonomyrmex	occidentalis	PoccidJG27	CoxI, CytB, EF1a	JG27	Seligman (AZ)	AY510667, AY538622, AY651906
Odontomachus	sp.	OdontomSEZ	CoxI. CytB, EF1a	CPS172	Mahé/Seychelles	AY510606, AY552485, AY651888
Ephebomyrmex	odoratus	E_odoratus	CoxI	PSW12731	Argentina	AY510624
Ephebomyrmex	pima	pima208m11	CoxI	CPS208	Tempe (AZ)	AY515288
Ephebomyrmex	pima	pima210_m7	CoxI	CPS210	Tempe (AZ)	AY515289
Ephebomyrmex	pima	pima219m20	CoxI	CPS219	Tempe (AZ)	AY515290
Ephebomyrmex	pima	pima220_m6	CoxI	CPS220	Tempe (AZ)	AY515285
Ephebomyrmex	pima	pima220_f1	CoxI	CPS220	Tempe (AZ)	AY515286
Ephebomyrmex	pima	EpimaTempe	CoxI	CPS222	Tempe (AZ)	AY510623
Ephebomyrmex	pima	pima225_f7	CoxI	CPS225	Tempe (AZ)	AY515291
Ephebomyrmex	pima	Epima226_L	CoxI, EF1a	CPS226	Tempe (AZ)	AY510622, AY651908
Ephebomyrmex	pima	PogEphpima	CoxI	JG48	Tempe (AZ)	AY510669
Ephebomyrmex	pima	pima6258AZ	CoxI	RAJ6258AZ (from SPC)	Yavapai (AZ)	AY510659

Pogonomyrmex	pronotalis	Ppronotal7	CoxI, CytB, EF1a	"P. pronotalis 7" (from RAJ and JG)	Argentina	AY646362, AY552523, AY651907
Pogonomyrmex	rastratus	Prastratu4	CoxI, CytB, EF1a	"P. rastratus 4"	Argentina	AY646363, AY647220, AY651916
Pogonomyrmex	rastratus	Prastra456	CoxI, CytB, EF1a	"P. rastratus 456"	Argentina	AY646364, AY647222, AY651917
Pogonomyrmex	rugosus	PrugoRmale	CoxI	CPS126 (from RAJ)	Phoenix (AZ)	AY510686
Pogonomyrmex	rugosus	PrugoBmale	CoxI	CPS127 (from RAJ)	Navajo (AZ)	AY510685
Pogonomyrmex	rugosus	Pogrugosus	CoxI, CytB	CPS151 (from RAJ)	n.a.	AY510676, AY552513
Pogonomyrmex	rugosus	PrugoFlats	CoxI	CPS235	Portal (AZ)	AY510691
Pogonomyrmex	rugosus	PrugStLnRd	CoxI	CPS238	Rodeo (NM)	AY510692
Pogonomyrmex	rugosus	Prugosu311	CoxI, CytB	CPS311	Sierra Ancha (AZ)	AY510688, AY552479
Pogonomyrmex	rugosus	PrugC56-18	CoxI	CPS56-18	Phoenix (AZ)	AY510684
Pogonomyrmex	rugosus	PrugCS56-9	CoxI, CytB	CPS56-9	Phoenix (AZ)	AY510690, AY552478
Pogonomyrmex	rugosus	PrugTucson	CoxI	CPS6	Tempe (AZ)	AY510687
Pogonomyrmex	rugosus	Prug-9whSL	CoxI	JG106	Tucson (AZ)	AY510683
Pogonomyrmex	rugosus	Prug-JG106	CoxI, CytB, EF1a	JG106	Tucson (AZ)	AY510689, AY552515, AY651909
Pogonomyrmex	rugosus	Prug-bicol	CoxI, CytB	JG97	Portal (AZ)	AY510693, AY552514
Pogonomyrmex	rugosus	18Cornelia	CoxI	PR10M1 (Hybrids)	Navajo (AZ)	AY510612
Pogonomyrmex	salinus	Pogsalinus	CoxI, CytB	JDP197	Oneide (ID)	AY510677, AY552490
Pogonomyrmex	salinus	Pogowyheei	CytB	PSW14121	Nevada	AY552491
Pogonomyrmex	salinus	Pogowyheew	CoxI, CytB	PSW14121	Nevada	AY510610, AY552492
Ephebomyrmex	saucius	Ephsaucius	CoxI	PSW11730-3	Dominican Republic	AY510621
Pogonomyrmex	snellingi	Psnelling2	CoxI, EF1a	RAJ2663	n.a.	AY510611, AY651910
Pogonomyrmex	subdentatus	Pogo_subdn	CoxI	PSW13540?	AY510675	
Pogonomyrmex	subdentatus	Pogosubdnn	CoxI	PSW13540	n.a.	AY510673
Pogonomyrmex	subdentatus	PsubdentYo	CoxI, CytB, EF1a	JG156	Yosemite (CA)	AY510696, AY552516, AY651911
Pogonomyrmex	subdentatus	Psubdensym	CoxI	PSW14124	Nevada	AY510695
Pogonomyrmex	subnitidus	Psubnitidu	CoxI, CytB	RAJ#CAL1043	Los Angeles (CA)	AY510697, AY552482
Pogonomyrmex	tenuispina	Ptenuisp16	CoxI, CytB, EF1a	RAJ#BC96-16	Baha Californica (MX)	AY510698, AY552518, AY651912
Pogonomyrmex	tenuispina	Ptenuisp29	CoxI, CytB	RAJ#BCS96-29A	Baha Californica (MX)	AY510699, AY552517
Ephebomyrmex	undet.	EpheboS-Am	CoxI, CytB	"1201(I)"	S-America	AY510620, AY552493
Pogonomyrmex	undet.	Pogomsp458	CoxI	"P. msp 458"	Argentina	AY646365
Pogonomyrmex	undet.	PoArgentin	CoxI, CytB, EF1a	CPS229	Argentina	AY510666, AY552494, AY651889
Ephebomyrmex	undet.	EpVzCPS316	CoxI, CytB	CPS316	Venezuela	AY646358, AY552521
Pogonomyrmex	uruguayensis	Poguruguay	CoxI, CytB, EF1a	CPS265 (from ALW)	Paraguay	AY510694, AY552519, AY651913
Pogonomyrmex	vermiculatus	Pvermiulat	CoxI, CytB, EF1a	"1234" (from RAJ)	Antofagasta/Chile	AY510700, AY552520, AY651914

9.9 Literature

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10. General Discussion

Effective multiple mating was demonstrated for *Pogonomyrmex rugosus* and *P. badius* using molecular markers (Gadau et al. 2003; Rheindt et al. 2004), corroborating behavioral observations of matings at the leks (Hölldobler and Wilson 1990). By their highly promiscuous mating behavior females produce an effectively lowered intracolonial relatedness, which in turn may positively affect colony organization (Cole and Wiernasz 1999; Wiernasz et al. 2004). For instance, morphological polymorphisms have an impact on the organization of colony members and so are important characters influencing the efficiency of whole colonies, e.g. in task performance (Davidson 1977) or reproduction (Wiernasz et al. 1995). It has been shown in *Solenopsis geminata* that worker polymorphism leads to a division of labor, i.e. majors always become seed millers (Wilson 1978). A similar effect might be assumed for the highly multiple mated (Rheindt et al. 2004) *Pogonomyrmex badius*. As in *P. badius* worker polymorphism is non-randomly distributed throughout the patrilines (Rheindt et al. 2005), an improved colonial organization and efficiency may favor polyandry in this species. The demonstrated selective advantage of low relatedness in *P. occidentalis* (Cole and Wiernasz 1999; Wiernasz et al. 2004) might be similarly supported. Thus probably yet undetected, genetically mediated behavioral polyethisms might be found in other *Pogonomyrmex* species. Genetically influenced worker polyethism has already been shown for the honeybee *Apis mellifera*, another highly polyandrous eusocial species (Oldroyd et al. 1993; Fuchs and Moritz 1998; Haberl and Tautz 1998; Page and Erber 2002; Pankiw et al. 2002; Fewell 2003).

Observations of interspecific slavery between the two sister taxa *P.* (*s.str.*) *barbatus* and *P.* (*s.str.*) *rugosus* (Hölldobler and Markl 1989) had previously been neglected, and the possibility of intraspecific slavery has up to now not been considered as an adaptive strategy for *P. rugosus* (Gadau et al. 2003). Brood raids together with incorporation of raided brood into the workforce of colonies can increase genetic variability (Kronauer et al. 2003). Intraspecific slavery was confirmed in *P. rugosus*, and therefore occasional brood raids represent an additional determinant of intracolonial relatedness (Gadau et al. 2003). As colonies of *Pogonomyrmex* are highly territorial (Hölldobler 1974; Hölldobler 1976b), "drifting" workers (Paxton 2000; Sanetra and Crozier 2001) are an unlikely event for additionally increasing the genetic variability of colonies. Therefore, beside polyandry, the incorporation of raided individuals might increase "the selective advantage of low relatedness" (Cole and Wiernasz 1999) in *Pogonomyrmex*.

Brood raiding presumably is advantageous especially during the founding stages, as it is not only increasing colony efficiency via an increased genetic variability, but also increasing colony size, and thus robustness during its most vulnerable phase. Assuming costs involved for raiding established colonies, this might be a rarely observed event and predominantly directed towards considerably smaller colonies. Probably mostly founding colonies might suffer from brood raiding, as own field observations (years 2000/2003, and 2003/2004) at Coon Bluff Rec. Area (chapter 2, Fig. 1) showed. Here the numbers of *P. rugosus* founding colonies were significantly reduced, with maximally 1 colony of originally 1,000 colonies remaining. Interestingly, in *P. barbatus* decreased mortality of nests was found in the presence of small neighboring nests (Gordon and Kulig 1998). Thus older colonies may take advantage of an accumulating incorporation of raided foreign genetic material from younger nests, because of increased genetic variability (Cole and Wiernasz 1999), decreased resource competition, and additional work force.

It was also shown for *Pogonomyrmex rugosus* and *P. badius*, that there are significant differences among the mates of a queen in their contribution to the female offspring (Gadau et al. 2003; Rheindt et al. 2004), and that this might be correlated with differences between the mates in copulation time (Hölldobler 1976a). Males, especially the first mating male, seem to prevent longer lasting copulations of others and therefore male-male competition might be assumed, e.g shaping male size (Davidson 1982; Abell et al. 1999). However, as transmission of genes is depending on colonies with genetically variable workforce, selection might drive males to join successful patrilineal combinations and spread their genes into as many future colonies as possible. Therefore, these males probably would act *against* those males who prevent female multiple mating. This trade-off might also be found in other ant genera, e.g. army ants, where big, wasp-like males are known, too (Hölldobler and Wilson 1990).

Pogonomyrmex females on the other hand should be reluctant to mate, because this increases the competition between males and might increase the probability for females to mate with the best, i.e. most competitive male(s). This is different from the "convenience"-hypothesis for the evolution of multiple mating (Alcock et al. 1978) stating a forced acceptance of additional copulations. Therefore, mating behavior in the ant genus *Pogonomyrmex* might have been proximately shaped by male-male competition and ultimately by cryptic female choice.

Polyandry might enable females to exploit postcopulatory mechanisms for reducing the risk and/or cost of fertilization by genetically incompatible sperm (Newcomer et al. 1999), or of producing diploid males due to inbreeding (Page 1980). Validity of these hypotheses might be found in the mating behaviors of *P. badius* (Van Pelt 1953; Harmon 1993), *P. barbatus* and *P. rugosus* (Hölldobler 1976a; Julian et al. 2002). In *P. badius* mating behavior produces the risk of inbreeding (Strehl and Gadau 2004), possibly opposed by one of the most extreme polyandrous systems among ants (Rheindt et al. 2004). In hybridizing *P. barbatus* x *rugosus* the incompatibility risk is minimized via a genetic caste determination system (Julian et al. 2002; Helms-Cahan and Keller 2003). In this system heterospecific individuals are exploited as the colonies workforce, thus probably producing a strong selective pressure in favor of polyandry due to the need for accumulating different genetic lineages (Volny and Gordon 2002).

As predicted by the genetic variability hypotheses, polyandry is less common among polygynous than monogynous species (Keller and Reeve 1994), but see: (Schmid-Hempel and Crozier 1999). Probably in accordance with this for *P.* (*E.*) *pima* a low number of matings per queen is found, whereas the presence of multiple matrilines is significantly lowering intracolonial relatedness (chapter 8). Polygyny is widespread throughout the whole genus *Pogonomyrmex*, although only rarely expressed in the subgenus *P. sensu stricto* (Creighton 1952; Taber 1998; Johnson 2004), and thus might be a plesiomorphic trait, which was lost in some taxonomic branches. If effective polygyny for this subgenus will be confirmed for additional North- and South-American species, a widespread effect of polygyny increasing genetic variability in *Ephebomyrmex* might be assumed.

Multiple mating of queens on the other hand is up to now only confirmed for the subgenus *P. sensu stricto*. Thus multiple mating may have either evolved early during the evolution of the genus *Pogonomyrmex* and has subsequently been lost in the subgenus *Ephebomyrmex* (plesiomorphic hypothesis) or it has first been evolved in the subgenus *Pogonomyrmex sensu stricto* (apomorphic hypothesis).

The smaller effective mating number of queens in *P. huachucanus* compared to its *sensu stricto* relatives (J. Gadau and C.-P. Strehl, *unpublished*) could mirror a change during the evolution of more advanced species and supporting the apomorphic hypothesis. However, it remains speculative if higher female mating frequencies evolved only within North American representatives of the subgenus *Pogonomyrmex sensu stricto*, or are a plesiomorphic character of the whole subgenus *sensu stricto*. Mating frequency analyses of South-American *Pogonomyrmex* species will be able to solve this question. Preliminary sociogenetic results showed effective multiple mating also in the South-American species *P. inermis* (J. Gadau and R. Pohl, *unpublished*), and therefore might indicate that polyandry is a plesiomorphic trait of the whole subgenus *sensu stricto*.

If multiple mating is plesiomorphic for the all North-American species of the subgenus *Pogonomyrmex sensu stricto*, it might also be assumed for those *sensu stricto* species with smaller colony sizes, e.g. for *P. apache*, a close relative of *P. barbatus* and *P. rugosus* (chapter 9; Parker and Rissing 2002), both polyandrous species (Volny and Gordon 2002; Gadau et al. 2003). The 'sperm need' hypothesis (Cole 1983) stated that especially those species with smaller colonies should show multiple mating less often. Although males of all species in the North-American *sensu stricto* complex seemingly possess enough sperm to father all of a queens offspring, the 'sperm need' hypothesis (Cole 1983) might still possess some validity, if mate number of queens is positively correlated with colony sizes.

Polygyny in *Pogonomyrmex* (*Ephebomyrmex*) *pima* is accompanied by the existence of intermorphic queens (Johnson et al. submitted), which probably have no monophyletic origin and thus are likely to result from a phenotypic plasticity in queen morphology (chapter 8). The widespread occurrence of intermorphic queens within *Pogonomyrmex* (Taber 1998) could suggest their invention several times. Phenotypic plasticity is usually depending on various factors like colony age, the colonies' condition, or environment (Herbers 1986; Buschinger and Heinze 2001). Because *P.* (*E.*) *pima* is living in an unpredictable desert-habitat (Johnson 2000; Johnson 2001) its queen size dimorphism likely is promoted as adjustment to favorable short and long range dispersal (Heinze and Tsuji 1995). Usually a "sequence of progressive physiological restrictions" (Wilson 1985) is leading to such a morphological flexibility, and might predict a differential treatment of brood by the workers. An indication for the existence of such a worker control over reproduction is the adjustment of sex allocation ratios in *P.* (*E.*) *pima* to intracolonial relatedness (chapter 8).

However, in *P.* (*E.*) *pima* queen size dimorphism might additionally be genetically mediated as queens are part of the same gene pool but produce only same-phenotype queens (Johnson et al. submitted; chapter 8). Hypothetically the polyphyletic origin of these queens could be maintained by attributes of the colonies' social environment alone, e.g. a differential treatment of genetically mediated odor cues peculiar to intermorphic or dealate queens and imprinted to their offspring, while indepencent outcrossing continues via the males. The importance of genes encoding recognition cues ('green beard genes') for shaping colony characteristics was previously demonstrated for polygyny in the Myrmicine ants *Solenopsis geminata* and *S. invicta* (Keller and Ross 1998; Krieger and Ross 2002; Ross et al. 2003).

Interestingly, for *P.* (*E.*) *pima* a split of the swarming males has been observed, with some of them staying on the ground (nearby their natal nests), and some with higher swarming prospect (Johnson et al. submitted). This might be comparable with morphological variability of male body and/or wing size in chironomid midges where ground mating is preferred by smaller males (Takamura 1999). Differences in male body sizes are reported only for some *Pogonomyrmex sensu stricto* species (MacKay 1980; Wiernasz et al. 2001). In social Hymenoptera smaller males have to face a reduced capability of sperm-transfer to females, as shown in *Pogonomyrmex occidentalis* (Wiernasz et al. 2001) and *Apis mellifera* (Schlüns et al. 2003). In addition, maybe also the 'gravity-hypothesis' of sexual size dimorphism (Moya-Larano et al. 2002) is applicable. Because of scramble competition and/or by escaping predation easier by running faster on vertical surfaces, males are assumed to get smaller with increasing female sizes and a need to climb to higher elevations to reach females (Moya-Larano et al. 2002). Future analyses in *P.* (*E.*) *pima* on mating behavior and sexual morphology might address these aspects.

The occurrence of high levels of polyandry in *Pogonomyrmex sensu stricto* (Volny and Gordon 2002; Gadau et al. 2003; Rheindt et al. 2004; Wiernasz et al. 2004) raises the question why this trait is absent in the sister group *Pogonomyrmex Ephebomyrmex* (chapter 8). It was shown for *Bombus terrestris* that the mating system can be affected by the risk of pathogen transmissions, such that singly mated females appear to be "constrained by an adaptive valley that needs to be crossed before high degrees of mating frequency can be reached" (Baer and Schmid-Hempel 2001). Generally, increasing parasite-loads are associated with a decrease in intracolonial relatedness (Schmid-Hempel and Crozier 1999). Hence, the possibly widespread occurrence of lowered intracolonial relatedness in *Pogonomyrmex* due to polygyny and polyandry species might mirror their need for facing parasite-loads and maybe also pathogen infestations. This risk might originate when the founding queen has contacted alien individuals (males) at the mating lek and may rise with a colonies' age due to increasing environmental contacts of the colonies members.

However, an increased transmissibility of pathogens during promiscuous mating behavior might affect only very young founding colonies, and be outweighed by the increased resistance of a genetically diverse social environment inside older colonies. Therefore, an increased efficiency in using resources (Keller and Reeve 1994), maybe accompanied by an improved detoxification ability of secondary plant compounds (Gadau et al. 2003), is more likely to explain the evolution of higher genetic diversities throughout *Pogonomyrmex* species.

The question if *Ephebomyrmex* should be elevated to generic status was not fully resolved, because a similar deep split in the phylogenetic gene-trees was observed between both, subgenera and geographic clades (chapter 9). Indications to elevate *Ephebomyrmex* to generic status in the presented gene trees came predominantly from a single nuclear gene; consequently, the incorporation of more information from different genes and additional species will be needed to resolve this difficulty. The morphologically founded subgeneric status of *Ephebomyrmex* was defended in the past, due to the existence of "taxa bridging the gap between subgenera" (Cole 1968, p.18). However, accumulating data on the distinct sociobiology and life histories of *Ephebomyrmex* species compared to *Pogonomyrmex sensu stricto* species seem to give additional hints for a reasonable distinction into separated genera. For instance, *P.* (*E.*) *pima* as a 'typical' *Ephebomyrmex* species (with 50-350 workers per colony; Johnson 2000; Johnson et al. submitted; chapter 8) has smaller-sized colonies than its *P. sensu stricto* congeners (600~12000 workers (Hölldobler and Wilson 1990).

In *P.* (*E.*) *pima* a weak trend of more sexuals produced in bigger colonies was observed (chapter 8). Similarly, in its *Pogonomyrmex* relatives size and/or age of colonies influence the production of sexuals. E.g. MacKay (1985) suggested that in *Pogonomyrmex* (*s.str.*) *montanus*, *P.* (*s.str.*) *subnitidus* and *P.* (*s.str.*) *rugosus*, where 8-16% of the energy is invested in the production of new individuals, worker brood care may be the most important determinant for brood production. He suggested that colonies of all three species invest a higher percentage of brood production in workers than in reproductives, with a rise in the number of male sexuals under food supplementation (MacKay 1985). In *P.* (*s.str.*) *barbatus* sexual output seems to correlate with colony age (Wagner and Gordon 1999). Therefore, the greater capability of brood care and fat storage in bigger sized colonies might correspond to this age-dependency. In *Myrmica rubra* (Elmes et al. 1999), but also in *P. badius* (Tschinkel 1998) the metabolic resources are stored as fat in the bodies of young workers, which are normally found in greater numbers in bigger colonies (Tschinkel 1998). Moreover, in *P.* (*s.str.*) *badius* metabolic and labor resources are needed for sexual production (Tschinkel 1998). Thus, only the well-fed, often older colonies might be able to shift into the production of female sexuals.

Fruitful fields for potential work on *Pogonomyrmex* harvester ants might be e.g. the explanation of the influence of worker control inside colonies due to the possibly widespread role of workers on resource allocation. The prospect for worker control is especially interesting as selection ultimately might have favored queens producing a low intracolonial relatedness via polyandry or acceptance of additional egg laying females, and thus lowering a possible kin conflict. Additionally, the function of males for reproduction and during mating should be further analyzed. Finally, a detailed and finer tuned phylogeny of *Pogonomyrmex* will allow to map and to discuss the evolution of colony sizes and multiple mating, but also other colony characteristics, e.g. foraging habits, territorial behavior, or polygyny, especially if knowledge on South-American species is added.

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11. Summary

The genus *Pogonomyrmex* is predisposed for analyzing the evolution of ant colony characteristics in general and the sociogenetic structure in particular, due to the renowned biology of several species and the diversity of mating frequency and queen number. This variation in the sociogenetic structure of colonies produces a high variance in intracolonial relatedness which can be a major component driving the evolution of various colony characteristics. To exactly determine the variability of the intracolonial relatedness in the genus *Pogonomyrmex* both were analyzed, the number of matrilines and patrilines, in selected members of *Pogonomyrmex*, namely *P.* (*sensu stricto*) *rugosus*, *P.* (*sensu stricto*) *badius* and *P.* (*Ephebomyrmex*) *pima* using DNA fingerprint techniques. The evolution of these colony characteristics were tried to be explained within a phylogenetic framework. For that purpose we constructed a gene-tree of 39 species of the genus *Pogonomyrmex*. The taxon sampling covered about 83 % of the North American species and 43 % of the South American species.

Effective multiple mating of queens was confirmed for *P. rugosus* (me=4.1) and *P. badius* (me=6.7). Additionally, both species are monogynous. These results corroborate behavioral observations of multiple mating for these species. Multiple mating is now known from 9 *Pogonomyrmex* species (behavioral evidence for 3 species – genetic evidence for 6 species).

However, in *P.* (*E.*) *pima* all queens that were analyzed were single mated (me=1.0). Therefore, multiple mating may have either evolved early during the evolution of the genus *Pogonomyrmex* and has subsequently been lost in the subgenus *Ephebomyrmex* (plesiomorphic hypothesis), or it has first been evolved in the subgenus *Pogonomyrmex sensu stricto* (apomorphic hypothesis). In *P. huachucanus*, a species basal to the North-American *sensu stricto* complex, smaller effective mating number of queens compared to its *sensu stricto* relatives (J. Gadau and C.-P. Strehl, *unpublished*) probably do mirror a change from monandry to polyandry during the evolution of more advanced *sensu stricto* species, which would support the apomorphic hypothesis.

The intracolonial relatedness in *P.* (*E.*) *pima* is however rather low. This is probably the result of multiple reproducing queens (polygyny). Polygyny is also documented for at least four other species of the subgenus *Ephebomyrex,* but so far *P.* (*E.*) *pima* is the only species with genetic evidence. It might be that there was an evolutionary trade-off

within the subgenus *Ephebomyrmex* between polyandry and polygyny. Therefore, both subgenera retained a high intracolonial genetic diversity. This high genetic diversity might be one cause for the success and radiation of the genus *Pogonomyrmex* in arid environments.

Evolution might have favored high genetic diversity of *Pogonomyrmex* colonies, because it helps colonies to improve their colonial organization and efficiency in performing external tasks. At least in *P. badius* a link between patrilines and physical polyethism was found, indicative of an improvement of colonial organization via polyandry. Furthermore, the documented extreme levels of polyandry might help *P. badius* females to overcome the possibility of inbreeding due to restricted dispersal.

Restricted dispersal is also found in *P.* (*E.*) *pima* due to wingless, intermorphic queens. However, in *P.* (*E.*) *pima* inbreeding is probably prevented by outcrossing via males because no significant inbreeding is found.

In the presented gene trees the subgenus *Pogonomyrmex Ephebomyrmex* was separated from the subgenus *Pogonomyrmex sensu stricto*. Therefore, *P. Ephebomyrmex* might be elevated to generic status, also due to its distinct morphological and life history characters. Nevertheless, for a precise taxonomic revision a broader complement of species has to be applied.

Regularly a low number of unrelated workers was found in *P. rugosus* colonies, which probably stem from brood raids between mature and founding colonies. It is well known that most founding colonies are destroyed by neighboring conspecific mature colonies, but so far it was assumed that the brood of these colonies was also destroyed. This often neglected aspect might be an important fitness token for mature colonies.

11. Zusammenfassung

Die Gattung *Pogonomyrmex* ist besonders gut geeignet, um die Evolution der Charakteristika von Ameisenkolonien allgemein zu untersuchen, und insbesondere deren soziogenetische Struktur, da die Biologie für einige ihrer Arten sehr gut bekannt ist, und eine Diversität an Paarungshäufigkeiten und Könniginnenzahlen vorkommt. Diese Variation in der soziogenetischen Struktur der Kolonien erzeugt eine hohe Varianz an Verwandschaftsgraden innerhalb von Kolonien, und kann eine Hauptkomponente darstellen, welche die Evolution verschiedenster Koloniecharakteristika vorantreibt. Um die Variabilität intrakolonialer Verwandschaftsgrade innerhalb der Gattung *Pogonomyrmex* genau zu bestimmen, wurde für ausgewählte Mitglieder der Gattung, nämlich für *P.* (*sensu stricto*) *rugosus*, *P.* (*sensu stricto*) *badius* and *P.* (*Ephebomyrmex*) *pima*, mit Hilfe der Technik des DNA-Fingerabdruckes die Anzahl an Matrilinien und Patrilinien bestimmt. Es wurde versucht die Evolution dieser Koloniecharakteristika vor dem Hintergrund einer

Phylogenie zu erklären. Zu diesem Zweck wurde ein Gen-Stammbaum für 39 Arten der Gattung *Pogonomyrmex* erstellt. Die Artabdeckung betrug 83% bei den Nord-Amerikanischen, und 43% bei den Süd-Amerikanischen Arten. Effektive Mehrfachpaarung von Königinnen wurde für *P. rugosus* (me=4.1) und *P. badius* (me=6.7) bestätigt. Zusätzlich wurde gezeigt, dass beide Arten monogyn sind. Diese Ergebnisse bestätigen Verhaltensbeobachtungen von Mehrfachpaarungen in diesen Arten. Mittlerweile ist Mehrfachpaarung in 9 *Pogonomyrmex* Arten bekannt (bei 3 Arten durch Verhaltensbeobachtungen – bei 6 Arten durch genetischen Nachweis).

In *P.* (*E.*) *pima* hingegen waren alle der untersuchten Königinnen einfach gepaart (me=1.0). Daher könnte es sein, dass Mehrfachpaarung entweder früh in der Evolution der Gattung *Pogonomyrmex* entstand und nachträglich in der Untergattung *Ephebomyrmex* verloren wurde (Plesiomorphie-Hypothese), oder sie entstand zum ersten mal in der Untergattung *Pogonomyrmex sensu stricto* (Apomorphie-Hypothese). In *P. huachucanus*, einer Art, die basal zu dem Nord-Amerikanischen *sensu stricto* Komplex ist, könnten die im Vergleich zu ihren *sensu stricto* Verwandten geringeren effektiven Paarungshäufigkeiten der Königinnen (J. Gadau and C.-P. Strehl, *unveröffentlicht*) einen Wechsel von Monandrie zu Polyandrie im Verlauf der

Entstehung der fortschrittlicheren *sensu stricto* Arten widerspiegeln, was die Apomorphie-Hypothese unterstützen würde.

Die intrakolonialen Verwandtschaftsgrade sind dennoch in *P.* (*E.*) *pima* niedrig. Dies ist möglicherweise auf mehrere reproduktive Königinnen (Polygynie) zurückzuführen. Polygynie ist ebenfalls dokumentiert für mindestens vier weitere Arten der Untergattung *Ephebomyrmex*, mit genetischer Evidenz allerdings bisher nur für *P.* (*E.*) *pima*. Es könnte sein, dass es einen evolutionären Ausgleich (trade-off) zwischen Polyandrie und Polygynie innerhalb der Untergattung *Ephebomyrmex* gab, und daher beide Untergattungen eine hohe genetische Vielfalt innerhalb der Kolonien behielten. Diese hohe genetische Vielfalt könnte einer der Gründe sein für den Erfolg und die Radiation der Gattung *Pogonomyrmex* in Trockengebieten.

Evolution könnte eine hohe genetische Vielfalt von *Pogonomyrmex* Kolonien begünstigt haben, da sie den Kolonien hilft die Organisation der Kolonie und die Effizienz mit der externe Aufgaben ausgeführt werden zu verbessern. Wenigstens in *P. badius* konnte eine Verknüpfung zwischen Patrilinien und physischem Polyethismus gefunden werden, was auf eine Verbesserung der Kolonieorganisation mit Hilfe von Polyandrie hindeutet. Darüber hinaus könnten die dargelegten extremen Polyandrie-Werte den *P. badius*-Weibchen helfen die Möglichkeit der Inzucht aufgrund eingeschränkter Ausbreitung zu bewältigen.

Eine eingeschränkte Ausbreitung wird auch in *P.* (*E.*) *pima* durch flügellose, intermorphe Königinnen beobachtet. Jedoch wird bei *P.* (*E.*) *pima* die Inzucht durch Auskreuzen mittels Männchen möglicherweise verhindert, da keine signifikante Inzucht gefunden wurde.

In den vorliegenden Gen-Stammbäumen war die Untergattung *Pogonomyrmex Ephebomyrmex* von der Untergattung *Pogonomyrmex sensu stricto* getrennt. Daher könnte es sein, dass *P. Ephebomyrmex* in den Status einer Gattung erhoben wird, auch aufgrund distinkter morphologischer und lebensgeschichtlicher Charaktere. Für eine präzise taxonomische Revision müsste allerdings eine breite Ergänzung an Arten vorgenommen werden.

Es wurde in *P. rugosus* Kolonien normalerweise eine geringe Anzahl von unverwandten Arbeiterinnen vorgefunden, die möglicherweise aus Brutraub ausgewachsener Kolonien auf Gündungs-Kolonien stammen. Es ist allseits bekannt, dass die meisten Gründungskolonien von benachbarten, ausgwachsenen Kolonien der eigenen Art zerstört werden, aber es wurde bisher angenommen, dass die Brut dieser Kolonien ebenfalls zerstört wurde. Dieser oft vernachlässigte Aspekt könnte einen wichtigen Stärke-Bonus für ausgewachsene Kolonien darstellen.

13. Curriculum Vitae

Persönliche Angaben

Name	Christoph-Peter, Karl, Helmut Strehl
Titel	Dipl.-Biol. (Univ.)
Wohnort	90461 Nürnberg
Strasse	Pflugstr. 10
Telefon	0911 4312658 oder 0171 5348540
Email	christoph_strehl@yahoo.de
Geburtstag und –ort	22.03.1972 in Nürnberg
Nationalität	deutsch

Schulausbildung

09/1982-07/1992	Neues Gymnasium Nürnberg, Abschluss: Abitur (Leistungskurse Biologie, Altgriechisch; Nebenfächer Deutsch, ev. Religionslehre; Facharbeit in Biologie zu „Aufzucht von Kaspar-Hauser Guppy-Männchen: Beobachtung und Vergleich des Balzverhaltens mit ‚normalen' Guppy-Männchen“)

Zivildienst

07/1992-09/1993	Apotheke des Städtischen Klinikums Nürnberg

Studium

09/1993-05/2000	Diplomstudiengang Biologie an der Friedrich-Alexander Universität Erlangen-Nürnberg
10/1995	Vordiplom in Organischer Chemie, Anorganischer Chemie, Physikalischer Chemie, Botanik und Zoologie
01/ 2000	*Diplomprüfung* in den Fächern Zoologie (HF), Genetik, Pharmazeutische Biologie und Rechtsmedizin; *Diplomarbeit* am Institut für Zoologie 1 der Friedrich-Alexander Universität Erlangen-Nürnberg (Betreuer: Prof. Dr. Jürgen Heinze) über „Experimentelle Untersuchungen zur Manipulation der Sex Ratio bei Leptothorax nylanderi“
ab 03/2000	*Doktorarbeit* am Lehrstuhl für Verhaltensphysiologie und Soziobiologie (Zoologie 2) des Thedor-Boveri Institutes, Biozentrum der Universität Würzburg über „Evolution of colony characteristics in the harvester ant genus *Pogonomyrmex*“; Abgabe: 02/2005
05/2000-11/2003	Promotionsstudiengang Biologie an der Julius-Maximilians Universität Würzburg

Berufspraxis

1998/1999	Wissenschaftliche Hilfskraft an der Universität Erlangen-Nürnberg (Zoologie 1)
03/2000-02/2004	Wissenschaftlicher Angestellter am Lehrstuhl für Verhaltensphysiologie und Soziobiologie (Zoologie 2) des Theodor-Boveri Instituts, Biozentrum der Universität Würzburg
Seit 03/2004	Angestellter bei der Winicker Norimed Gesellschaft für medizinische Forschung mbH, Nürnberg (junior Monitor)

Soziale Aktivitäten

1986-1999	Ehrenamtliche Mitarbeit und Ehrenämter in der Jugendarbeit der evang.-luth. Kirchengemeinde Nürnberg-Lichtenhof und auf Dekanatsebene
1996-2000	Mitglied der Dekanatsjugendkammer des evang.-luth. Dekanates Nürnberg
1998-2000	Vorsitzender der Evangelischen Jugend Nürnberg (EJN), dem größten Nürnberger Jugendverband; Delegierter der EJN im Kreisjugendring der Stadt Nürnberg; Mitglied der Synode des evang.-luth. Dekanates Nürnberg

Besondere Kenntnisse und Interessen

Fremdsprache	Englisch (5 jähriger Schulunterricht; Arbeit mit biologischer Fachliteratur; Während der Promotion praktische Konversation mit Gastwissenschaftlern, bzw. während Auslandsaufenthalten); Latinum und Graecum
EDV-Kenntnisse	sehr gute Kenntnisse in gängigen Windows-Programmen (Word, Excel, Powerpoint), im Statistik-Programm Statistika, Programmen zur Verwandtschaftsanalyse
Führerschein	Klasse 3

Auslandsaufenthalte

2000-2003	jeweils einmonatige USA-Aufenthalte: Freilandarbeiten; Besuche der UC Davis (Kalifornien), der Archbold Biological Field Station (Lake Placid, Florida), der Arizona State University (Tempe) und der Southwestern Research Station des American Museum of Natural History (Portal, Arizona); Exkursionsbetreuung (Sierra Ancha, Arizona).
02/2002	Workshop in Oulu, Finnland

Ort, Datum

Unterschrift

14.Publikationsliste

Veröffentlichungen

Strätz M, Strehl C-P, Heinze J (2002) Behavior of Usurping Queens in Colonies of the Ant species Leptothorax nylanderi (Hymenoptera: Formicidae). Entomologia Generalis 26:073-084

Gadau J, Strehl C-P, Oettler J, Hölldobler B (2003) Determinants of intracolonial relatedness in *Pogonomyrmex rugosus* (Hymenoptera; Formicidae): mating frequency and brood raids. Molecular Ecology 12:1931-1938

Rheindt FE, Gadau J, Strehl C-P, Hölldobler B (2004) Extremely high mating frequency in the Florida harvester ant (*Pogonomyrmex badius*). Behavioral Ecology and Sociobiology 56:472-481

Rheindt FE, Strehl C-P, Gadau J (2005) A genetic component in the determination of worker polymorphism in the Florida harvester ant *Pogonomyrmex badius*. Insectes Sociaux 52:in press

Strehl C-P, Gadau J (2004) Cladistic Analysis of Paleo-Island Populations of the Florida Harvester Ant (Hymneoptera: Formicidae) Based Upon Divergence of Mitochondrial DNA Sequences. Florida Entomologist 87:576-579

Eingereichte Veröffentlichung

Johnson RA, Strehl C-P, Gadau J (submitted) Colony Structure and Morphometrics in the Queen Dimorphic Harvester Ant *Pogonomyrmex pima*. Insectes Sociaux

Veröffentlichungen in Vorbereitung

Strehl C-P, Feldhaar H, Gadau J, Johnson RA, Hölldobler B (in preparation) Phylogeny of the Harvester Ant Genus *Pogonomyrmex* (Hymenoptera: Formicidae) in North-America and its Relation to South-American *Pogonomyrmex* species. in preparation

Strehl C-P, Johnson RA, Gadau J (in prep.) Colony Structure and Sociogenetics of the Queen Dimorphic Harvester Ant, *Pogonomyrmex* (*Ephebomyrmex*) *pima*.

Konferenzbeiträge

Strehl C-P, Strätz M, Heinze J (1999) Behavior of "intraspecifically parasitic" queens in the ant Leptothorax nylanderi - Abstract. In: Rosenkranz P, Garrido C (eds) IUSSI-Tagung Hohenheim 1999. IUSSI Internationale Union zum Studium der Sozialen Insekten (Deutschsprachige Sektion), Graz/Hohenheim/Würzburg

Strätz M, Strehl C, Foitzik S, Heinze J (2001) Ecological constraints affect colony founding tactics in the ant Leptothorax nylanderi. INSECT Symposium, Granada, 19-22 April 2001, Granada

Strehl C-P, Gadau J (2001) Distribution and evolution of polyandry in the harvester ant genus *Pogonomyrmex* (Hymenoptera; Formicidae). In: Menzel R, Rademacher E (eds) Berlin Meeting of the European Sections of IUSSI - the International Union tor the Study of Social Insects. 2001 Berlin Meeting of the European Sections of IUSSI - the International Union tor the Study of Social Insects, Berlin (pp. xiv + 251)

Strehl C-P, Gadau J, Hölldobler B (2001) High genetic variability in colonies of *Pogonomyrmex rugosus*: polyandry and brood raids. In: Zissler D (ed) Proceedings of the 94. Annual Meeting of the Deutsche Zoologische Gesellschaft, vol 104. Urban&Fischer, Osnabrück, p. 37

Strehl C-P, Johnson B, Gadau J (2001) Phylogeny of the harvester ant genus Pogonomyrmex, analyzed by mitochondrial DNA-sequences. In: Workshop on Ant Biodiversity and First Symposium of INSECTS - Integrated Studies of the Economy of Insect Societies, financed by the European Commision via the IHP programme, Granada, 19-22 April 2001, Granada

Strehl C-P, Gadau J, Hölldobler B (2002) Are the variances in colony founding success and colony growth rates in *Pogonomyrmex rugosus* (Hymenoptera: Formicidae) correlated with intracolonial genetic diversity? In: Zissler D (ed) 95. Annual Meeting of the Deutschen Zoologische Gesellschaft, vol 105, Supplement V (DZG 95.1) edn. Urban&Fischer, Halle (Saale, Germany), p. 85

Strehl C-P, Gadau J, Anderson KE, Johnson RA, Fewell JH (2003) Preventing Introgression in Ants by Shunting F1 Hybrids into the Sterile Caste. In: 9th Congress o f the European Society for Evolutionary Biology (ESEB). ESEB, Leeds (UK), talk no. 4.9

15. Danksagung

Einer Vielzahl von Menschen möchte ich für ihre Beiträge danken, durch die vorliegende Arbeit entstehen konnte.

In erster Linie möchte ich hier Herrn Prof. Dr. Bert Hölldobler und Herrn PD Dr. Jürgen Gadau nennen, die eine Durchführung der Studien am Institut für Zoologie 2 der Universität Würzburg ermöglicht haben. Bei beiden möchte ich mich auch für die vielen hilfreichen Anmerkungen und Kommentare bedanken, welche die Arbeit inhaltlich vorangebracht haben.

Insbesondere PD Dr. Jürgen Gadau möchte ich für seine geduldige, vielfältige Unterstützung danken, die er mir während der langen Phasen der Laborarbeit, der erhellenden Phasen der Feldarbeiten und der langwierigen Phase des Schreibens zukommen ließ. Durch seine wissenschaftliche Dynamik und menschliche Stärke entstand ein motiviertes Umfeld in dem ein erfolgreiches Arbeiten möglich wurde.

Herrn PD Dr. Jürgen Gadau und Herrn Prof. Dr. Erhard Strohm möchte ich für die Erstellung der Gutachten danken.

Meiner Zimmerkollegin Dr. Heike Feldhaar möchte ich für die vielen gemeinsamen Stunden und Gespräche danken, die meine Zeit in Würzburg zu einem wertvollen Teil meines Lebens gemacht haben, und an den ich bestimmt immer gerne zurückdenken werde.

Karin Möller, der Seele des Labors, danke ich von ganzem Herzen für die immer freundlichen Gespräche, und die viele Unterstützung bei der Durchführung der genetischen Analysen.

Meinem Zimmerkollegen Christof Pietsch, und der „Außenstelle Ost“, Karl Falk und Annett Endler, danke ich für die kollegialen und motivierenden Gespräche. Annett danke ich zudem für die spannende Feldarbeit in Florida, und Karl danke ich besonders für Kommentare zum Manuskript dieser Arbeit.

Dr. Robert Johnson und Kirk Anderson waren wichtige Anlaufstellen für die Sammel- und Freilandarbeiten in Arizona; für ihre großzügige Bereitschaft zur Beherbergung und für viele impulsgebende Abende möchte ich mich hier herzlich bedanken.

Weiterhin danke ich folgenden Menschen: Annette Laudahn für die Bereitschaft den aus den USA mitgebrachten, heimatlos gewordenen Pogo-Königinnen eine Zuflucht für den Aufbau neuer Kolonien zur Verfügung zu stellen; Frank Rheindt für die Umsetzung von Ideen zu Arbeiten über die Ernteameise aus Florida; Frank Stelzer für das Füttern von Ameisen und das Durchführen von Vorversuchen im Labor; Andrea Herb, Bernd Grillenberger, Vanessa Nieratschker, Matthias Mösl, Johannes Penner, sowie Susanne Hoyer für Unterstützung beim Ameisensammeln – Bernd und Matthias dabei auch für „Blitzschläge und andere Abenteuer“; und last but not least Stefan Grau für die seelische Unterstützung während der unruhigen Zeiten dieser Doktorarbeit.

Zum Schluss möchte ich mich bei der Deutschen Forschungsgemeinschaft bedanken, die meine Arbeit finanziell unterstützt hat.

16.Ehrenwörtliche Erklärung

Gemäß §4 Absatz 3 Ziffern 3, 5 und 8 der Promotionsordnung der Fakultät für Biologie der Bayerischen Julius-Maximilians-Universität Würzburg

Hiermit erkläre ich, Christoph Strehl, ehrenwörtlich, die vorliegende Dissertation selbständig angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben.

Die Dissertation wurde bisher weder vollständig noch teilweise einer anderen Hochschule mit dem Ziel einen akademischen Grad zu erwerben vorgelegt.

Ich erkläre weiterhin, dass ich außer meines Diploms in Biologie an der Friedrich-Alexander Universität Erlangen-Nürnberg keine weiteren akademischen Grade erworben habe oder zu erwerben versucht habe.

Ort, Datum	Unterschrift