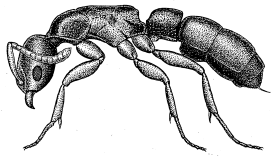
'Safer without Sex?'

Thelytokous Parthenogenesis and Regulation of Reproduction in the Ant

Platythyrea punctata



Dissertation

at the

Julius-Maximilians-University Würzburg

submitted by

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Bremen

Würzburg, 1999

Erklärung:

Hiermit erkläre ich ehrenwörtlich, dass die vorliegende Dissertation von mir selbständig und nur unter Verwendung der angegebenen Quellen und Hilfsmittel angefertigt wurde.

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Acknowledgements

First of all, I would like to express my thanks to Prof. Dr. Jürgen Heinze for his encouragement to pursue the ways of the ant *Platythyrea punctata*, for his support during all stages of the work, for discussions and ideas, as well as detailed comments and suggestions on manuscripts and on the dissertation. His support enabled me to travel twice to Florida and Puerto Rico to work in the natural habitat of this fascinating ant.

Similarly, I'm thankful to Prof. Dr. Bert Hölldobler for his support during all stages of the research. In his department he provided excellent facilities and a lively scientific atmosphere that made this research possible.

During my field work in Florida and Puerto Rico, Mark Deyrup, Lloyd Davis and Juan Torres provided invaluable advise on the natural history of the species. Both Dr. Jürgen Gadau and Olav Rüppell did a great job in helping to collect *P. punctata* and keeping company.

Many thanks are due to Prof. Dr. Roy Gross for providing facilities to develop microsatellites at the Department of Microbiology. Thilo, Jochen, Heike and Barbara patiently answered all my molecular-biological questions and were great fun to work with in the lab. Thanks to Klaus and Indrajit, Department of Human Genetics, for help with karyotype analysis.

Thanks for fruitful scientific discussions to Kasuki Tsuji, Pia Gertsch, Stan Braude and Michael Haberl.

Of course, I would like to thank all my colleagues at the Department of Zoology II for help in many aspects of the work, for good company and friendship. Thanks, Corinna, Jürgen, Jürgen, Olav, Diethe, Brigitte, Anja, Christoph, Johannes, Annette, Roman, Flavio and Uli.

Finally, I would especially like to thank my parents for their patience, understanding and support not only during my dissertation, but during my whole academic career.

For my parents

Contents

Front cover: Drawing of *Platythyrea parallela* after Wilson and Taylor 1967.

1 Introduction

Ants are everywhere (Hölldobler and Wilson 1990). They probably evolved some 100-130 million years ago (Agosti *et al.* 1998) and subsequently have been remarkably successful across a broad range of habitats. Being eusocial (truly social), ants show some of the most complex social organizations currently known. Most of the colony members have given up own reproduction in favor of helping nestmates to rear their offspring. The evolution of altruism is the central problem underlying the evolution of eusociality (Trivers 1971; Alexander 1974; West-Eberhard 1975; Gadagkar 1994). Wilson (1971) defined eusociality as the possession of all of the following traits: a) cooperative brood care of conspecifics, b) reproductive division of labor with sterile or subfertile individuals working on behalf of fecund individuals, and c) overlap of generations with offspring helping their parents.

In addition to the eusocial Hymenoptera (ants, bees and wasps) and Isoptera (termites), within the last years eusociality has been found in aphids (Aoki 1977; Ito 1989), ambrosia beetles (Kent and Simpson 1992), thrips (Crespi 1992), possibly in spiders (Vollrath 1986), in shrimps (Duffy 1996) and in several naked mole rats (Jarvis 1981; Sherman *et al.* 1991; Burda and Kawalika 1993). Within the Hymenoptera alone, eusociality evolved several times, possibly as often as 12 times (Wilson 1971). Since eusociality also evolved several times independently within aphids and naked mole-rats (Jarvis *et al.* 1994; Stern 1994), the phenomenon is not such a rare evolutionary achievement as it was once perceived (Hölldobler and Wilson 1990).

Eusociality is often subdivided into primitively and highly eusocial species depending on the presence of a morphologically differentiated reproductive and a non-reproductive caste, with the later present only in highly eusocial species (e.g. Gadagkar 1994). In ants in particular, female caste dimorphism is pronounced between winged queens, which mate and disperse during nuptial flights, and workers, which are wingless and typically lost their reproductive potential. Accumulating evidence of social organization and reproductive behavior in a number of species has, during the last years, blurred the distinction between eusociality and other social systems (Sherman *et al.* 1995). In an effort to unify definitions of cooperatively breeding societies (mostly birds and mammals) and eusocial societies Crespi and Yanega (1995) took the presence of morphological castes as sole criterion for eusociality. This approach has subsequently been criticized as being too narrow for excluding several eusocial species. To overcome the problem of how to quantify the varying degrees of reproductive division of labor, it has instead been suggested to expand the scope of eusociality to include semisocial, primitively eusocial and highly eusocial species, as well as cooperatively breeding vertebrates, where some individuals give up on reproduction to help others (Gadagkar 1994). Partitioning of lifetime reproduction among family

or colony members is the criterion used for placing species along a continuum of reproductive skew, i.e. a standardized measure of reproductive variance, ranging from egalitarian reproduction (skew of zero) to complete monopolization of reproduction by one individual (skew of one) (Keller and Vargo 1993; Reeve and Ratnieks 1993; Sherman *et al.* 1995).

The evolution of eusociality has been one of the great puzzles of evolutionary biology. Even Darwin realized that the presence of sterile worker ants represents "*...one special difficulty, which at first appeared to me insuperable, and actually fatal to the whole theory*" (Darwin 1859: 268-273). About a century later, Hamilton's famous theory of kin selection provided an elegant explanation for the widespread occurrence of altruism among social insects (Hamilton 1964a, 1964b). The theory states that altruistic behavior should be favored when the product of benefits of altruistic behavior for the recipient (b) and relatedness to the donor of the behavior (r) is larger than the costs (c) accruing to the donor of the altruistic act. This so-called "Hamilton's rule" ($br > c$) has subsequently been used to explain altruistic behavior and sterile individuals in a broad range of species by the added benefits of inclusive fitness. The male-haploid genetic system of the Hymenoptera has been interpreted as a pre-adaptation for the evolution of eusociality since high sister relatedness in social insect colonies favors the evolution of altruism through kin selection in females (e.g. West-Eberhard 1975).

Subsequently, there has been some criticism of the haplodiploidy hypothesis (reviewed in Bourke and Franks 1995). Polyandry (multiple mating of queens) and polygyny (multiple reproductives in a colony) often lower the genetic relatedness below the eusociality threshold favoring selfish worker reproduction (Wilson 1971; Wade 1982; Anderson 1984). Alternative hypothesis favoring the evolution of eusociality in social insects have been suggested including nest-building and nest-provisioning (Alexander 1974), the possession of a sting (Starr 1985), female arrhenotoky (Seger 1991), and advantages of group life (Gamboa 1978). It has to be kept in mind that factors important during the evolution of eusociality may be different from those important for its maintenance (Bourke and Franks 1995).

The major components of reproductive structure in social insect societies are the reproductive division of labor (worker sterility), the number of reproducing individuals (gyny level) and the variance in reproduction among cohabiting reproductives (reproductive skew) (Keller and Vargo 1993). Haplodiploidy introducing relatedness asymmetry within the colony is expected to lead to queen-worker conflict over male production, queen-rearing, and sex allocation (Ratnieks and Reeve 1992). However, actual reproductive conflicts observed could be diminished compared to the potential conflicts when the resulting costs, e.g. in terms of colony productivity, are high. Therefore, an understanding of the proximate mechanisms maintaining eusociality in ants requires the detailed investigation of reproductive regulation and female caste specialization.

Ponerine ants in particular are well suited for the study of reproductive conflict since female caste differentiation is less pronounced than in higher formicine subfamilies. As in higher ants, workers are able to produce male offspring from unfertilized eggs. They additionally retain a spermatheca and are thereby able to mate and produce female progeny. Reproductive conflicts occurring in this group will resemble the ancestral condition, under which eusociality evolved in ants, more closely. The ponerine ant *Platythyrea punctata* shows a remarkable complexity of reproductive modes including both the potential for bisexual and asexual reproduction (Heinze and Hölldobler 1995; Schilder *et al.* 1999a). The ability to produce diploid offspring from unfertilized eggs by thelytokous parthenogenesis is rare among the Hymenoptera and provides a challenge to the theories mentioned above that try to explain the maintenance of eusociality (Stern and Foster 1997). *P. punctata* therefore is especially well suited for the investigation of altruism and reproductive conflicts in ants.

Figure 1.1 Worker of the small ponerine ant *Platythyrea punctata* Smith tending pupae.

The first objective of this investigation is to determine the degree of morphological and physiological specialization found within the female caste of *P. punctata* (Fig. 1.1). The degree of female dimorphism will provide general insight into the reproductive options available to both queens and workers in this species as compared to other ponerine ants. In a second step, the proximate mechanisms underlying the regulation of reproduction under thelytokous worker parthenogenesis and its implications for the social organization of the colony are investigated. Thelytoky, in addition to

sexual reproduction by queens and mated workers, quite dramatically enlarges the repertoire of reproductive options available to ponerine ants, and has profound effects on the genetic structure of colonies. Asexual reproduction is expected to reduce within-colony relatedness and thereby to alter the potential of reproductive conflict among colony members (Stern and Foster 1997). Molecular microsatellite markers are applied to determine intra- and intercolonial genetic variability and thereby deduce the proportion of thelytokous reproduction in *P. punctata*. The implications of thelytokous parthenogenesis in *P. punctata* are finally compared to the mechanisms and evolutionary consequences of thelytoky within other Hymenopteran societies. In a final step, the mechanism of reproductive regulation and the importance of thelytokous parthenogenesis for the evolution of social life in *P. punctata* are integrated with current knowledge available from other thelytokous Hymenopterans.

2 Life history traits

2.1 Introduction

Ant societies typically are characterized by a reproductive division of labor between two distinct female castes: queens, who are inseminated and reproduce, and virgin workers, who forage, defend and maintain the nest, and nurse the brood (Hölldobler and Wilson 1990). In the majority of species, the two castes are morphologically specialized for their respective tasks: queens, which mate and disperse during nuptial flights, are winged and often well-endowed with reserves for starting their new society. Workers, on the other hand, usually are energetically less costly all-purpose tools, which lack wings and whose ovaries are often strongly reduced and typically do not have a spermatheca for the storage of sperm. The simple ovarian anatomy leaves workers with few options concerning the maximization of their fitness. In most species they can increase their reproductive success directly only by occasionally producing haploid males via arrhenotokous parthenogenesis from unfertilized eggs either in the presence of the queen or after her death (Bourke 1988; Choe 1988; Bourke and Franks 1995) and indirectly in colonies containing a singly mated queen by biasing sex allocation towards more female sexuals (Sundström 1994) or towards males when the relatedness asymmetry in their colony is smaller than the population average (Boomsma and Grafen 1991). The conflict of interest over reproduction is discussed in detail in chapter 3.

The Ponerinae is a phylogenetically primitive subfamily within the Hymenoptera whose members are distributed within tropical and sub-tropical habitats. Morphology, life history and behavior are a mixture of primitive and highly derived characters (Peeters 1997). In ponerine ants reproductive caste dimorphism is much less pronounced than in most species of the more advanced subfamilies. Ovarian morphology, regarding size and number of the ovarioles, is rather similar in queens and workers. Generally colony size is low in ponerine ants (usually reaching only few hundred individuals) which is reflective of the low fecundity of queens (which lay between 1 and 5 eggs per days) in the whole subfamily (Peeters 1991b, 1993). The tergosternal fusion of abdominal segment IV, being characteristic for the whole group, further constrains the evolution of a higher number of ovarioles. Queens of some species may partially compensate for this constraint by widening the IVth abdominal segment and by enlarging their spermatheca as compared to workers (Ito and Ohkawara 1994). In *Leptogenys* species reaching colony sizes of several thousand workers, gaster enlargement is facilitated by the physogastric stretching of intersegmental membranes (Maschwitz *et al.* 1989). In some species, workers have a spermatheca, they mate and produce diploid offspring, which gives them an additional option to increase their fitness (Peeters 1991b). These factors often increase the opportunity for reproductive conflict (chapter 3). On the other hand, ponerines exhibit derived social traits such as large colony size (e.g. several thousand workers in *Brachyponera lutea* (Haskins and Haskins 1950), *Pachycondyla luteola* (Verhaagh 1994) or *Paltothyreus tarsatus* (Braun *et al.* 1994)), trophobiosis with homopterans and exchange of liquid food droplets or group predation and migratory behavior (Peeters 1997). The small number of workers in most species limits the foraging strategies employed. Usually ponerine ants forage by solitary hunting (Peeters and Crewe 1987). Only in some *Leptogenys* species army ant-like raiding behavior has evolved (e.g. Maschwitz *et al.* 1989). Limitation in nest space may be an important factor regulating colony size (Wilson 1959). Ponerine ants living in rotten branches or natural cavities may not be able to substantially enlarge their nest. Nesting preferences reflecting the adaptation to a specialized ecological niche may therefore limit colony size (Peeters 1997). Only some *Leptogenys* species that build bivouac nests in the leaf litter evolved much larger colony sizes (Maschwitz *et al.* 1989).

In a number of ponerine species, different types of female reproductives may co-occur and compete for reproduction. In many species permanently wingless or ergatoid queens have replaced winged queens completely (reviewed in Peeters (1991a) and (Heinze 1998)). In other species queens co-occur or have been replaced by workers that mate and reproduce (Peeters 1991b). Ordinary, originally winged queens and mated, egg-laying workers (also called "gamergates“ (Peeters and Crewe 1984)) coexist in *Harpegnathos saltator* (Peeters and Hölldobler 1995) and *Pachycondyla tridentata* (Sommer and Hölldobler 1992). In the latter species, gamergates and queens engage in dominance contests by rapid antennation bouts and pulling at the opponent’s mandibles. Their hierarchy rank determines who

will reproduce and who will not. In other species, gamergates have completely replaced the queens (Peeters 1991).

In several ant species wingless female individuals occur that are morphological intermediate between reproductive queens and non-reproductive workers. Ergatoid (or permanently wingless) queens are believed to have evolved as a specialized reproductive caste in some species (reviewed in Peeters 1993). Ergatoid queens are morphologically invariable and replace queens completely to regularly serve the reproductive function (Peeters 1991a; for a discussion of exceptions see Heinze 1998). Interestingly most species with ergatoid queens are monogynous. Other morphological intermediates termed 'intercastes' expressing both queen and worker traits sporadically occur in other species (Brian 1955; Ohkawara *et al.* 1993; Düssmann *et al.* 1996). In contrast to ergatoid queens the thorax morphology of intercastes is quite variable from an almost queen-like to a worker-like structure (reviewed in Heinze 1998). They are generally not winged but their thorax is distinguished from that of workers by simplified flight sclerites. Erratically produced intercastes may coexist with winged queens (Peeters 1991a; Düssmann *et al.* 1996) but under natural conditions rarely serve a specific reproductive function. In other species morphological intermediates regularly reproduce (Francoeur *et al.* 1985; Heinze and Buschinger 1987; Tsuji *et al.* 1991; Ohkawara *et al.* 1993). Due to their regular occurrence, the well-coordinated development of morphology, the stability of ovarian anatomy and their specialized reproductive function these individuals however should be referred to as 'intermorphic queens' (Heinze 1998) to clearly separate them from sporadically occurring intercastes. Caste determination in ants in general depends largely on physiological rather than on genetic factors and is therefore strongly influenced by environmental variation (Oster and Wilson 1978). Several factors contribute to the determination of individual larvae to the worker or queen caste (Hölldobler and Wilson 1990): Larval nutrition, winter chilling and variation in ambient microclimate, rearing temperature, caste self-inhibition as a negative feedback loop, egg size and yolk content, and the age of the queen. Caste-determining factors interact along a series of decision-points the developing egg passes through. Intercastes are likely to result from environmental disturbances during individual ontogeny and do not serve a regular reproductive function (Wheeler 1928). Malnutrition of queen-presumptive larvae, inhibitory pheromonal effects, parasitization with mermithid nematodes or treatment with insect growth regulators may stimulate their development (Peeters 1991a; reviewed in Heinze 1998).

Colony founding in most ant species is usually done by queens who disperse on the wing to found new colonies alone (haplometrosis) or with several other queens thus reducing the risks of the foundation phase (pleometrosis) (Hölldobler and Wilson 1990). Additionally, queens may found solitarily (independent) or be accompanied by workers (dependent) (Hölldobler and Wilson 1977). There is large variation both in the mode of colony foundation and in the number of individuals involved. In queenless ponerine ants mated workers do not possess the body reserves necessary to found a new colony solitarily, instead colonies reproduce by budding, i.e. departure of a small colony fragment

containing mated and unmated individuals (e.g. in *Ophthalmopone berthuodi* (Peeters and Crewe 1985)), or by fission, i.e. splitting of the colony in two or more parts each of which contains potential reproductives. Fission is particularly frequent in species with ergatoid queens (Hölldobler and Wilson 1990; Peeters, 1991b). While budding in queenless species seems to be associated with frequent movement of workers and brood and can accidentally occur during colony emigration, colony fragmentation or fission may be the result of the destruction of nests due to environmental conditions. Virgin workers in fission groups will usually start to develop their ovaries after inhibition from the current reproductive has ceased. Depending on the time of year however there may no males be available for mating. Colony fission may therefore be followed by a period of male production by virgin workers before one or more of them mates. Fission will be favored over alate dispersal when the species either experiences highly competitive environments or a large number of individuals is needed for successful colony foundation (e.g. Seeley 1985).

Social insects are known for their well-developed capabilities to distinguish nestmates from alien conspecifics since commonly they attack non-nestmates from other colonies (Breed and Bennett 1987; Hölldobler and Wilson 1990). Nestmate recognition has also been described as a form of kin discrimination since it ensures that beneficial behaviors are primarily directed towards related individuals. However, kin discrimination could also be seen as a mere by-product of nestmate recognition because recognition labels are simultaneously specific and anonymous (Hölldobler and Carlin 1987). The adaptive value of kin discrimination therefore remains controversial (Frumhoff and Schneider 1987; Carlin 1989; Grafen 1990). Variation in within-colony relatedness does not seem to strongly influence recognition abilities in ants (e.g. Peeters 1988; Crosland 1990; Carlin *et al.* 1993; Bourke 1994). Instead the available evidence suggests that nestmate recognition cues are both genetically encoded and acquired from the environment (Hölldobler and Carlin 1987). In most cases recognition will rely on a mixture of intrinsic (genetic) and extrinsic (environmental) cues but the relative proportions of each may vary (examples are reviewed in Hölldobler and Wilson 1990). Thelytokous parthenogenesis will strongly influence the genetic component of nestmate recognition systems up to the replacement by a 'clonemate' recognition system. One would expect that depending on the importance of intrinsic cues, recognition capabilities may be diminished with increased degree of genetic similarity of colonies inhabiting the same habitat. Nestmate recognition in *P. punctata* is investigated both in the field and in the laboratory.

Division of labor is an important feature of eusocial insect colonies (Wilson 1971). Task allocation in ponerine ants is usually similar but not as pronounced as in higher ants due to the lack of the caste polymorphism found in many higher subfamilies (e.g. Fresneau and Dupuy 1988; Corbara *et al.* 1989, Corbara *et al.* 1990; Villet 1992b). In most ponerine species the exchange of liquid food by trophallaxis is absent (Hölldobler and Wilson 1990; Peeters 1997; for an exception see Liebig *et al.* 1997), but ponerines employ an external social bucket in social food exchange (Hölldobler 1986).

Therefore, most individuals regularly have to leave the nest to forage outside. For efficiency reasons a small colony size may not allow for a highly specialized division of labor among nestmates. Nonetheless the reproductive individual within the colony is usually characterized by a distinct behavioral profile. While foundresses usually exhibit the full range of behaviors observed in her nestmates later in colony ontogeny, their repertoire decreases as they concentrates on their reproductive role (Peeters 1997). Similarly gamergates usually have a behavioral profile that is distinct from sterile workers in the colony. They usually do not start to forage once they become older. In genus *Platythyrea* the division of labor has been studied in great detail within several queenless African species containing gamergates (Villet 1990, 1991a, 1991b; Villet and Wildman 1991). In many cases, multivariate analysis reveals few, but different social roles that are characterized by different individual behavioral profiles reflecting different tasks. However, functional groups of workers appear to be more flexible and variable in composition than in the higher ants (Peeters 1997).

This chapter investigates several aspects of the natural history of *P. punctata* and examines the importance of these traits for the evolution of its life cycle.

2.2 Materials and methods

Field work was conducted in fall 1994, spring 1996 and spring 1997 in various parts of Florida, in spring 1996 at the El Verde Field Station located in Luquillo Experimental Forest, Puerto Rico, and in January 1995 in Turner's Hall Woods on Barbados, West Indies (Fig. 2.1). The major study sites in Florida are located at Archbold Biological Station ('ABS', Highlands County, Florida, at an elevation of 43m). Additional colonies were collected in subtropical hardwood hammocks at Vero Beach and Ft. Pierce, St. Lucie county, Florida ('VB' and 'FP'; 5 colonies) and in mangrove vegetation on No Name Key, Monroe county, Florida ('NNK'; 1 colony). The northernmost occurrence of *P. punctata* is known from Indian River county just north of St. Lucie county (Deyrup *et al.* 1989). Colonies nested in rotting logs on the ground. In El Verde, Puerto Rico ('PR'), most colonies were found on the ground nesting in hollow rotting stems of the pioneer shrub *Psychotria berteriana* in a subtropical rain forest at an elevation of 350m. Colonies were collected by breaking twigs and windfall branches on the forest floor into plastic dishes and rapidly picking up individuals with forceps. Additional colonies were found by baiting and following individual foragers back to their nests. Colony composition and number of brood was examined right after collection. Since the nests usually had only one entrance hole it is reasonably assumed that all colonies could be obtained almost completely assuming the absence of polydomy.

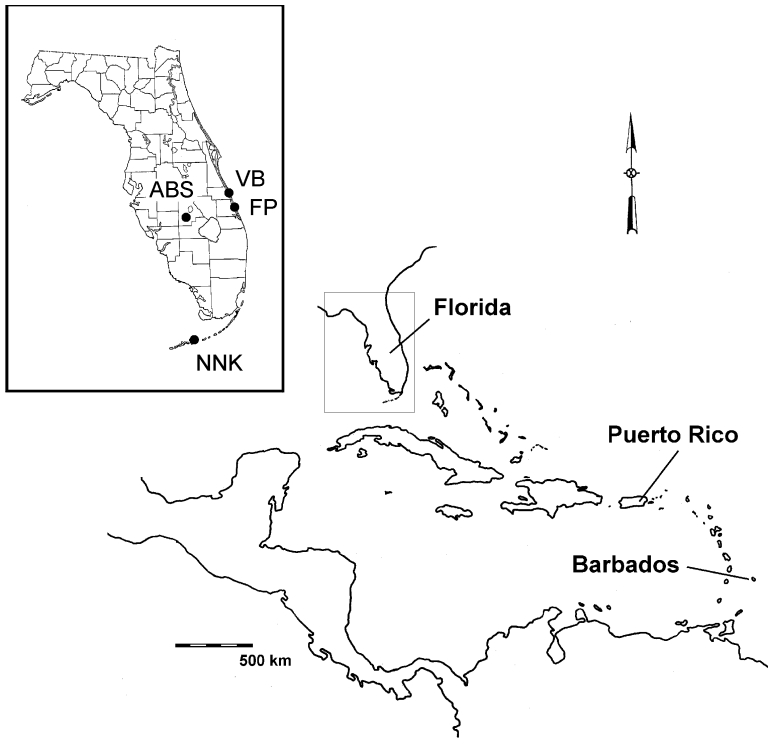

Figure 2.1 Location of the sampling sites of *P. punctata* colonies in Florida (ABS, Archbold Biological Station; VB, Vero Beach; FP, Fort Pierce), on the Florida Keys (NNK, No Name Key), and on two locations within the Caribbean.

Colonies were transferred to the laboratory and housed at 25°C under a 12h light / 12h dark cycle in plastic boxes with artificial plaster nests. Colonies were fed twice a week with a mixed diet of diluted honey and frozen crickets. Under these conditions, all colonies collected continued to produce new eggs, larvae and pupae, from which numerous workers and a few female and male sexuals eclosed.

Morphometric examination of individuals *of P. punctata* included measuring Weber's thorax length, thorax width, length of meso- and metanotum and length of meso- and metanotal suture, if present. Measurements were either made using a Wild M3Z stereo microscope with ocular micrometer scale or taken from dorsal SEM micrographs of the alitrunk of individuals (all intercastes).

Nestmate recognition in the field was examined in a limited number of tests on intercolonial aggression that were carried out directly after collection of colonies at Archbold Biological Station and Ft. Pierce involving three colonies (ABS8, ABS9 and FP2). Pairs of workers were placed in a circular plastic arena (4.5 cm in diameter) separated by a removable sheet of plastic both coated with Fluon® to prevent ants from escaping. The floor of the arena was covered with filter paper that was changed after each trial. Dyadic encounters lasted for 5 min. During this period all incidences of aggressive interaction were recorded ad libitum. Tests of nestmate recognition in the lab consisted of dyadic encounters between two workers from five colonies originally collected in Florida, Puerto Rico and Barbados that were kept in the lab for about 3 years (ABS24, FP2, BAR1, PR4 and PR15). Ants were dot-marked on the thorax using color paint markers (Edding paint marker) on the day prior to the experiment to facilitate individual recognition. Ants from colony PR15 were not dot-marked but similarly handled with forceps to control for an influence of the color-marking. Individual pairs of ants were placed into a circular plastic arena as described above. Each dyadic encounter test lasted for 7 min with 2 - 3 min prior to the actual test to allow for the ants to inspect the arena and acclimate. Ants were either taken from nests belonging to the same population (type I tests) or to a different population (type II tests). For each of the 10 possible combinations five replicate tests were performed resulting in a total of 50 dyadic encounters (Type 1: n = 10; type 2: n = 40). No individual was tested twice. Dyadic encounters were video-taped using time-lapse video recordings (Panasonic F15HS and AG-6730) for subsequent behavioral analysis. The following non-agonistic inspection behaviors were observed and subsequently considered:

Brief antennal contact: Both workers very briefly touched each other with the tips of their antennae for mutual investigation.

Antennation: Brief inspection of different parts of the body with the tips of the antennae.

Withdrawal of antennae: Upon inspection the antennated worker withdraws its antennae and subsequently walks backwards to avoid further inspection.

For each behavior the identity of the initiating individual and the total duration was recorded and the mean frequency of interaction calculated for all tests. Agonistic behaviors like antennal boxing, biting, dragging, leap or immobilization, all being observed within an aggressive context, and ignore, standing still, crouching and withdrawal, typically observed within a submissive context, are described in detail in chapter 3.2.3 in the context of reproductive regulation.

Division of labor was studied in one small colony collected at Archbold Biological Station. Ad libitum sampling was used to construct an initial behavioral catalog of *P. punctata* including 32 individual behaviors. For clarity these behaviors were subsequently grouped into the following eight behavioral categories:

Locomotion in the nest:	Movement inside the nest that did not involve carrying of food
Inactivity in the nest:	Motionlessly standing within the nest
Self-grooming:	Self-directed grooming of various body parts
Foraging:	Searching for food in the arena outside the nest cavity, carrying food particles back into the nest
Brood care:	Any grooming or holding behavior directed towards eggs or brood
Inactivity on brood pile:	Motionlessly standing on parts of the brood pile
Guarding:	Standing at the inside of the nest entrance, frequently antennating arriving nestmates
Grooming:	Any grooming directed towards nestmates or received from them.

Based on these categories scan sampling (Altmann 1974) was conducted to compile an ethogram of every individual within the colony. Aggressive interactions and egg laying were not observed during the sampling period. Subsequently the behavioral organization of this colony was analyzed using multivariate hierarchical cluster analysis based on the single linkage algorithm (Sneath and Sokal 1973). Principal component analysis employing the centroid method (Überla 1977) was used to determine which of the 8 behavioral categories were responsible for the observed individual behavioral differences.

All statistical analysis were carried out using the Statistica 5.1 software package (Statsoft 1999). All means are given with standard deviation, if not otherwise indicated. In all figures and tables significance levels are indicated by stars (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). Where applicable, comparisons of means were adjusted according to Bonferroni (with an adjusted critical value of $\alpha' = \alpha / k$; $\alpha = 0.01$ and k being the number of tests, Sokal and Rohlf 1995).

2.3 Distribution and habitat

The ancient ponerine tribe Platythyreini contains two genera: *Probolomyrmex* and *Platythyrea*. The genus *Platythyrea* consists of some 37 species worldwide (Bolton 1995) most of which live in tropical habitats. In the New World tropics including Central and South America at least 8 species are currently described (ref. in Bolton 1995). Fossil specimens of *Platythyrea primaeva* in Baltic amber have been dated back to the Oligocene (Wheeler 1915). *Platythyrea punctata* Smith is the only New World member of the genus reaching as far north as the southern USA, including the southern parts of Florida and Texas (Brown 1975; Deyrup *et al.* 1988; Brandao 1991), with the center of its distribution in Mexico, Central America, and the West Indies (Kempf 1972; Smith 1979).

Figure 2.2 Typical bayhead habitat ('West Bayhead') of broad-leaved evergreen trees and diverse shrubs with a thick layer of organic mater on the ground at Archbold Biological Station, Florida.

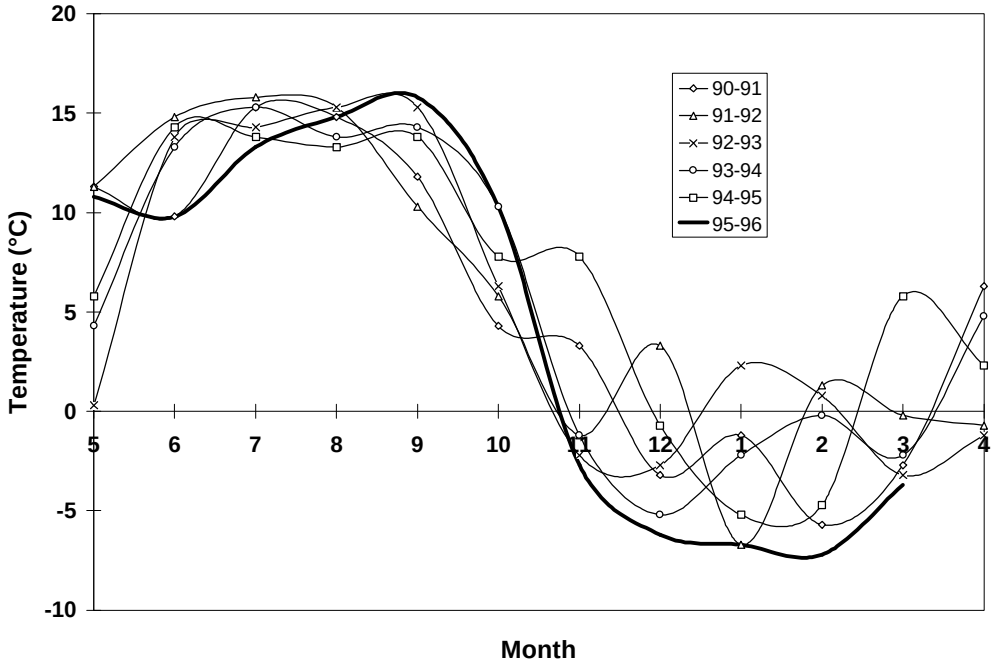

Figure 2.3 Mean minimal monthly temperatures measured at Archbold Biological Station, Florida, for the winters 1990/91 to 1995/96. Mean temperature for the winter 1995/96 is shown in bold (data courtesy of Archbold Biological Station).

Figure 2.4 Dense subtropical rain forest habitat at the El Verde Field Station, Luquillo, Puerto Rico. Colonies were found in stands of the pioneer shrub *Psychotria berteriana.*

The habitat at Archbold Biological Station is characterized by scrubby flatwoods dominated by xerophytic oaks and palmettos, and mesic bayhead forests (called 'hammocks') of broad-leaved evergreen trees subject to periodic flooding (Fig. 2.2). Coastal hammocks in Florida consisted of a much lighter and dryer forest including subtropical hardwood species. At both locations an abundance of leaf litter and decaying wood covered the forest floor. One colony (NNK 1) was located in a rotten stump in mangrove vegetation on No Name Key, Florida. The climate at Archbold Biological Station is seasonal with mean monthly minimal temperatures ranging from -8 to 17 C° (Fig. 2.3). Vegetation at El Verde in Puerto Rico is characterized by a subtropical rain forest (Fig. 2.4). Climate is warm and moist throughout the year with a mean annual precipitation of 3,456 mm and mean monthly temperatures between 21 - 25 C°.

The majority of colonies in Florida inhabited dead wood located on the forest floor. Nest entrances usually were small, inconspicuous openings in dead twigs and branches on the forest floor (Fig. 2.5). The structure of the nests inside branches was rather simple and may depend on the size of the colony, varying from one to several small chambers with interconnecting tunnels (Fig. 2.6). Nest cavities were lined with a mixture of organic material containing dead plant material, remnants of prey and empty cocoon cases. Sometimes the entrance was lined with this material to decrease its diameter. *P. punctata* is not likely to be capable of substantially enlarging the nest site but rather moves to a larger preexisting nest cavity during colony development. Colonies collected at El Verde in Puerto Rico

Figure 2.5 Nest entrance of a colony of *P. punctata* collected at Archbold Biological station, Florida with a cent for size comparison.

were peculiar in that they inhabited hollow stems of the pioneer shrub *Psychotria berteriana* patchily

distributed within small open patches within denser forest. Due to its ephemeral nature these nesting sites may force *P. punctata* to frequently relocate colonies. For practical reasons the canopy region could not be screened for further nests. Several foragers baited with tuna chunks were observed to move up the trunks of nearby trees and subsequently disappearing in higher canopy layers. Therefore it seems reasonable to assume that an unknown proportion of the nests of *P. punctata* are located in higher strata of the canopy or understory regions. This may equally apply to the populations sampled in Florida. Other species within the genus *Platythyrea* are well-known to nest arboreally (Dejean *et al.* 1990; Majer and Delabie 1994). Nest structure and composition of canopy-nesting colonies are unknown. Foragers hunt alone for small arthropod prey, raid insect brood (especially lepidopteran larvae) and scavenge on dead arthropods. Larvae are partly fed on insect prey. Foraging was not limited to any particular time of day, although foraging activity was observed to increase at dusk and lasted during most of the night. When disturbed, foragers hid under vegetation cover. Species within the genus are known to be insectivorous (Torres 1984).

Figure 2.6 Opened empty nest chambers of medium-sized colony ABS 8 collected from a dry oak branch 1.5 m above the ground near the entrance to Archbold Biological Station, Florida.

2.4 Composition of colonies

The composition of all 61 colonies at date of collection is summarized in Tab. 2.1. Total colony size ranged from 11 to 167 individuals with a mean of 59.5 ± SD 40.1 counted directly after collection. According to locality, mean number of individuals in a colony was 50.3 ± SD 35.2 for colonies obtained from Florida (n = 34), 51 and 148 workers for two colonies obtained from Barbados and 69.0 ± SD 42.1 for colonies obtained from Puerto Rico (n = 25). Mean colony sizes were not significantly different among the three populations (Kruskal-Wallis-ANOVA, $H_{(2, 61)} = 4.27$; p = 0.12). In 29 percent (n = 10) of colonies collected in Florida, between one and five queens (both winged and dealate) were found. Florida colonies containing queens were not different in size from queenless colonies (Mann-Whitney U-test, Z = -0.983, p = ns). Queens were absent from all colonies from Barbados and Puerto Rico. One male each was present in five colonies from Florida and one from Puerto Rico. Both queens and males are known from various other locations in Florida (Deyrup, pers. comm.). In addition to queens, workers and males, morphological intermediates between workers and queens ("intercastes") were found in 16 colonies from Archbold Biological Station (see below).

2.5 Morphology

In 16 colonies from Florida (47.1 percent) a total of 66 individuals was found which differed morphologically from workers or queens. Their thorax morphology differed greatly between individuals, varying from a worker-like to an almost queen-like thorax structure (Fig. 2.7). Scutum and scutellum were fused to a variable degree and the length of the mesonotal and metanotal sutures differed strongly between individuals (n = 13 for all comparisons; mesonotal suture mean 0.38 ± SD 0.18 mm, rel. coefficient of variation CV = 47.4 percent; metanotal suture mean 0.12 ± SD 0.12 mm; CV = 100 percent; see Fig. 2.8). All other parameters measured showed rather little morphological variation (Weber's thorax length 2.40 ± SD 0.06 mm, CV = 2.5 percent; thorax width 0.73 ± SD 0.03 mm, CV = 4.1 percent; length of mesonotum 0.61 ± SD 0.07 mm, CV = 11.5 percent; length of metanotum 0.21 ± SD 0.03 mm, CV = 14.3 percent; length of propodeum 0.40 ± SD 0.07 mm, CV = 17.5 percent). Like workers and queens, these individuals usually had six ovarioles and a spermatheca (see below). Because of their broad morphological variability, the lack of evidence for a specialized function in reproduction, and because they occurred together with queens in at least eight colonies these individuals are referred to as "intercastes" (sensu Peeters 1991a; concerning the terminology see also Heinze 1998). The proportion of intercastes within a nest was negatively correlated with the total number of workers in the Florida colonies, i.e., larger colonies had a significantly smaller proportion of intercastes than smaller colonies (n = 16, r = - 0.652, p < 0.001).

Location	Colony	Workers	Callows	Pupae	Larvae	Eggs	Queens[1]	Males	Intercastes
Florida	ABS 1	24	2	6	14	9	3 (2)		3
	ABS 2	75	8		16	10			2
	ABS 3	75	13		8	14			
	ABS 4	22	5	1	4	10			
	ABS 5	10	5		7	4			2
	ABS 6	58	11	2	37	14	1 (1)		5
	ABS 7	18	14	2	16	10	1 (0)	1	1
	ABS 8	43	31	2	30	20	5 (3)		8
	ABS 9	17	7			6			
	ABS 10	21	9	7	12	5		1	2
	ABS 11	11	5	7	4	1			2
	ABS 12	19	7	12	26	6			
	ABS 13	14		2	10	5	1 (0)		
	ABS 14	43	9	3	17	13	1 (1)	1	9
	ABS 15	15	4	13	9	4			
	ABS 16	17	5	8	7	6			
	ABS 17	57	8	5	36	15	1 (1)		5
	ABS 18	64	17		27	20			6
	ABS 19	34	12	1	42	20			
	ABS 20	64	9	6	31	21	1 (1)		6
	ABS 21	87	39	1	48	21		1	5
	ABS 22	17	5	2	11	15			
	ABS 23	47	16	10	15	6			
	ABS 24	41	17	4	16	20	1 (1)		4
	ABS 25	9	5	7	17	2			
	ABS 26	44	16	4	20	8			
	ABS 27[2,3]	161							1
	ABS28	18	8	10	33	30			5
	VB 1	28	3	3	11	7			
	FP 1	18	17	2	8	5			
	FP 2	43	30	8	20	20		1	
	FP 3	8	3	6	2	3			
	FP 4	36		28	14	15	1 (0)		
	NNK 1	16	8	11	10	3			
Barbados	BAR1[2]	31	20	1	29	10			
	BAR2[2]	81	67	49	45	15			
Puerto Rico	PR 1	33	14		6	8			
	PR 2	47	1	6	47	10			
	PR 3	33	4	32	24	5			
	PR 4	49	4	47	43	10			
	PR 5	18	2	12	24	14			
	PR 6	71	39	138	57	20			
	PR 7	15	4	7	19	6			
	PR 8	90	20	77	73	25			
	PR 9	94	45	132	61	20			
	PR 10	46	20	36	55	15			
	PR 11	9	2	1	13	6			
	PR 12	29	12	3	13	10			
	PR 13	87	34	80	54	20			
	PR 14	37	16	30	35	10			
	PR 15	47	21	32	33	18		1	
	PR 16	57	14	86	85	18			
	PR 17	106	9	61	58	5			
	PR 18	36	10	3	30	24			
	PR 19	41	11	14	46	13			
	PR 20	10	2	10	19	6			
	PR 21	50	34	77	55	23			
	PR 22	90	28	54	75	25			
	PR 23	118	49	110	85	15			
	PR 24	31		4	8	25			
	PR 25	43	41	48	70	15			
Mean		43.8	15.3	24.5	29.5	12.7	1.6	1.0	4.1
± SD		31.0	14.0	33.3	22.0	7.1	0	0.3	2.2

[1]: number of dealate queens is given in parenthesis
[2]: colony composition determined one week after collection
[3]: number of callows and brood not counted

Table 2.1 Composition of 61 colonies of *P. punctata* immediately after collection from different field locations (ABS = Archbold Biological Station, FL; VB = Vero Beach, FL; FP = Ft. Pierce, FL; NNK = No Name Key, FL; BAR = Turner's Hall, Barbados; PR = El Verde, Puerto Rico). Means and SD are provided for each category.

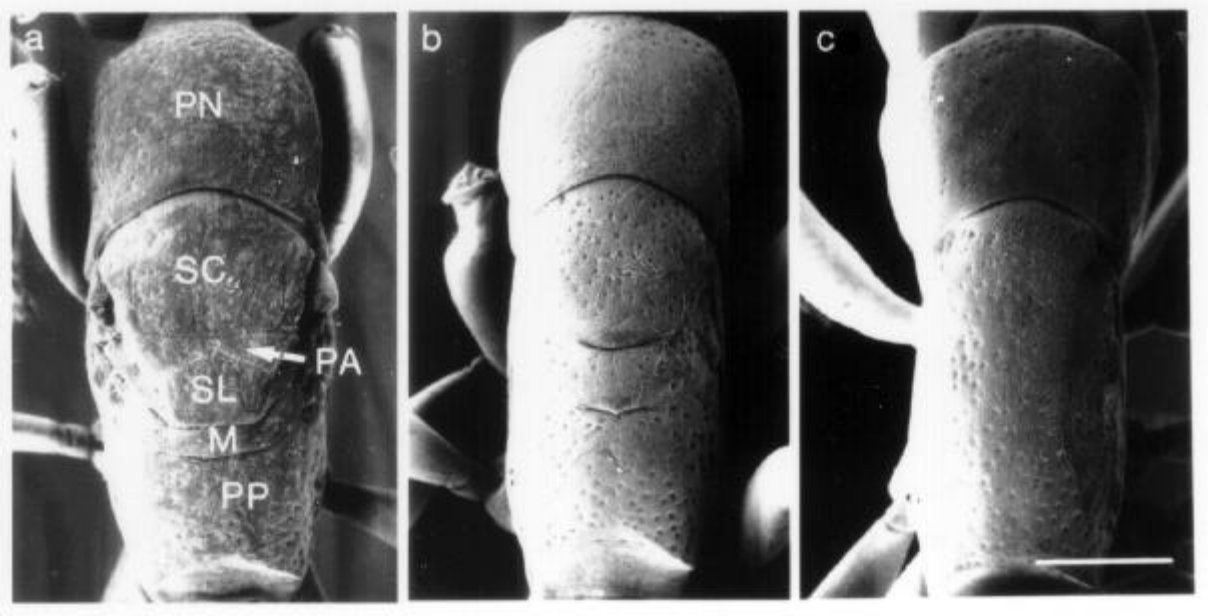

Figure 2.7 Scanning electron micrograph in dorsal view showing the alitrunk of *P. punctata*: a) dealate queen, b) intercaste and c) worker. Note the variable degree of fusion in the thoracic sclerites and their relative proportions. Mesonotum consists of scutum, scutellum and parapteron. Meso- and metanotum and propodeum of workers are fused to one 'megasclerite'. (PN = pronotum; SC = scutum; PA = parapteron; SL = scutellum; M = metanotum; PP = propodeum). Bar length is 500 μm.

No intercastes were found in colonies collected in Barbados and Puerto Rico but have been collected from various other locations in Florida (Deyrup, pers. comm.). To test whether rearing temperature would have an effect on intercaste production, 4 colonies (ABS 4, ABS 6, ABS 10 and PR 13) were kept at 10°C for several month. All colonies continued to produce eggs. However mortality was extremely high and no workers eclosed.

Upon closer inspection in one colony from Florida (ABS 18) one mosaic individual was found with a worker thorax morphology on one side and queen characteristics on the other size. Its cuticle was dark brownish and not fully hardened characteristic of callows. The individual possessed wing buds (wings had already been shed or have been lost during transport) on the queen side which was sculptured into the thoracic sclerites as described above. The rest of the body was clearly worker-like. Following current nomenclature a female containing patches of tissue of both queen and worker is called 'gynergate' (Hölldobler and Wilson 1990). Dissection of the gynergate revealed undeveloped ovaries with 6 ovarioles as found in other females.

Worker size polymorphism is not very pronounced in *P. punctata*. Worker size was unimodally distributed with a mean Weber's thorax length (WTL) of 2.33 ± SD 0.09 mm (n = 231) (Fig. 2.9). Intercaste WTL (2.40 ± SD 0.06 mm, n = 13) was not different from that of workers ($t = 0.371$; $df = 239$; $p = ns$). Similarly, queens were not larger than an average worker (WTL 2.31 ± SD 0.05, n = 7; $t = 0.280$; $df = 236$; $p = ns$).

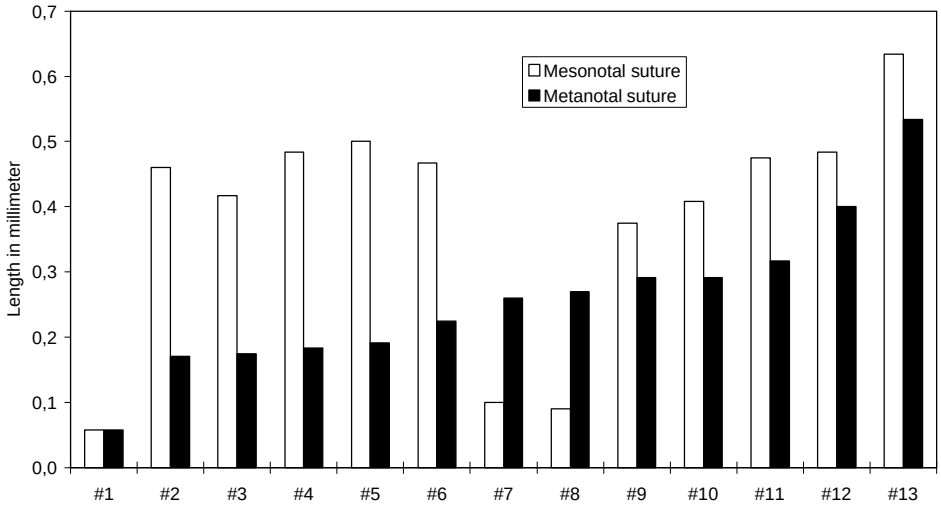

Figure 2.8 Variation in length of mesonotal suture and metanotal suture in 13 intercastes of *P. punctata*. Individuals are arranged according to increasing length of the metanotal suture.

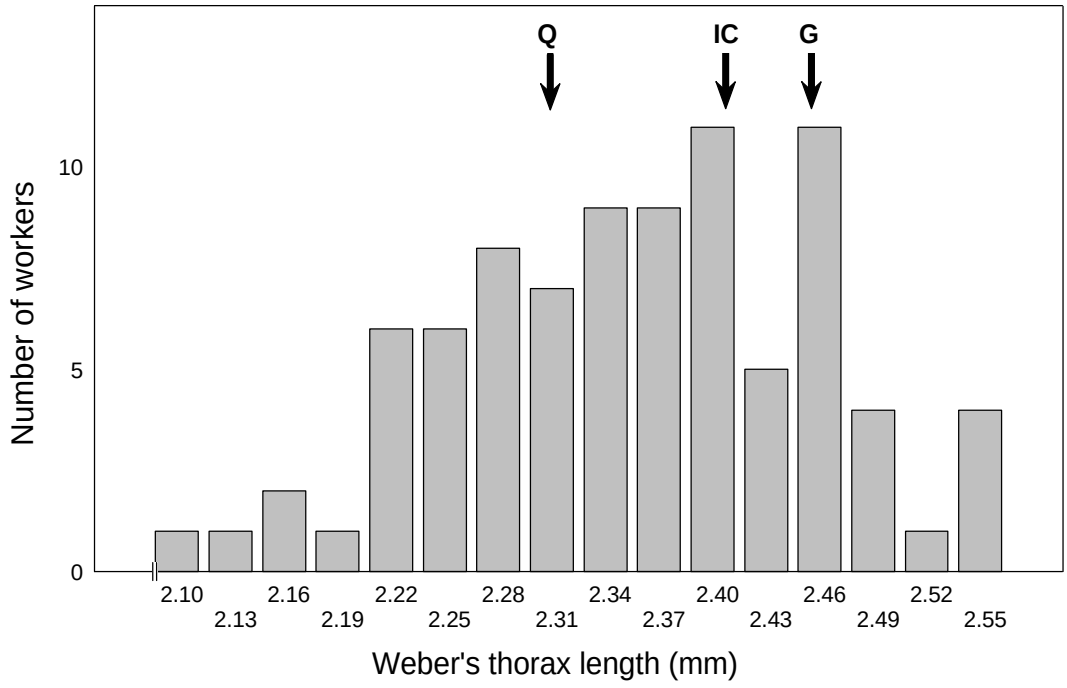

Figure 2.9 Worker size distribution measured as Weber's thorax length (n = 231). Arrows indicate the mean thorax length of intercastes (IC), of queens (Q) and of one gamergate observed in colony ABS 13 (G) for comparison.

2.6 Nestmate recognition

Some workers originating from freshly collected colonies were tested for the occurrence of nestmate discrimination in a small plastic arena several days after collection from the field. Pairs of workers originating from two colonies collected at Archbold Biological Station (ABS 8 and ABS 9) did not engage in any aggressive interactions but showed mutual inspection behavior. Pairs of workers drawn from ABS 8 and a colony collected at Ft. Pierce on the East coast of Florida however attacked each other. Aggressive behaviors consisted of frequent antennations and biting targeted at legs and other body parts of the opponent. In order to investigate whether the ability for colony recognition is influenced by environmental homogeneity workers from several colonies were tested more systematically under laboratory conditions.

In all of 50 dyadic encounters conducted in the laboratory the ants interacted in the arena. In none of the encounter tests however agonistic interactions, as see directly after collection above, could be observed. Dyadic encounters were rather brief and characterized by reciprocal avoidance. Individuals interacted on average 8.7 ± SD 3.2 times within the observation period. Most of the interactions observed (n = 435) involved only brief antennal contact of the initiating ant with a body part of the other individual before the first continued exploration of the arena. The antennated individual usually continued either with its current behavior (73.1 percent) or remained motionless during the encounter (21.4 percent). In the remaining 5.4 percent the antennated worker withdrew her antennae or crouched down on the ground. The encounters rarely lasted for more than 1 or 2 seconds. Individuals instead spend most of the time exploring the arena or remaining inactive.

The frequency of interactions was rather homogeneous between the various combinations of colonies, ranging from a maximum of 17 during an encounter involving individuals from colonies ABS24 and FP2 (type I) to 4 during several type II encounters. In each of five replicates performed for any combination of colony encounters both individuals were equally likely to initiate an encounter (Mann-Whitney U-test, p = ns in each case). Mean percentage of antennal contacts, antennation and other behaviors such as withdrawal of the antennae between individuals taken from colonies within the same population (type I tests) was not significantly different from the frequency of interaction between individuals taken from alien colonies originating from different populations (type II tests; Mann-Whitney U-test, p = ns) (Fig. 2.10).

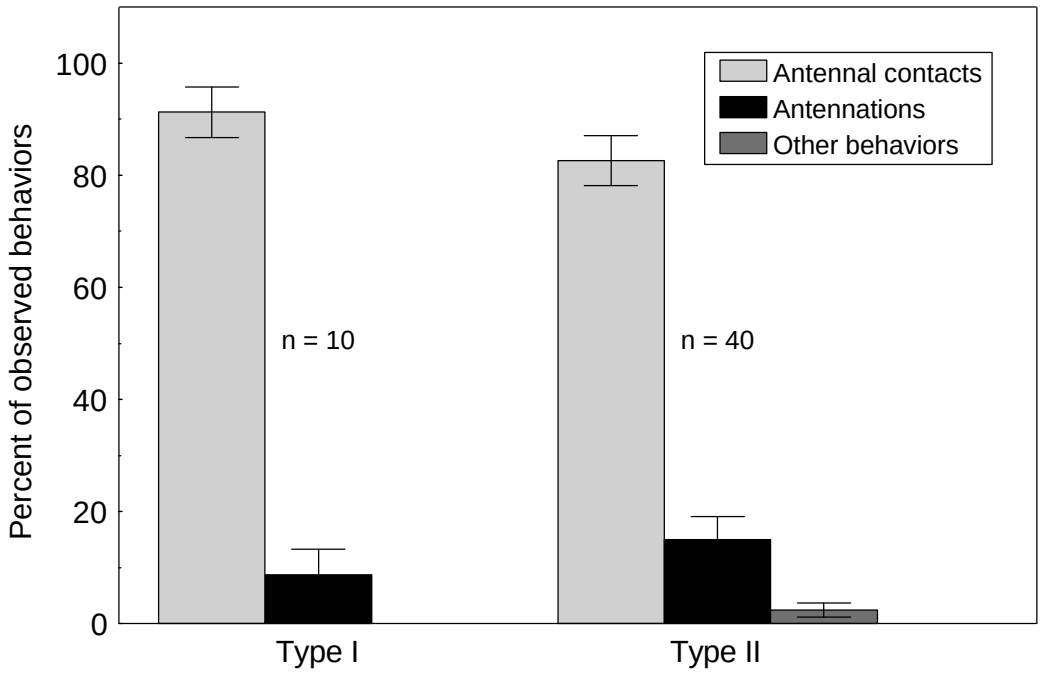

Figure 2.10 Percentage of interactions by antennal contacts, antennations or other behaviors such as crouching or withdrawal for type I and type II encounters in the laboratory (given is the mean ± SE). Differences are not significant.

2.7 Division of labor

The division of labor was examined in colony ABS A collected from Archbold Biological station, a small queenless colony containing 14 workers at the beginning of observations. A total of 36 scans were conducted during a period of 28 days. Within this period observations were conducted on 9 different days. No aggressive interactions were observed among colony members. Similarly no oviposition was observed but worker BBB once carried an egg that might have been recently laid by her or another colony member. Observed behaviors were grouped into 8 behavioral categories covering all observed scans. Following cluster analysis with a Euclidian distance of 11 arbitrarily defined as cut-off level two main groups A (n = 11) and B (n = 5) could be identified based on individual's behavior (Fig 2.11). The smaller group B was less clearly defined and included worker RRG who was exceptional in that she remained inactive for 61.1 percent of total scans.

More detailed analysis of individual profiles using principal component analysis revealed further differences: Using the first and second of three principal components extracted (total combined variance explained: 60.8 percent) the two groups already distinguished in the cluster analysis were re-identified. Individuals were most clearly separated by the degree with which they performed brood care and the time they spend close to the brood pile (principal component 1), with workers within the smaller group B performing most of these behaviors (Tab. 2.2). Additional differences between groups existed according to grooming behavior (principal component 2) or general inactivity (principal component 3) but were less pronounced. RRG, although remaining close to the brood pile most of the time (25.0

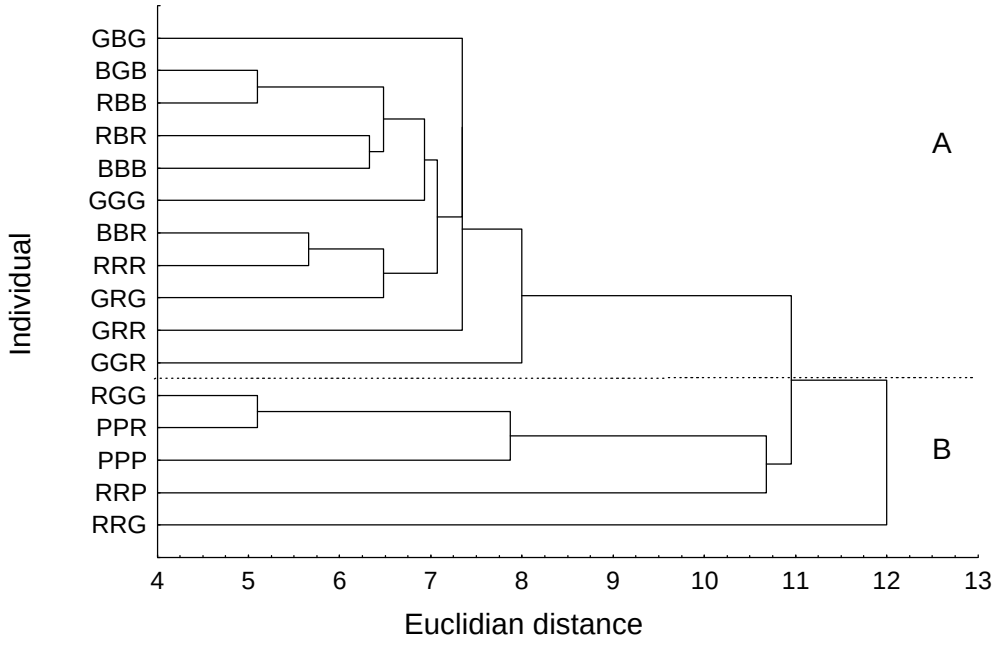

Figure 2.11 Division of labor in a small queenless colony of *P. punctata*, based on hierarchical cluster analysis of individual ethograms comprising 8 behavioral categories (single linkage). Group A represents domestic workers and foragers, group B domestic workers and nurses.

percent of observations), was exceptional in showing a high percentage of inactivity (61.1 percent). Workers within the larger group A represent individuals mainly performing domestic tasks (39.3 ± 12.9 percent), nursing (14.7 ± 19.7 percent) and foraging (6.4 ± 4.3 percent), members of group B concentrated on nursing (61.2 ± 39.3 percent) and to a lesser extend on domestic tasks (15.7 ± 21.9 percent). The reproductive individual within the colony however became not obvious through a distinct behavioral profile. Since the colony was not sacrificed to dissection after the termination of the observations, the reproductive individual in ABS A could not be identified. During video observations in thelytokous worker groups (chapter 3), the reproductive worker usually remained inactive in close proximity of the brood pile, did rarely interact with nestmates and upon colony disturbance behaved timidly.

	Principal Component		
Behavior	1	2	3
Locomotion in the nest	-0,57	-0,03	-0,59
Inactivity in the nest	-0,32	-0,33	**0,80**
Self-grooming	-0,06	**-0,86**	-0,17
Foraging	-0,66	0,49	0,31
Brood care	**0,83**	0,18	-0,43
Inactivity on brood pile	**0,90**	0,06	0,26
Guarding	**-0,82**	0,41	-0,18
Grooming	-0,56	-0,48	-0,18
Eigen value	3,3	1,5	1,4
Percent variance explained	41,8	19,0	17,9
Percent cumulative variance	41,8	60,8	78,7

Table 2.2 Factor loadings of 8 behavioral categories in a small queenless colony of *P. punctata*, based on principal component (PC) analysis (centroid method). Characteristic factors loading strongly on single variables are shown in bold. Groups A and B were most clearly separated by PC1.

2.8 Discussion

The neotropic ponerine ant *Platythyrea punctata* shows a remarkable complexity of reproductive tactics. In addition to reproduction by mated workers (gamergates) and presumably also queens, thelytokous parthenogenesis occurs, i.e., unmated workers produce diploid offspring from unfertilized eggs. Whether intercastes (morphological intermediates between workers and queens), unmated or mated queens are also capable of thelytokous reproduction, is unknown. Unmated queens of the Cape honeybee *Apis mellifera Capensis* will produce females by thelytoky, but it is unknown whether fertilized queens do so as well (Ruttner and Mackensen 1952). Furthermore, as in most other species of Hymenoptera, at least some unmated individuals are capable of producing males from unfertilized eggs by arrhenotokous parthenogenesis.

The life cycle of *P. punctata* in the investigated populations, however, is characterized by the predominance of queenless colonies. Due to the absence of queens in most colonies collected in Florida and all colonies obtained from Barbados and Puerto Rico, colony fission appears to be the predominant mode of colony propagation in *P. punctata*. Queens have not been observed to fly in the field nor in the lab although they have occasionally been found in light traps (Torres, pers. comm.). The lack of ocelli, a rather slender thorax and poor wing development (Brown 1975; Heinze and Hölldobler 1995) at least suggests that they are not very good dispersers. Males, on the contrary, are perfectly able to fly and may

provide for outbreeding opportunities. A high risk of frequent destruction of nest sites in dead branches or tree cavities due to flooding or tropical storm damage may have additionally let to the evolution of colony fission. In Florida and within the Caribbean tropical storms and hurricanes frequently damage forest habitat thereby destructing nesting opportunities suitable for *P. punctata*. Usually ponerine ants inhabit simple nests that can be easily abandoned to emigrate to a new nest site (Peeters 1997). Fission has the advantage that in risky environments new nests start off with an already established work force. An additional advantage arises regarding the risks of foraging. The founding stage in ponerine ants is usually semiclaustral, i.e. foundresses will have to leave the nest to forage. The risks of post-dispersal predation, intercolonial aggression and parasitism during foraging are quite considerable (Schmidt-Hempel and Schmidt-Hempel 1984; Hölldobler and Wilson 1990). In fission groups the risky task of foraging could be fulfilled by non-reproductives thus enabling reproductives to remain within the shelter of the nest.

Both workers and queens have a spermatheca and are capable of mating, but the frequency of inseminated individuals in the four studied populations was low, suggesting that sexual reproduction is only of minor importance. Whether queens or workers mate regularly is therefore still unknown. Furthermore, it still has to be investigated whether inseminated workers and queens use the sperm in their spermathecae to fertilize their eggs. The significance of sexual reproduction in *P. punctata* will be discussed in greater detail in chapter 6.

In *P. punctata* colony fission seems to be linked to a reduction in the production of sexuals in a large part of the species' distribution. The majority of ponerine ant species which exhibit colony fission either have ergatoid, worker-like queens, or completely lack the queen caste (reviewed in Peeters 1993). Once fission has evolved, the necessity to produce winged reproductive units may be diminished. In the Austral-Pacific *Rhytidoponera impressa* complex for example, queens are absent in some species while in others both queenright and queenless colonies co-occur (Ward 1981a, 1981b, 1983a, 1983b). Due to special idiosyncrasies of this group such as a simple a nest structure, the need for frequent nest relocation and outbreeding by workers in this species complex exhibits an evolutionary inclination for the loss of queens. Thelytoky may pose an additional evolutionary advantage to species which undergo colony fission: In times when no males are available, e.g. after a sudden colony fragmentation or destruction of the natal nest due to adverse environmental conditions or following a natural disaster, workers capable of thelytokous reproduction have a strong selective advantage because they directly add to the work force of the colony. Virgin workers in gamergate species still would have to find a suitable (outbred) mate to start sexual reproduction. Harsh winter climates in the Florida population and tropical storms regularly occurring throughout the range of *P. punctata* may have provided the selective regime under which colony fission and possibly thelytoky were established in this species. Nest relocation is frequent in the Japanese thelytokous ant *Pristomyrmex pungens* with an average half life of the nest of 8.4 days (Tsuji 1988b). Although mean colony size is about 22.000 workers, frequent relocation is

possible because nest are of low value and emigration includes a low risk of loss of reproductives since all workers are potential replacement reproductives. In the facultatively thelytokous ant *Cataglyphis cursor* it has been suggested that workers groups leaving the mother nest could produce females by thelytoky who would subsequently mate with males from neighboring colonies (Lenoir *et al.* 1987). Orphaned colonies however have never been observed in the field. In general colony dispersal by fission is limited. Daughter colonies will remain in the vicinity of their natal nests and therefore enhance local competition for resources (Bourke and Franks 1995). In addition workers do not accumulate storage proteins as do queens before they leave their natal nest. Founding success after colony fission is expected to depend on a certain critical size of the worker group: Experimental single worker foundations in *Harpegnathos saltator* were less successful than groups of three workers (Liebig *et al.* 1998).

So far there is only little information available on the natural frequency of colony fission and on the size of newly budded fragments in *P. punctata*. In theory even small groups of workers may be able to successfully found a new colony thelytokously. This has been equally suggested for isolated worker groups in the thelytokous ant *Cataglyphis cursor* (Lenoir and Cagniant 1986). The smallest complete colony of *P. punctata* (FP 3) was collected from Fort Pierce, Florida and contained eight workers (Schilder *et al.* 1999a). Almost 30 percent of all colonies had less than 20 workers although colony fission may lead to much larger fragments. In the literature there is only little evidence for the ability of single workers to successfully rear offspring (Ward 1981b; Liebig *et al.* 1998). Given the high risks during the independent founding stage this strategy evidently would not be evolutionary stable. Colonies of *P. punctata* containing queens were generally larger indicating that a certain critical size may be necessary for queen production after fission has taken place. Especially in small colonies an extreme energetical burden would select against producing sexuals. This is consistent with patterns of sexual production in several ant species in which only large colonies produce queens and males (Hölldobler and Wilson 1990). Likewise in *P. quadridenta* from Western Malaysia only the largest colonies contained queens (Ito 1994). Colony fission in *P. punctata* likely serves as the usual mode of colony propagation but occasionally the production of winged sexuals could facilitate the colonization of new habitats through long distance dispersal and semiclaustral founding (Hamilton and May 1977). Fission by itself would lead to the reduction of new queens produced because an existing colony will only be able to divide in two or three parts (Franks and Hölldobler 1987). Once fission regularly occurs the production of winged sexuals is no longer adaptive (summarized in Tinaut and Heinze 1992). Reproduction by ergatoid queens, gamergates or even by thelytokous parthenogenesis thus could have evolved to channel more of the colony resources towards the production of workers.

In 16 of a total of 34 Florida colonies a rather large number of individuals was found, which were morphologically intermediate between the two castes. Peeters (1991a) suggests to refer to wingless females with a morphologically variable phenotype more or less between those of normal queens and

workers as "intercastes". In most species, intercastes arise from accidental deviations from normal caste differentiation (Wheeler 1928). In contrast, the regularly occurring female reproductives in some Ponerinae (e.g. *Ponera pennsylvanica* (Holiday 1903); *Hypoponera bondroiti* (Yamauchi *et al.* 1996) or *Pachycondyla obscuricornis* (Düssmann *et al.* 1996)) and other subfamilies have also been referred to as intercastes. Recently, Heinze (1998) has questioned whether a distinction between "reproductive intercastes" and "ergatoid queens" is helpful and instead suggested to distinguish between accidental deviations from normal caste differentiation and regularly occurring wingless female reproductives. The replacement of winged queens by wingless reproductives seems to depend more on environmental conditions than on the developmental pathway involved (Heinze 1998). On the basis of the data currently available, one cannot finally judge whether morphological intermediates in *P. punctata* regularly serve a specific reproductive function or are merely an accidental by-product resulting from deviations during normal caste differentiation. Similar to regular workers they may at least occasionally reproduce by thelytoky, complicating the decision. Their relative paucity in the Florida samples and their complete absence from all Puerto Rican colonies nevertheless argues in the latter direction. These individuals are therefore referred to as intercastes following Heinze (1998). More pronounced seasonal variation in climatic conditions with temperature below freezing during the winter months may lay a stronger energetic burden on brood development in Florida as compared to the two other field sites Barbados or Puerto Rico and may therefore lead to an increase in intercaste occurrence. Brood rearing experiments under controlled temperature conditions however failed to show whether low ambient temperatures during critical brood rearing periods may cause an increase in intercaste production.

Mean monthly temperature measured at Archbold Biological Station however provide indirect evidence for a strong effect of minimal winter temperatures on colony development and survival. While colonies of *P. punctata* were abundant at Archbold Biological Station following the winter of 1993/94, only one colony was collected two years later from a location on the east coast. The average minimal monthly temperature for the period from November 1995 to February 1996 was much lower as compared to the five preceding winters. Extreme winter climates may be limiting the distribution of *P. punctata* further north and seriously diminishing marginal populations such as those in Central Florida. *P. punctata* has until now not been recorded from any location north of a line connecting St. Petersburg and Vero Beach (Deyrup *et al.* 1989; Deyrup, pers. comm.). Whether further distribution is limited by climate or by the lack of suitable habitat is however unknown. Interestingly, intercastes have also been found in several colonies of *Odontomachus clarus* colonies (Hölldobler and Heinze, unpubl. obs.) from Central Florida equally suggesting a temperature effect. In addition, larger colonies may be able to allocate more resources to brood rearing compared to smaller colonies resulting in a decrease in intercaste number as suggested by the negative correlation between colony size and intercaste number in Florida.

Gynergates (female mosaics with both queen and worker characteristics) are one of several rarely cited examples of a variety of developmental abnormalities found in social insects. Gynandromorphs (mosaics of both male and female tissue) are more frequent and have been described from over 42 ant species in 22 genera (Jones and Phillips 1985). The frequency of their occurrence in natural populations seems rather low (for an exception see Kinomura and Yamauchi 1994). The physiological process of gynergate formation is still unclear. In contrast to a haploid-diploid mosaic of male and female tissues in gynandromorphs, gynergates are all-diploid. Explanations assuming the initial presence of both an unfertilized and a fertilized nucleus in an egg cell or chromosomal non-disjunction in males resulting in female cell lines at a later stage may not be able to explain a pure female mosaic (Crozier 1975; Taber and Francke 1986). Mosaic females presumably arise from fertilization of both nuclei in a binucleate egg - a very rare event given the rarity of binucleate eggs and the low incidence of bispermy (Crozier 1975). Gynergates in *P. punctata* and in other species may equally arise when queen-determined individuals experience averse temperature conditions or a nutrient shortage during their ontogeny (quite similar to intercastes). This may interfere with the regulation of those genes responsible for the development of queen characteristics. Therefore gynergates should be more frequently occurring in marginal habitats of a species distribution. Limited sample size in most cases does however not allow a solid conclusion. As in most other ants, the mechanism of caste differentiation in *P. punctata* still remains subject to discussion (Oster and Wilson 1978; Hölldobler and Wilson 1990).

Although workers of field colonies showed agonistic behaviors towards alien conspecifics right after collection, intercolonial intolerance was virtually absent in lab-reared colonies both in intrapopulation (type I) and interpopulation (type II) encounters. Under laboratory cultivation the ability to discriminate non-nestmates on the basis of environmental cues is normally greatly reduced due to the uniformity of external conditions including diet, ambient odors and nesting material (Obin 1986). This is quite similar to what is observed in *P. punctata*: Nestmate recognition based on environmental cues, although existing in field-collected colonies, is obviously lost after prolonged lab culture. In contrast, workers of other ponerine ants in which nestmate recognition was studied aggressively reacted to the introduction of non-nestmates. Nestmate discrimination has been examined only in few species including *Odontomachus bauri* (Jaffe and Marcuse 1983), *Pachycondyla crassinoda* (Silveira-Costa and Moutinho 1994), *Rhytidoponera confusa* (Crosland 1989) and *Rhytidoponera* sp. 12 (Peeters 1988). The existence of strong nestmate recognition even under uniform lab conditions (i.e., in the absence of strong environmental cues) is generally taken as evidence for the importance of genetic determinants resulting in differential phenotypic expression of chemical cues (reviewed by Waldman *et al.* 1988). Are the reproductive peculiarities of *P. punctata* responsible for the obvious reduction in nestmate recognition? If discrimination of conspecifics is genetically based, related nests (in the case of budding or fission being identical to neighboring nests) should share more recognition cues and therefore behave

less aggressive towards each other. It has to be mentioned that for polygyne colonies quite the opposite has been suggested: Following the discriminator hypotheses nestmate recognition should be reduced as a consequence of increased within-colony genetic variation due to a possible dilution of nestmate discriminators (e.g. Breed and Bennett 1987). Supporting the first scenario, in *Formica polyctena* and *F. pratensis* genetic similarity has a strong negative influence on aggressive behaviors towards non-nestmates (Beye *et al.* 1997, Beye *et al.* 1998). Both Nei's genetic distance and spatial distance correlated with observed agonistic behaviors. Additionally it has been shown quite recently that nestmate recognition systems are likely to rely on cuticular hydrocarbons that serve as colonial recognition discriminators (Liu *et al.* 1998; Vander Meer and Morel 1998; Lahav *et al.* 1999). The cuticular chemistry is closely linked to the physiology of the postpharyngeal gland (Bagnères and Morgan 1991). In a number of species the secretions from the postpharyngeal gland proximately mediate nestmate recognition (Soroker *et al.* 1994; Hefetz *et al.* 1996). As will be discussed in chapter 4, no pronounced intrapopulation genetic differences are present between colonies of *P. punctata* on which such a heritable recognition system could be based. Populations are virtually clonal. Lack of intercolonial nestmate recognition may, by inference, be attributed to the lack of genetic variation resulting in the homogeneous expression of cuticular hydrocarbon constituents and the subsequent failure to express nestmate recognition in *P. punctata*.

The absence of a morphologically distinct reproductive caste in *P. punctata* does not lead to an unusual division of labor but rather follows a pattern described for several ponerine ants (e.g. Fresneau and Dupuy 1988; Corbara *et al.* 1989, Corbara *et al.* 1990; Villet 1992). All workers within the colony remain quite flexible in the performance of the respective tasks. In *P. punctata* only two different social roles could be identified: Those that performed predominantly brood care and domestic work and those that performed various domestic tasks and foraging-related activities. The observed pattern of task allocation in *P. punctata* resembles that found in other ponerines: In *Pachycondyla apicalis* for example most workers fit one of only three task groups (brood care, non-social activities inside the nest and foraging) with only few workers remaining that have intermediate profiles (Fresneau and Dupuy 1988). In *Ponera pennsylvannica* workers can be roughly classified into three behavioral groups (brood tenders, foragers and those performing both grooming and nest maintenance) although they were highly variable in their composition (Pratt *et al.* 1994). Individual workers appear to respond rather flexibly to colony needs. Variation and flexibility in the role individual workers fill, caused by differences in the worker's ages, sizes or genotypes, results in a flexible and efficient system of self-organization (Bourke and Franks 1995). In general great individual variability as is conspicuous in several ponerine ants, may be due to the small colony sizes (Peeters 1997). The lack of a temporal division of labor in *Amblyopone pallipes* has been attributed to its small colony size and the peculiar age structure of cohorts of similarly aged workers (Traniello 1978). Subsequent observations however detected some behavioral task groups similar to those of other ponerine ants (Lachaud *et al.* 1988). The reproductive worker in colonies of *P.*

punctata was not clearly behaviorally distinct from non-reproductive nestmates. However, she appeared to participate only little in colony labor and to remain in close association with the brood. More detailed investigations on the division of labor are necessary to substantiate these preliminary observations.

Within the tribe Platythyreini similar social roles have been identified in the queenless African species *P. lamellosa* (Villet 1990), *P. cribrinodis* (Villet 1991a) and *P. schultzei* (Villet 1991b). In all of these species however the reproductive role is filled by gamergates. Gamergates replacing queens similarly resemble ponerine queens in their social roles and have a behavioral profile distinct from that of sterile workers (Peeters 1997). In the genus *Platythyrea* they monopolize the care of eggs, rest in characteristic posture on the egg pile or tend larvae more often than their nestmates (Villet 1992a). Even in the phylogenetically most primitive subfamily of ants, the division of labor, although more flexible as compared to higher ants, seems to play a major role in the regulation of social organization and colony task allocation.

3 Reproductive regulation

3.1 Introduction

The capacity of producing diploid female offspring by thelytokous parthenogenesis is rather rare among the social Hymenoptera (Bandara and Walter 1993, see chapter 5 for a review). It appears to evolve sporadically at the distant tips of phylogenies and therefore is generally assumed to be a transient phenomenon because it is likely to constitute an evolutionary dead end (Maynard Smith 1978). Only in a small minority of ant species, workers have evolved a way to produce diploid offspring without the need for insemination: they are capable of producing workers or queens from unfertilized eggs by thelytokous parthenogenesis (the production of female, diploid offspring from unfertilized eggs). In addition to *Platythyrea punctata* (Heinze and Hölldobler 1995), obligate or facultative thelytokous parthenogenesis is known from only five other ant species, *Pristomyrmex pungens* (Itow *et al.* 1984; Tsuji 1988a, 1994; Tsuji and Yamauchi 1990), *Cerapachys biroi* (Tsuji and Yamauchi, 1995), *Cataglyphis cursor* (Cagniant 1979, 1982, 1983; Wenseleers pers. comm.), *C. piliscapus* (Lenoir and Cagniant 1986) and, less convincing, in *Messor capitatus* (Grasso *et al.* 1998). Only in *C. biroi* and *P. pungens* thelytoky is obligatory (Itow *et al.* 1984; Tsuji and Yamauchi 1995). There are some earlier reports on thelytoky among the Formicidae in the literature (summarized in Crozier and Pamilo 1996) which have not been confirmed by subsequent experiments (e.g., see Ledoux 1950 and Crozier 1970 for discussion of thelytoky in *Oecophylla longinoda*). Villet (1991a) observed several virgin workers of *Platythyrea* cf. *cribrinodis* to lay eggs of average size in addition to the only gamergate in the colony. Number of eclosing males however was smaller than might be expected from the number of eggs laid (in one colony only nine percent instead of an expected 83 percent males eclosed). Heinze and Hölldobler

(1995) suggested that thelytoky might be involved although this has not yet been investigated in *P. cribrinodis* or any other *Platythyrea* species. Depending on nutritional conditions, workers of *Crematogaster scutellaris* have also been implied to lay diploid eggs yielding queen larvae by deuterotoky (development of unfertilized eggs into either sex), although this observation remains to be confirmed (Soulié 1960).

Reproductive conflict between queens, queens and workers, and among workers is characteristic of many social insect societies. These conflicts arise because in the Hymenoptera the haplodiploid sex determination mechanism causes divergent reproductive interests among nestmates. In particular there is conflict between queens and workers about the relative investment in both sexes and the production of males (Heinze *et al.* 1994). Assuming monogyny and monandry, workers on average are related to sisters by 0.75 but to brothers by only 0.25. Therefore they should favor a colony sex ratio of 3:1 in favor of their sisters. The queen, however, is equally related by 0.5 to her male and female offspring resulting in her preference for equal investment in daughters and sons. If workers would be able to win this conflict, they would be expected to compete for male production. However, in the queenright situation only in a small proportion of ant species workers contribute significantly to the production of males (Bourke 1988b). Queens therefore seem to be well equipped to monopolize reproduction. This queen control could be achieved either by direct physical aggression towards nestmates that initiated the development of their ovaries or through pheromones that chemically inhibit nestmates from egg laying (Wilson 1971). Conflict by physical aggression seems to be limited to species with small colony sizes since it requires repeated interactions among nestmates. In large colonies direct aggression supposedly is not a suitable means to regulate reproduction because agonistic interactions of several hundreds or thousands of individuals would seriously disturb colony efficiency (Heinze *et al.* 1994). Indeed, in *Ropalidia marginata* mean dominance rates of queens decline as colony sizes increases (Ito 1993). In some cases both regulatory mechanisms may occur depending on the social context (e.g. Peeters and Higashi 1989). Physical aggression should be selected against when it becomes detrimental to the overall performance of the colony (Ratnieks and Reeve 1992). In larger colonies, therefore, reproduction is assumed to be regulated by primer pheromones or other chemical cues. There is however a controversy as to whether these pheromones constitute an active manipulation by the reproductive (Wilson 1971) or are honest signals correlating with ovarian activity that are perceived by nestmates and cause them to refrain from reproduction in their own interest (Keller and Nonacs 1993). Active manipulation however does not appear to be evolutionary stable since workers would be selected to evolve countermeasures to esCape reproductive domination. Along these lines it has been suggested that workers may actively police nestmates' reproduction to maximize their inclusive fitness (Ratnieks 1988).

Reproductive conflicts through aggressive interactions among colony members are generally assumed to determine dominance. Individual variation in dominance results in the establishment of dominance hierarchies in which only one or a small group of top-ranking individuals monopolize reproduction (although compare Lambert *et al.* (1994) for a different view). Since the first description of a linear dominance hierarchy in the paper wasp *Polistes dominulus* (Pardi 1948), dominance hierarchies have been regarded as one of the major factors regulating the social structure of Hymenopteran societies (Keller 1993a; Ross and Matthews 1991). In *Polistes* the social rank of an individual is positively correlated with ovarian development. Aggression is expected to be especially pronounced in species were most or all individuals have the same reproductive potential thereby rendering the ratio between potential reproductives and non-reproductives high (Heinze *et al.* 1994). This is the case in polistine wasps (Reeve 1991; Röseler 1991) and some bumble bee species (e.g. Cameron and Jost 1998; Röseler and van Honk 1990), where all females have equal possibility to mate and lay eggs but only few dominant individuals actually reproduce. More recently, in a number of ant species agonistic interactions leading to the establishment of reproductive dominance orders have been observed (Cole 1981). A high rank is usually positively correlated with egg laying frequency although reproductive success may additionally depend on other factors such as oophagy or differential mortality during larval development (Keller 1993b). Only genetic markers can reliably determine the correlation of social rank and reproductive success (e.g. Trunzer 1999).

Conflicts over reproduction lead to the partitioning of direct reproduction among potential reproductives (being it queens or workers). The range from total monopolization by a single female to an (almost) egalitarian partitioning of reproduction among members of the reproductive caste is termed reproductive skew (Vehrencamp 1983a, 1983b; Reeve and Ratnieks 1993; Reeve and Keller 1995). Functional monogyny, i.e. reproduction by a single mated queen in the presence of several mated queens in the colony, reflects one extreme end of the continuum with a reproductive skew index of 1. The partitioning of reproduction among two or more potential reproductives in the colony, termed polygyny, reflects the other end with a skew index of less than 1. Generally, under the optimal skew model, reproductively dominant individuals may allow direct reproduction in subordinates to prevent them from either leaving the colony, the so-called "staying incentive" or to prevent them from aggressively challenging the own dominant position, the so-called "peace incentives" (Reeve and Ratnieks 1993). Both effects can act in combination and are expressed as proportion of overall reproduction yielded to subordinates. A general prediction emerging from the theory states that dominance testing is more likely to evolve as skew indices increase. This should be especially pronounced in situations where ecological constraints (such as high risks of dispersal and low founding success) are strong. The effect of relatedness on skew is less clear. Dominance testing however should be particularly favored in a high relatedness, high skew colony because subordinates refraining from reproduction may still gain high

inclusive fitness benefits (Reeve and Ratnieks 1993). Recently, both additions to optimal skew models (e.g. Clutton-Brock 1998; Johnstone *et al.* 1999; Kokko and Johnstone 1999) as well as alternative models, arriving at contrasting predictions to the original models, have been developed (Cant 1998; Reeve *et al.* 1998).

In *Polistes versicolor* only the dominant female in a nest has fully developed ovaries and monopolizes reproduction while co-founding females possess ovaries in various stages of development (Nascimento *et al.* 1997). In functionally monogynous colonies of *Leptothorax gredleri* and *L.* sp. A, several mated queens compete for reproduction but finally only the *alpha* queen monopolizes reproduction (Buschinger 1968; Heinze 1993; Ortius and Heinze 1999). In several queenless ponerine ants where the reproductive function is filled by mated workers, or gamergates, it is well documented that the colony is functionally monogynous even if more potential reproductives are available (e.g. in *Dinoponera quadriceps* (Monnin and Peeters 1998), *Hagensia havilandi* (Villet 1992); *Pachycondyla sublaevis* (Peeters *et al.* 1991; Higashi *et al.* 1994), *Pachycondyla krugeri* (Wildman and Crewe 1988), *Pachycondyla* sp. (Ito 1993a) and *Streblognathus aethiopicus* (Ware *et al.* 1990)). In the extreme case of *Diacamma* only the single mated reproductive worker retains its gemmae (tiny thoracic appendages). All other workers are mutilated by the gamergate shortly after eclosure and cannot mate or develop their ovaries (Fukumoto *et al.* 1989; Peeters and Higashi 1989; Peeters *et al.* 1992; Tsuji *et al.* 1998).

Within species in the primitive subfamily Ponerinae, different types of female reproductives may co-occur and compete for reproduction. Ordinary, winged queens and mated, egg-laying workers (also called "gamergates“ (Peeters and Crewe 1984)) coexist in *Harpegnathos saltator* (Peeters and Hölldobler 1995) and *Pachycondyla tridentata* (Sommer and Hölldobler 1992). In the latter species, workers (almost all of which are inseminated) and queens engage in dominance contests by rapid antennation bouts and pulling at the opponent’s mandibles. Their rank and not their caste determines who will reproduce and who will not. Similarly among queens of the functionally polygynous ant *Odontomachus chelifer* dominance structure is linked to reproductive status (Medeiros *et al.* 1992).

In a large number of ponerine ant species however mated, reproductively active workers (so-called gamergates) have completely replaced queens (Peeters 1991). Potential for reproductive conflict is maximized since a specialized reproductive caste is eliminated and morphologically identical workers compete for reproduction (Peeters 1993). Here, too, dominance rank may determine the reproductive success of an individual as in other social insects. In the functionally polygynous ant *Amblyopone* sp. from West Java reproduction among several gamergates is regulated by frequent antennations resulting in a linear hierarchy (Ito 1993b). Gamergates additionally inhibit ovarian development in virgin workers. In *Dinoponera quadriceps* (Monnin and Peeters 1998), *Hagensia havilandi* (Villet 1992) and *Pachycondyla sublaevis* (Peeters *et al.* 1991; Higashi *et al.* 1994) workers form almost linear hierarchies but only the top-ranking worker is mated and reproduces. In *Pachycondyla* sp. most of the

colonies contain multiple gamergates but only one high-ranking gamergate, usually a younger individual, reproduces in the colony (Ito 1993a). Lower ranking gamergates were usually older suggesting that these individuals will not be able to take over reproduction even when the dominant gamergate dies. In worker groups of *Pachycondyla villosa* only the top position in the social hierarchy seems to guarantee a high reproductive success with the correlation breaking down at lower ranks (Trunzer 1999).

The neotropic ponerine ant *Platythyrea punctata* Smith, 1858 shows one of the most complex pattern of reproduction so far known in ponerine ants in which unmated workers can reproduce by thelytokous parthenogenesis, but in which also queens, morphological intermediates between queens and workers ("intercastes") and gamergates can co-occur. Therefore there is a multi-fold of possibilities for reproductive competition over the production of female progeny. Queens may compete with mated workers and possibly also mated intercastes for sexual reproduction. Simultaneously one or more females in the colony may reproduce parthenogenetically and thereby competing with mated queens or workers in the colony. Finally, when the colony is orphaned following a period of sexual reproduction, there can be intense competition about asexual reproduction of female offspring between unmated workers. Thelytokous workers, which have been produced by a sexual mother, would profit more from raising own female offspring, to whom they are clonally related ($r = 1$), than to aid sisters in raising their sons ($r = 0.75$). Orphanage and / or colony fission, resulting in a high proportion of all-worker colonies, seems to be frequent and important in colony foundation in *P. punctata* (see chapter 1).

The following experiments are aimed at determining whether aggression leading to social dominance hierarchies regulates reproduction in *P. punctata* in a way similar to that discussed for other ponerine ants. There is preliminary evidence that aggressive interactions indeed result in linear dominance hierarchies, however, their relation to individual reproductive rank remains unclear (Heinze and Hölldobler 1995). To this point, there are only few detailed observations available on the reproductive division of labor in thelytokous systems, e.g. in *P. pungens*, where no dominance interactions have been observed (Tsuji 1988a, 1995).

In more detail, the following hypotheses will be evaluated in this chapter: In thelytokous, i.e. potentially clonal societies, levels of reproductive conflict due to genetic relatedness asymmetries should be diminished and therefore aggression at least be greatly reduced. If aggression still exists among nestmates in *P. punctata*, does it regulate reproduction in thelytokous colonies through the establishment of linear dominance hierarchies? Alternatively the observed aggression could well serve other purposes, one being the social regulation of colony organization and division of labor. Before turning to reproductive regulation this chapter will establish whether thelytokous parthenogenesis exists in a broader sample from three natural populations of *P. punctata* and whether it constitutes a viable mode of reproduction in all-worker colonies under laboratory conditions.

3.2 Materials and methods

3.2.1 Colony dissection

The reproductive organization of field colonies was examined by completely dissecting 9 colonies both from Florida and Puerto Rico kept in the laboratory for 4 to 9 month. An effort was made to collect all individuals found dead. They were frozen at -20 °C and later dissected in insect ringer (8.8 g NaCl, 0.2 g KCl, 2.3 g TES, 8.6 g Sucrose, ad 1 l water) or de-ionized water under a stereo microscope (Wild M3Z) to determine reproductive status of the individuals. For dissection forceps were used to gently pull the last three abdominal plates with the attached reproductive tract and sting apparatus out while holding the tubulate abdominal segment IV with another forceps. Ovarian activity was assessed by recording the following parameters: number and length of ovarioles, number of mature (basal oocytes as long as an egg, with a distinct chorion) and immature yolk-containing oocytes inside the ovaries, and presence of yellow bodies (corpora lutea) at the base of paired ovaries. Yellow bodies are remnants of eggs already laid or resorbed. Only the longest ovariole in the left and right ovary was measured since the length of ovarioles differed only slightly within an individual. Combining all parameters, individual ovarian development was scored into four types modified after categories introduced by Heinze *et al.* (1992):

Type I completely undeveloped ovaries without any oocytes or yellow bodies,

Type II ovaries containing one or more immature yolky oocytes, but no yellow bodies,

Type III degenerated ovaries with remnants of resorbed oocytes, and

Type IV reproductively active ovaries containing maturing yolky oocytes and yellow bodies at the basis of the ovarioles (no mature oocyte may be present right after oviposition).

3.2.2 Experimental worker groups

Thelytokous parthenogenesis among workers was examined by isolating groups of 10 laboratory-reared callow workers together with three pupae and two last instar larvae in plaster nests. The callow workers (i.e., newly eclosed workers with soft and lightly pigmented cuticle) certainly were virgins because no males occurred in the source colonies as long as the callows were present. A total of 21 isolates were set up, 15 from colonies collected at ABS, 1 from Barbados, and 5 from Puerto Rico.

Subsequently, brood development was recorded twice per week over a period of 18 weeks. Individuals found dead during observation were frozen for future dissection. After observations ended, 18 of 21 isolated worker groups were frozen and later dissected. Dissections were carried out as described in the previous section.

3.2.3 Behavioral observations

Reproductive regulation in clonal societies was investigated in 10 established groups of laboratory-reared virgin workers initially set up as described above. 3 originated from colonies collected at ABS, Florida, 1 from VB, Florida, and 6 from Puerto Rico. All experimental groups were subsequently monitored for the occurrence of males to exclude the potential insemination of the reproductive worker through intranidal mating. All individuals including eclosing callows were individually color-marked (Edding paint marker) dorsally with three color spots (codes used: B blue, G green, O orange, P purple, R red, W white, Y yellow) on thorax and petiolus (Fig. 3.1). Eclosed workers were marked as long as their cuticle was still brownish, however re-identification of those unmarked callows that were observed to perform agonistic behaviors during video observations was not possible in all cases. Behavioral observations were conducted using time-lapse video recordings (Panasonic F15HS and AG-6730). 24h time-lapse video recording yielded a resolution fine enough to distinguish individuals, record their behavior and track the fate of eggs laid in the colony. Additional ad

Figure 3.1 Individually marked experimental group of *P. punctata*.

libitum observations were carried out to help identify the current egg-laying individual if it could not be determined unambiguously from the time-lapse observations. Observations were conducted before and after experimental removal of the dominant egg-laying individual in each worker group. Individuals found dead during the whole observation period were frozen for future dissection. After observations ended, all experimental groups were frozen to determine individual reproductive status.

During the course of an initial real-time observation period five distinct agonistic behaviors with dominant aggressive character were identified to occur in *P. punctata*. These agonistic interactions were subsequently used to describe agonistic dominance interactions in the virgin worker groups in the lab and therefore are described in detail in the following section. These behaviors are regarded as being reflective of the dominance of the initiating individual (the one that started the interaction) because they usually were directional and not retaliated by the opponent within the course of the agonistic interaction. On subsequent agonistic interactions of the same individuals however dominance may be reversed repeatedly. Since no case of severe injury resulting in the death of one of the opponents was observed these interactions appear to be ritualized and therefore may be considered as a dominance display rather than an actual fight.

Antennal boxing:

An individual approaches and rapidly pummels typically the head, the thorax or on fewer occasions the gaster of the opponent in short bouts with the tips of its antennae. The opponent may stand still, crouch down with her antennae drawn back- and sideways towards the head, withdraw at a later point or ignore the display and carry on with its current activity. After the display is terminated the initiating ant may groom various parts of the opponent's body while she remains motionless.

Biting:

An individual bites the flagellum of one antenna, a leg or less frequently another part of the opponent's body with its mandibles while antennal boxing on the head and/or thorax is performed. The initiating ant usually frontally targets the distal antennal segments of the opponent. During the course of this behavior the initiating ant may release its grip, move along the body of the opponent and bite another body part such as a leg. On some occasions a more intense form of biting was observed with the initiating individual bending its gaster forward between her legs, exposing its dorsal region towards the opponent The opponent may stand still, crouch down, withdraw at a later point or ignore the antennation and carry on with its current activity. After the behavior is terminated the initiating ant may groom various parts of the opponent's body.

Dragging:

While this behavior starts out similar to the biting sequence described above it gains intensity since the initiating ant will drag the opponent for a variable distance through the colony while retaining a firm grip onto the opponent's body. The opponent usually crouches down and may withdraw at a later point.

Leap:

The initiating individual rapidly lunges towards the opponent with its mandibles open typically across a distance of more than half a body length. Usually this behavior is followed by antennal boxing or biting described above.

Immobilization:

One individual may also stand motionless on top of another for variable time span. The immobilization may follow any of the other agonistic behaviors described above. If immobilization follows biting the initiating individual may stop antennal boxing on the opponent but may still retain a firm bite. The opponent usually crouches down and may withdraw at a later point.

In addition to these five agonistic behaviors with a clear dominant character four submissive behaviors were recorded. Although after antennal boxing and biting the receiving individual could be performing any of these behaviors the more intense agonistic interactions involving dragging and leaps led to crouching or withdrawal of the submissive individual.

Ignore:

The opponent ignores the agonistic behavior of the initiating ant and carries on with its current behavior. This behavior often leads to the termination of the agonistic behavior after a brief time span.

Standing still:

Upon initiation of an agonistic interaction the opponent stops its current activity and stands motionlessly while the initiating ant may perform any of the agonistic behaviors. This behavior may be followed by a withdrawal.

Crouching:

Upon initiation of an agonistic interaction the opponent retracts its antennae and legs and crouches down on the ground adopting a pupal-like posture. The opponent often remains frozen in this posture for a variable time span after the initiating individual stopped its behavior. This behavior may be followed by a withdrawal.

Withdrawal:

Rapid movement of the opponent away from the initiating individual at any time in the course of the interaction. Usually the opponent moved away over a distance of more than one body length, often through most of the nest.

Only those agonistic interactions where one individual (in most cases the initiating one) clearly dominated the other were subsequently included in the analysis. This was however true for more than 99.5 percent of the observed interactions. Interactions shorter than 20 seconds were equally excluded from further analysis because some short agonistic episodes might have been missed in the analysis of the time-lapse observations and thereby introduce an unwanted bias in the estimation of frequency of individual aggressive behaviors. Based on the number of agonistic interactions, rank matrices were constructed for each of the experimental groups. Individuals that initiated aggressive interactions were ranked in all experimental groups according to the number of initiated interactions in decreasing order. Individual identity and frequency of targeting is noted for individuals that received aggression but did not themselves initiate agonistic interactions. Resulting rank orders for aggressive individuals were checked for the presence of linear dominance relationships according to the measure of linearity of hierarchies using Kendall's rank correlation (Appleby 1983). This test assumes dominance relationships within the group to be transitive. To appropriately treat hierarchies with unknown dominance relationships modifications by de Vries (1995) were applied. Additionally individual aggressive profiles for 5 agonistic behaviors (antennal boxing, biting, dragging, leap and immobilization) were calculated for each individual involved in agonistic interactions before and after the experimental removal of the egg-layer.

All individuals in the experimental groups were assigned to one of three different age classes according to the following classification:

Older workers (A) - Adult individuals that were older than two month at the point when the respective observation period started.

Younger workers (B) - Adult individuals that were up to two month old at the beginning of the respective observation period.

Callows (C) - Callow workers without a completely hardened brownish cuticle that were either marked right before the respective observations period or eclosed during the observations.

3.3 Results

3.3.1 Partitioning of reproduction

A total of 824 individuals from 9 colonies collected from Florida (8 colonies) and Puerto Rico (1 colony) were dissected in the laboratory to examine their reproductive status (Tab. 3.1). Due to high mortality (average 28.1 %) during transfer to the laboratory, the majority of the Puerto Rican colonies were retained alive for future behavioral observations. For 16 individuals, ovarian condition could not be determined because their deaths were noticed too late for dissection. The ovaries of workers, queens and intercastes of *P. punctata* typically consisted of a total of six ovarioles with a spermatheca. Variation in ovarian morphology among workers was not pronounced: Only a small proportion of the 808 individuals for which ovarian condition could be recorded (n = 19, 2.3 %) had less than six ovarioles, typically five or four, but all retained a spermatheca. In 8 of the 9 colonies examined, only a single uninseminated worker (or one intercaste in ABS27) had strongly elongated ovarioles with maturing eggs and yellow bodies (ovarian development type IV). The ovaries of other individuals did not contain mature eggs. In one colony, ABS11, in addition to the fertile worker a second worker was present, whose ovaries contained several yellow bodies and a few developing oocytes (Tab. 3.1). This individual might have been the previously active egg-laying individual before a recent supersedure.

Colony #	# Individuals	Caste			Ovarian development[3]					Max. ovariole length (mm)	
		worker	intercaste	queen	I	II	III	IV	n.a.[2]	I - III	IV
ABS 5[1]	33	31	2		26	5		1	1		
ABS 11	97	95	2		62	26	4	2	3	1.06 ± 0.22	3.03[4]
ABS 12	91	91			66	20	2	1	2	1.36 ± 0.09	3.57
ABS 13[1,5]	32	31		1	23	3		1	5		
ABS 14[1]	99	89	9	1	80	13	4	1	1		
ABS 15	68	68			50	12	1	1	4	1.27 ± 0.20	3.70
ABS 16	147	147			100	40	6	1		1.16 ± 0.20	1.89
ABS 27	162	161	1		109	40	12	1		1.20 ± 0.21	4.28
PR 16[1]	95	95			59	28	7	1			
Total	824	808	14	2	575	187	36	10	16		

[1]: ovariole length not measured
[2]: ovarian development not identified
[3]: for index of ovarian development see text
[4]: value for reproductive worker with longer ovarioles shown
[5]: The gamergate had fully developed ovaries (IV), the inseminated queen undeveloped ovaries (I)

Table 3.1 Reproductive organization of 9 colonies of *P. punctata* collected from the field in Florida and Puerto Rico. Means and standard deviation are indicated in mm. For further explanation see text (n.a. = not available).

Alternatively, reproduction may have been shared between these two workers. Furthermore, 187 individuals (23.1 %) showed ovaries in less pronounced stages of development, containing one or more immature oocytes but no yellow bodies (II). However, behavioral observation with the aid of time-lapse video recordings of additional colonies confirmed that there exists only one egg-layer per colony at a particular time. An additional 36 individuals (4.5 %) had resorbed ovaries (III). Maximal ovariole length was measured in 556 individuals from 5 colonies. Here, the reproductive individuals (IV) had longer ovarioles than non-reproductives (I, II and III, see Tab. 3.1; Kruskal-Wallis ANOVA, $H_{[1, 556]} = 14.821$; $p < 0.001$).

Five of the 9 field colonies dissected contained intercastes and / or queens in addition to workers. The two intercastes in colony ABS 11 could not be dissected. In colony ABS 5, the ovaries of both intercastes were undeveloped (I). In colony ABS 14, 8 of 9 intercastes had undeveloped ovaries (I), the ovaries of one intercaste were clearly resorbed and did not contain yellow bodies (III). Prior reproductive activity for this individual is therefore ruled out. The ovaries of the dealate virgin queen in this colony were undeveloped (I). The intercaste of colony ABS 27 had fully developed ovaries with dark yellow bodies at the base of its ovarioles (IV), and at least in this colony the intercaste monopolized the reproduction by thelytokous parthenogenesis. In colony ABS 13, an inseminated, reproductive worker (gamergate) with fully developed ovaries (IV) was found to monopolize reproduction in the presence of an inseminated, alate, but non-laying queen with undeveloped ovaries (I). The gamergate did not differ from unmated workers in morphology or size. All other workers in this colony were unmated and infertile. All additionally dissected intercastes (three intercastes each from ABS 1, ABS 17, ABS 18, and one intercaste each from ABS 20 and ABS 24), two alate queens (from ABS 7 and FP 4) and two dealate queens (from ABS 1 and ABS 8) had undeveloped ovaries (I) and were not mated. Dissections of few callow males eclosed in the laboratory (2 to 3 weeks of age) revealed that testicular follicles (each testis contains 3 follicles) were still swollen indicating that degeneration of testes may not yet be complete. Whether meiotic divisions of spermatogenesis are complete (as is the case in most other ants at the time of emergence) could however not be judged.

3.3.2 Thelytokous parthenogenesis

The number of offspring produced by thelytokous parthenogenesis in experimental groups of unmated workers is shown in Fig. 3.2 for 20 isolates (15 from Florida and 5 from Puerto Rico). The mean number of callows produced in each replicate during 18 weeks was 7.8 ± 2.7 (mean ± SD). Virgin workers in the Barbados isolate were equally capable of producing workers but here no data on colony growth were recorded. No male production was observed in these experimental groups.

A total of 858 individuals from 18 experimental groups were dissected after the termination of the experiment. The ovarian status of 42 individuals could not be identified unambiguously and they were omitted from further investigation. As in natural colonies, typically only one individual per worker group had fully developed ovaries (IV) and monopolized reproduction at the time of dissection. In one isolate, however, the ovaries of two individuals were classified as type IV although one had only slightly elongated ovaries and showed very few yellow bodies. This individual clearly had not the same reproductive capacity as the co-occurring worker with fully developed ovaries and conspicuous yellow bodies. Ovaries had degenerated in 19 of the dissected individuals (2.3 %, III). These individuals may have contributed to callows produced in the isolates prior to reproduction by the current reproductive. Another 94 of the individuals (11.6 %) had ovaries in various stages of development containing one or more immature yolky eggs (II). One of these individuals may be a candidate to take over reproduction at a later time. The ovaries of 684 individuals (83.8 %) were completely undeveloped (I). As expected, the spermathecae of all individuals were found to be empty. Ovariole length, which was measured in 816 individuals, was significantly different between individuals assorted into the four different ovary

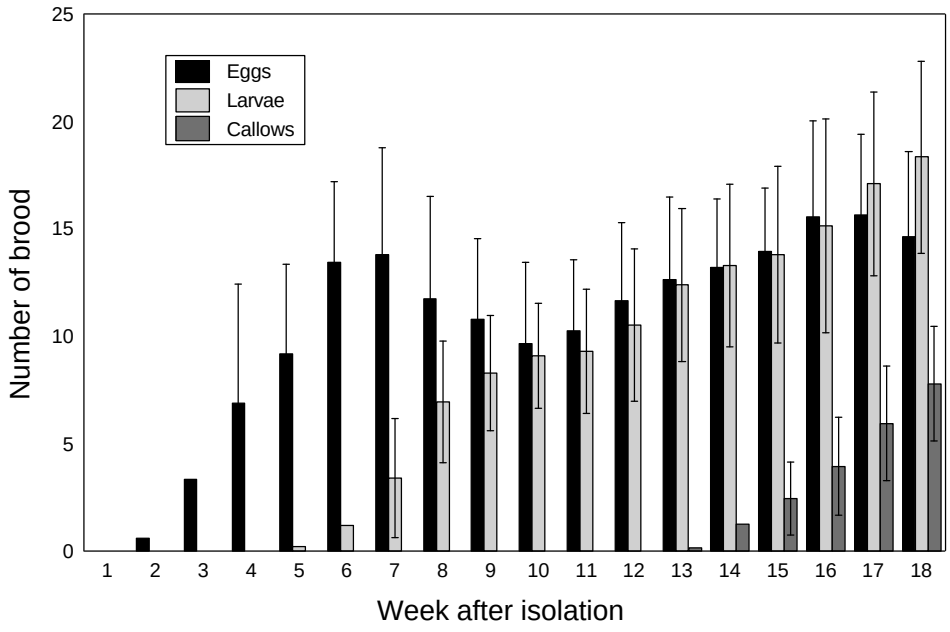

Figure 3.2 Production of brood and callow workers by thelytokous parthenogenesis in 20 isolates of *P. punctata* (15 derived from colonies collected in Florida and 5 from colonies from Puerto Rico). Similar results were obtained for an isolate derived from a colony collected in Barbados (data not shown). Error bars indicate standard deviation.

categories (Fig. 3.3; Kruskal-Wallis ANOVA: $H_{[3, 816]} = 140.051$; $p < 0.001$). More detailed post-hoc analysis using Bonferroni-adjusted Mann-Whitney U-Test for all 6 possible pairwise comparisons showed significant differences between some of the types. Reproductively active individuals (IV) had significantly longer ovaries than either of the three other categories I, II or III ($p < 0.001$ in all cases),

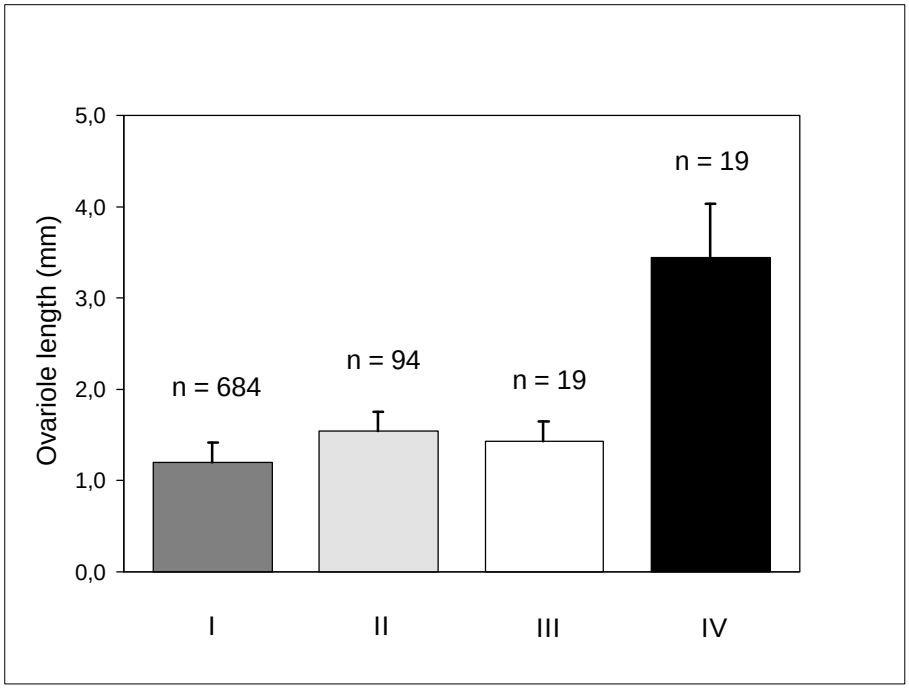

Figure 3.3 Maximum ovariole length (mm) for 816 individuals with varying degree of ovarian development in 18 isolates of *P. punctata*. Categories comprise individuals with undeveloped ovaries (I), those with developing ovaries in various stages (II), individuals whose ovaries were resorbed and inactive (III) and reproductively active individuals (IV). Overall differences in ovariole length are significant (Kruskal-Wallis ANOVA, $H_{[3, 816]} = 140.05$; $p < 0.001$).

and ovaries of individuals in the undeveloped condition (I) were significantly shorter than those of individuals with developing or completely resorbed ovaries ($p < 0.001$ in both cases). Comparison of ovariole length between these two latter categories did not reveal a significant difference ($p = 0.255$).

Reproductive organization was similar in 10 additional colonies collected in Florida and Puerto Rico that were kept in the lab for more than 3 years but were not used for direct observations (Tab. 3.2). At the time of dissection all colonies contained only workers. Although some of the colonies grew much larger in the lab than field-collected colonies (mean 110.4 ± 43.3 workers; range 42 to 180) in all colonies still only one worker monopolized reproduction by thelytokous parthenogenesis. None of the other workers (n = 1,094) was inseminated although some of the colonies could have contained inseminated individuals at the time of collection that might have subsequently died in the lab but were not collected for later dissection. In colony FP4 originally an alate queen was collected that was not mated (see 3.3.1). 78 workers (7.1 %, II) had developing ovaries containing one or more immature oocytes, the ovaries of another 100 workers (9.1 %, III) were clearly resorbed. As described above individuals with different degree of ovarian development differed in ovariole length (Kruskal-Wallis ANOVA: $H_{[3, 1072]} = 241.101$; $p < 0.001$). This was mainly due to the thelytokous workers having much longer ovarioles than any of the other workers (Mann-Whitney U-test, $p < 0.001$ in all cases). Again

maximal ovariole length in individuals that did not develop their ovaries was significantly shorter than both in individuals with developing or degenerated ovaries ($p < 0.001$).

Colony	# Individuals	Ovarian development[2]					Max. ovariole length (mm)	
		I	II	III	IV	n.a.[1]	I - III	IV
ABS 2	114	96	15	2	1		1.40 ± 0.24	5.56
ABS 9	140	112	13	10	1	4	1.43 ± 0.26	4.16
ABS 19	124	94	11	14	1	4	1.41 ± 0.24	5.43
ABS 21	123	98	13	8	1	3	1.42 ± 0.24	4.46
ABS 23	155	125	15	7	1	7	1.26 ± 0.25	3.52
ABS 26	180	133	18	18	1	10	1.50 ± 0.27	4.64
FP 4	74	61	3	6	1	3	1.42 ± 0.22	4.31
PR 6	91	78	8	4	1		1.40 ± 0.18	4.54
PR 8	42	38		3	1		1.33 ± 0.22	3.98
PR 24	61	49	4	6	1	1	1.42 ± 0.24	3.01
Total	1,104	884	100	78	10	32		

[1]: ovarian development not identified
[2]: for index of ovarian development see text

Table 3.2 Reproductive organization of ten additional colonies that were kept in the lab for more than 3 years. Means and standard deviation are indicated in mm. For further explanation see text (n.a. = not available).

3.3.3 Reproductive regulation in worker groups

General characteristics

Intense agonistic interactions were observed during the course of the experiments to investigate thelytokous parthenogenesis in virgin worker groups between some of the workers and callows that eclosed in the experimental groups (section 3.3.2). In this section the significance of these agonistic behaviors for the regulation of reproduction in all-worker groups is examined.

Time-lapse video observations amounted to a total of 1,638 hours and varied from 54.0 to 182.5 hours for 10 individual groups. Extensive observations were necessary because of the low frequency of interactions observed in initial ad libitum observations. Other studies on dominance relations in ants could use shorter observation periods (range 8 to about 100 hours per colony) due to a higher rate of interactions (e.g. Oliveira and Hölldobler 1990; Ito and Higashi 1991; Medeiros *et al.* 1992; Sommer and Hölldobler 1992; Ito 1993a, 1993b; Sommer *et al.* 1993; Heinze *et al.* 1996; Monnin and Peeters 1999). During the observation period a total of 4,367 agonistic interactions was

observed. Characteristics of the observed experimental worker groups are given in Tab. 3.3. Size of the experimental groups prior to first observation varied from 13 to 74 with a mean of 33 ± 20 (mean ± SD). This is slightly smaller than mean size of naturally collected colonies (44 ± 31 (mean ± SD), compare section 2.4) but still well in the range encountered in the field. Since most of the colonies obtained from the field did not contain sexuals, this setup reflects a natural situation orphaned worker groups may find themselves in. During the observation period the size of experimental groups increased to 39 ± 14 (mean ± SD). All experimental groups were monitored for the occurrence of males to exclude the possibility of intranidal matings. No males eclosed and later investigation confirmed that none of the workers dissected was inseminated. Observations were initiated after a mean of 408 ± 207 (mean ± SD) days allowing experimental groups to become well established. VB 1/1 is an exception in that behavioral observations started 2 days after the experimental group was initiated and all individuals were callows at that time. This group is discussed in greater detail below. Mean observation period of individual experimental groups was 142 ± 69 days (mean ± SD).

Oviposition and ovarian development

In all ten experimental groups the current egg-laying individual could be determined unambiguously from the time-lapse observations or additional ad libitum observations although fecundity of reproductive workers was rather low. Additionally the egg-layer could be identified by its unique behavioral profile typical for reproductive workers in this species, i.e. the individual that spends most of

Experimental group	Size at 1st observation	Size at end of observation	Initial period [d]	Observation period [d]	Total observation time [hh:mm]	
					Before removal	After removal
ABS 2/2	51	62	525	149	68:46	63:07
ABS 27/1	74	58	606	93	73:04	72:35
ABS 8/4	18	33	477	176	111:35	73:00
VB 1/1	13	27	2	105	121:42	63:20
PR 11/1	22	22	391	29	73:20	72:05
PR 19/1	35	41	279	65	72:20	73:40
PR 2/1	16	36	309	196	92:34	73:25
PR 21/1	31	21	549	139	54:05	72:58
PR 22/1	48	45	703	230	77:30	73:10
PR 4/2	20	41	239	234	182:25	73:30

Table 3.3 Characteristics of ten experimental workers groups set up to investigate reproductive regulation. Group size at end of observation includes all individuals that died during the observation period. Initial period denotes the time span before observations started.

the time close to the brood pile, did not actively engage in aggressive interactions and generally remained inactive most of the time.

Reproductive status (type IV) of all removed egg-layers was confirmed by immediate dissection upon removal. A total of 38 ovipositions were observed during both time-lapse and real-time observations (Tab. 3.4). Typically the egg-laying individual bent its gaster forward and under the thorax at the initiation of the egg-laying sequence. After a few minutes the sting was partially extruded until the egg appeared at the tip of the gaster. While ovipositing, the ovipositing worker usually turn away from nestmates who approached and antennated her body. Egg-laying took between 6:10 and 36:20 minutes

Experimental group	Old reproductives			New reproductives		
	Egg layer	Age group	# eggs laid	Egg layer	Age group	# eggs laid
ABS 2/2	RRR	A	2	BOG	B	-
ABS 27/1	GGB	A	4	YYB	A[1]	1
ABS 8/4	GGR	A	4	YRR	C	2
VB 1/1	BBG	B	4	GRG	A[2]	1
PR 11/1	RRR	A	1	GRO	C	1
PR 19/1	GGR	A	2	GBR	A[2]	-
PR 2/1	GGR	A	2	RYY	A[3]	1
				GRB	B	-
PR 21/1	GGB	A	2	GRO	B	4
	PGG[4]	A	2	GOB	C	-
PR 22/1	RBB	A	1	BOB	B	-
PR 4/2	GRR	A	4	YYG	B	-

[1] - 4 month old; [2] - 2.5 month old; [3] - 3 month old; [4] - ovaries were resorbed at time of dissection

Table 3.4 Characteristics of old and new reproductive workers in 10 experimental groups of *P. punctata* before and after removal of the dominant reproductive. In addition, information on age and observed ovipositions is provided.

with a mean of 18:21 ± 2:53 minutes (mean ± SD, n = 34). Once the egg was fully visible the ant grasped the egg with its mandibles and deposited it immediately on the brood pile or held it for up to 19:40 minutes before the egg was deposited (mean 13:25 ± 6:39 minutes, n = 33) on the brood pile. Sometimes nestmates were attracted to the freshly laid egg, antennated it while it was held by the egg layer and on four occasions took it from the mandibles of the egg-layer (11.8 percent). Yet no incidence of egg cannibalism was observed. Individuals grasping an egg from the reproductive worker rather deposited the egg on one of the egg piles scattered throughout the nest themselves. Only on one instance an egg layer (BBG in group VB 1/1) was observed to remain over the egg pile after egg deposition which did either occur by chance or could be interpreted as guarding behavior. Once deposited on the brood pile, eggs eventually became reshuffled by other nestmates not allowing for further individual

recognition. The egg-layer was not observed to actively place the egg under others in an attempt to mask its identity. Nestmates were never observed to grasp the egg directly from the abdomen of the egg-layer. No incidence of oophagy could be observed following 38 ovipositions as well as during the remaining observation time.

Generally after the experimental removal of the reproductive individual (which was the sole egg layer in the experimental group with the exception of PR 21/1 were two workers were reproductive) a single younger worker took over reproduction. Only in two experimental groups PR 2/1 and PR 21/1 a second individual started to reproduce. Whereas in PR 21/1 both individuals (GRO and GOB) had equally well developed ovaries with lightly colored yellow bodies at the basis of the ovarioles, in PR 2/1 only GRB had yellow bodies suggesting that RYY had not oviposited before. The latency from the time of removal until the day these individuals (which will be referred to as new reproductives) were first observed to lay an egg was short (19 ± 13 days (mean ± SD), n = 5) indicating that their ovaries at least in some individuals were developed before the old reproductive was removed. Since observations after removal were not continuous however this time may actually have even been shorter. Generally the new reproductives were either callows that were born within the observation period (n = 3) or younger workers born within a 2-month period before (n = 5). Although in four cases the new reproductives were older than two month, none of the individuals was older than four month (Tab. 3.5).

Experimental group	Date	Individual	Duration of oviposition	Fate of egg
ABS 2/2	22.09.98	RRR	10:00	deposited on egg pile
	25.11.98	RRR	09:40	YGY takes egg, deposits it on egg pile
ABS 27/1	15.05.97	GGB	n.a.	not observed
	05.08.97	GGB	18:45	not observed
	03.03.99	GGB	09:10	deposited on egg pile
	03.03.99	GGB	07:45	deposited on egg pile
	11.03.99	YYB	27:10	deposited on egg pile
VIR 8/4	01.07.98	GGR	16:30	deposited on egg pile
	30.09.98	GGR	10:00	deposited on egg pile
	15.12.98	GGR	18:00	deposited on egg pile
	17.12.98	GGR	22:00	deposited on egg pile
	15.01.99	YRR	25:25	deposited on egg pile
	15.01.99	YRR	17:00	deposited on egg pile
VB 1/1	07.05.97	BBG	24:00	deposited on egg pile
	07.05.97	BBG	18:00	deposited on egg pile
	05.06.97	BBG	20:00	deposited on egg pile, guarding egg pile afterwards
	10.06.97	BBG	13:00	deposited on egg pile
	05.08.98	GRG	15:17	deposited on egg pile
PR 11/1	13.12.98	RRR	26:20	deposited on egg pile
	18.01.99	GRO	12:30	deposited on egg pile
PR 19/1	05.08.98	GGR	09:30	deposited on egg pile
	14.09.98	GGR	13:00	deposited on egg pile
PR 2/1	26.11.99	GGR	n.a.	not observed
	16.12.98	GGR	06:10	deposited on egg pile
	15.03.99	RYY	08:50	YGY takes egg, immediately deposits it on egg pile
PR 21/1	27.10.98	PGG	36:20	deposited on egg pile
	06.12.98	PGG	n.a.	not observed
	27.10.98	GGB	17:35	deposited on egg pile
	16.12.98	GGB	18:00	GBB takes egg, then BGG, who deposits it on egg pile
	13.01.99	GRO	09:40	BGG takes egg, deposits it on egg pile
	18.01.99	GRO	19:00	not observed
	19.01.99	GRO	17:00	deposited on egg pile
	20.01.99	GRO	16:50	not observed
PR 22/1	22.03.99	RBB	n.a.	deposited on egg pile
PR 4/2	26.06.98	GRR	18:25	deposited on egg pile
	02.09.98	GRR	21:00	deposited on egg pile
	03.02.99	GRR	13.50	deposited on egg pile
	04.02.99	GRR	09:45	deposited on egg pile

Table 3.5 38 observed worker ovipositions in 10 experimental groups of *P. punctata*. For each group date of egg-laying, identity of individual, duration of laying and fate of the egg are indicated.

After dissection differences emerged in the degree of ovarian development between older workers, younger workers and callows after the end of observations (Fig. 3.4). Although these differences are not statistically significant between age classes (Kruskal-Wallis ANOVA), the proportion of callows having developing ovaries (type II) is about twice as large as compared to both older and younger workers. Spearman rank correlations between the proportion of individuals and their respective class of ovarian development did however reveal significant differences: Callows less frequently had degenerated ovaries (type III; $R = -0{,}39$, $p < 0.05$) as compared to the other two age

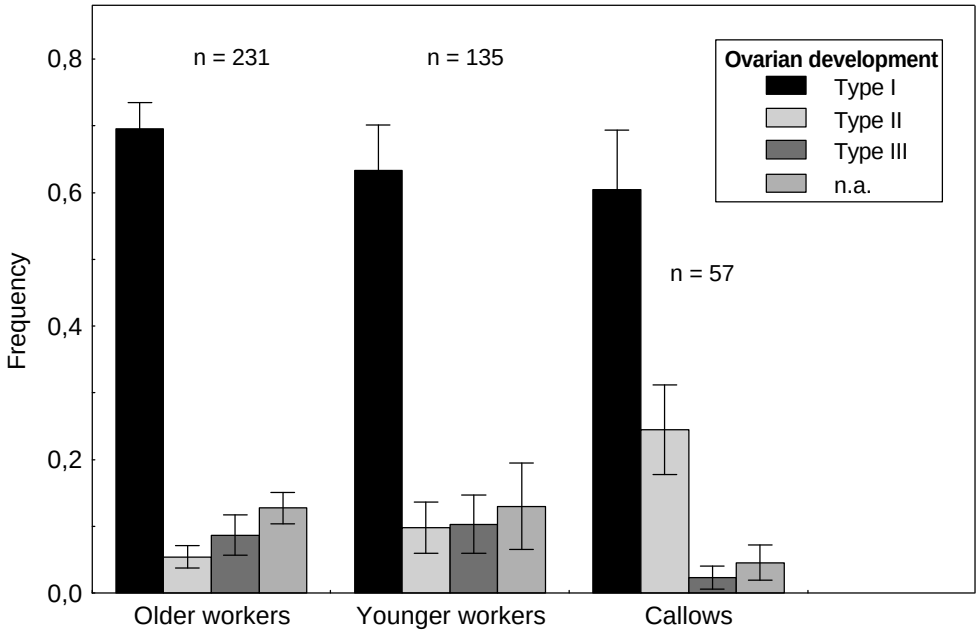

Figure 3.4 Mean frequency of older workers, younger workers and callows in 10 experimental groups belonging to each class of ovarian development. Included are both workers that were present at the termination of the experiment and those that died during the post-removal period (n=423). Reproductives are omitted from the analysis (n.a.: ovarian status could not be determined).

classes. However the apparent difference in the proportions of workers with developing ovaries (type II; R = 0.34, ns) and with undeveloped ovaries (type I; R = -0.18, ns) were not significant between age classes. Contributing to the absence of a significant trend is the high inter-group variation in the proportion of workers belonging to the same age class but with different ovarian development that is most pronounced in callows. Whereas in three groups almost half of the callows had developing ovaries in another four groups none of the callows had started to develop its reproductive organs. Low number of callows present in the groups (between 6.3 and 21.5 percent) may contribute to this high variation. It appears that workers of all ages will develop their ovaries after the removal of the old reproductive although the category older workers does not allow further differentiation among individuals older than two month.

Agonistic interactions

Agonistic interactions were observed in a consistent pattern in all experimental groups although the frequency of interactions was rather low: Only 2.7 interactions occurred per hour. Interactions lasted from 20 seconds (since shorter episodes were excluded from the analysis) up to 24 minutes. Most interactions were shorter than two minutes (77.4 percent) with only few lasting for a prolonged period of time. Due to this skewness towards shorter interactions the median instead of the mean duration of interaction was calculated: Before removal interactions lasted on average 55 seconds (n = 2,041), after

removal duration of interactions was slightly shorter with 45 seconds (n = 2,289). The median length of interaction in all 10 experimental groups however did not differ significantly before and after removal (Mann-Whitney U-test, U = 32, p = 0.174).

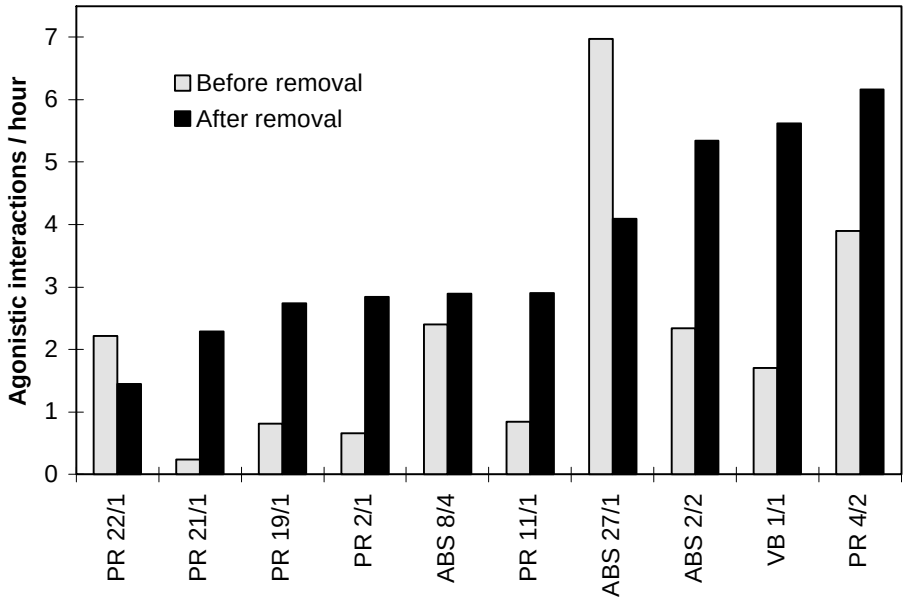

Figure 3.5 Aggressive interactions (antennal boxing, biting, crouching, leap and immobilization) in 10 experimental groups prior and after the removal of the dominant reproductive. Differences are only significant if ABS 27/1 is omitted (Wilcoxon matched pairs test, Z = 2.43, $p < 0.05$), see discussion in text.

In 8 out of 10 experimental groups the level of aggression measured as frequency of agonistic interactions per hour increased significantly after the removal of the dominant egg-layer (Fig. 3.5). Mean frequency of agonistic interactions increased on average from 2.21 ± 2.00 to 3.63 ± 1.58 interactions / hour (mean ± SD, Mann-Whitney U-test, U = 17.0, $p < 0.05$). Only in groups ABS 27/1 and PR 22/1 less agonistic interactions were observed after the manipulation. Whereas in PR 22/1 differences in interaction rates were not so pronounced ($\chi^2 = 3.19$, p = ns), in ABS 27/1 observed interactions dropped from 6.98 / hour to 3.62 / hour ($\chi^2 = 12.54$, $p < 0.001$). This reverse trend in ABS 27/1 may be due to the presence of a larger number of newly eclosed callows prior to the manipulation than in any other group: 63.8 percent of all individuals observed to be involved in agonistic interactions were callows. Mean proportion of callows in all groups was much lower (31.2 ± 19.2 (mean ± SD), n = 10). In ABS 27/1 intense fighting among these callows contributed to a high frequency of aggression in the group even before the reproductive GGB was removed.

If it is generally true that callows are the most aggressive individuals than the proportion of aggressive callows present in the group should be positively correlated with the hourly rate of aggression observed. However the expected correlation was not significant both before (Spearman rank

correlation: R = 0.57, n = 10) and after the old reproductive was removed (R = 0.17, n = 10) due to high inter-group variability. Consequently, although highly aggressive in some groups (e.g. ABS 27/1, ABS 8/4) callows did not show similar aggressive behaviors in other groups (e.g. ABS 2/2, PR 19/1).

Antennal boxing alone accounted for 40.0 percent of all interactions prior removal as compared to only 29.5 percent after removal (χ^2 = 3.90, $p < 0.05$). A similar decrease in the performance of antennal boxing was observed in two other groups (ABS 2/2 and PR 11/1). Biting frequency however remained unchanged after the dominant reproductive was removed. This was typical for all experimental groups (average percentage of pre-removal biting 39.3 ± 12.5 percent and post removal 40.2 ± 10.9 percent (mean ± SD), n = 10) with the exception of ABS 2/2. Additionally, since the old reproductive GGB had been reproductively dominant for a period of almost 24 month after the assembly of the experimental group, the raised level of aggression likely reflects a period of social instability during GGBs reproductive status was already challenged by the future reproductive YYB. YYB was observed to lay its first egg only 6 days after GGB was removed from the group. Since latency until the first oviposition by the new reproductive was observed was much larger in all other groups (19 ± 13 days (mean ± SD), see above) it is likely that YYB already had well-developed ovaries at the time GGB was removed from the group. Removal of GGB in ABS 27/1 therefore probably coincided with a natural replacement of the dominant reproductive in this group. Due to this social instability pre-removal level of aggression probably has been elevated similar to a post-removal situation.

The post-removal period in PR 22/1 was characterized by frequent but brief agonistic interactions lasting shorter than 20 seconds that were not included in the analysis. These interactions involved rapid antennal boxing among the individuals that equally initiated longer interactions and therefore led to an actual underestimation of the rate of interactions per hour.

Equally, mean frequency of individual agonistic interactions per hour increased from 0.18 ± 0.03 (mean ± SD) in the period before the removal to 0.33 ± 0.48 (mean ± SD) in the period after the removal, the difference being significant (Mann-Whitney U-test, $U = 4295.5$, $p < 0.01$). Whereas the mean hourly rate of antennal boxing, leap and immobilization did increase slightly but insignificantly after the manipulation, an obvious increase occurred in dragging behavior which was more often observed after the old reproductive had been removed in nine out of ten experimental groups (Mann-Whitney U-test, $Z = -2.72$, $p < 0.01$). Whereas this behavior constituted only a mean of 0.7 percent of all interactions before the removal (range 0 to 3.6 percent) it increased to a mean of 6.8 percent (range 0.5 to 23.7 percent) after the removal took place. Similarly, but less pronounced, biting occurred significantly more often (Mann-Whitney U-test, $Z = -2.04$, $p < 0.05$) but its percentage increased in only six out of ten groups (Fig. 3.6). The mean percentage of immobilizations increased from 1.9 percent pre-removal to 2.5 percent post-removal which again reflects an increase in six out of ten

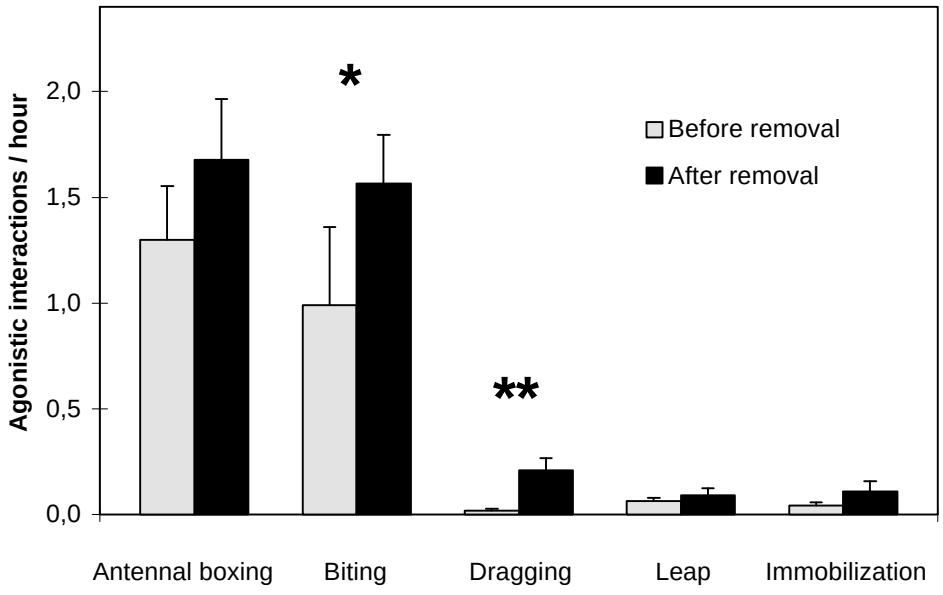

Figure 3.6 Mean hourly rates of antennal boxing, biting, crouching, leap and immobilization in 10 experimental groups prior and after the removal of the dominant reproductive. Given are mean and standard error (overall differences not significant: Kruskal Wallis ANOVA, $H_{[2,20]} = 2.286$, $p = ns$).

groups. This change however is not representative of the situation found in most groups: As summarized in Tab. 3.6, most aggressive behaviors in fact did not change in frequency after the old reproductive was removed. While the frequency of antennal boxing decreased significantly only in 3 out of 10 groups, the frequency with which biting was observed did not change in most groups and a significant increase in the frequency of dragging and immobilization was observed in only 3 out of 10 groups. An increase in frequency of behaviors such as dragging and immobilization therefore appear to

Experimental group	Antennal boxing	Biting	Dragging	Leap	Immobilization
ABS 2/2	↓ ★	↑ ★	↑ ★	ns	↑ ★★
ABS 27/1	↓ ★	ns	↑ ★★★	ns	↑ ★
ABS 8/4	ns	ns	ns	ns	ns
VB 1/1	ns	ns	ns	↓ ★	n.a.
PR 11/1	↓ ★	ns	↑ ★★	ns	ns
PR 19/1	ns	ns	ns	ns	ns
PR 2/1	ns	ns	ns	ns	ns
PR 21/1	ns	ns	ns	↓ ★	↓ ★
PR 22/1	ns	ns	ns	ns	ns
PR 4/2	ns	ns	ns	ns	↑ ★★★

Table 3.6 Effect of the removal of the old reproductive on the rate of agonistic behaviors per hour in 10 experimental groups. Arrows indicate the direction of change of aggressive frequency, differences were tested using the χ^2 -test (n.a. = not observed).

be indicative of an actual increase in the intensity of agonistic interactions in times when the social dominance order within the experimental group is disturbed by the artificial removal of the reproductive worker simulating the natural supersedure that occurs in natural all-worker colonies.

Differentiating according to the age of the interacting individuals reveals a similar picture: Levels of agonistic interactions increased within each of all three age groups (older workers, younger workers and callows) after the dominant egg layer was removed (Fig. 3.7). This is however confounded by the fact that both before (Kruskal-Wallis ANOVA, $H_{[2,116]} = 10.160$, $p < 0.01$) and after the removal (Kruskal-Wallis ANOVA, $H_{[2,96]} = 9.796$, $p < 0.01$) the frequency of agonistic interactions was negatively correlated with age of the initiating individuals. Separate examination within each age group revealed a significant increase however only among younger workers (Kruskal-Wallis ANOVA, $H_{[1,77]} = 9.506$, $p < 0.01$). Age therefore had a profound influence on the individual rate of agonistic interactions that individual workers initiated regardless of the degree of social stability in the group. Especially younger adults and callows were responsible for the increase in observed aggression after the supersedure of an old reproductive.

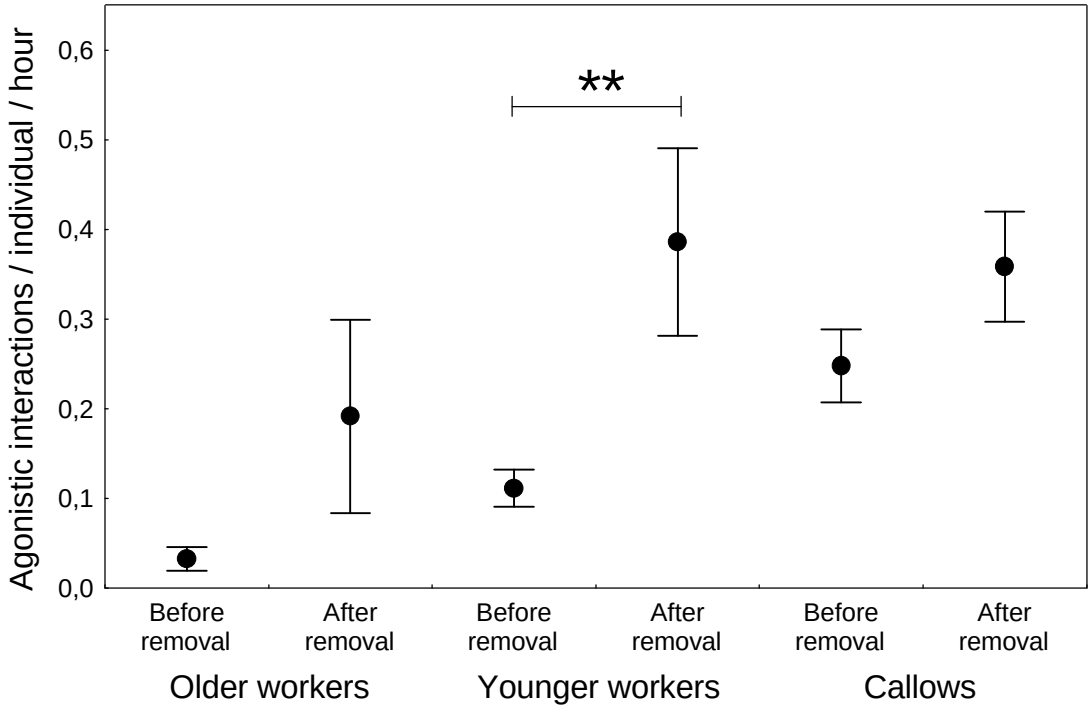

Figure 3.7 Rate of agonistic interactions per individual and hour for three age classes. Given is the mean and standard error over 10 experimental groups.

Social status

Based on a total of 4,367 agonistic interactions 20 rank matrices were constructed for each of the experimental groups both before and after experimental removal of the dominant egg-laying. A typical rank matrix before and after the removal of the old reproductives is shown for experimental groups ABS 2/2 in Tab. 3.7. (for complete data of all groups refer to the annex 1.1 to 1.8). Tab. 3.8 shows the rank matrices for group VB 1/1 which will be discussed below. Since frequently individuals were targeted but did not initiate agonistic interactions themselves, their identity and the number of observed interactions is additionally included (right of the dotted line). Their order is arbitrarily chosen. Two thirds of the aggressive behaviors, however, were directed towards nestmates that initiated aggressive interactions themselves (representing 65.3 ± 21.9 percent (mean ± SD) of total agonistic interactions observed).

a)

Dominant \ Subordinate	YGO	BPP	PGG	GGW	GGG	BBP	BBR	PGP	GRW	GBG	BRR	RGB	RWB	PBB	GPP	PPP	RWR	BGW	GGP	WGG	GWR	GGY	GBB	WGB	PPB	BWB	GRR	BPB	RBR	GGR	BRG	GGO	GYG	OGO	GYY	PPG	RGW	BRO	Total	Age
YGO	X	2	1	1	1	16	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	3	1	-	-	-	1	-	-	-	5	2	-	-	1	1	-	4	43	C
BPP	-	X	4	15	2	-	7	-	1	-	-	-	2	1	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36	B
PGG	-	-	X	-	-	-	-	-	1	-	-	-	-	1	2	1	2	2	2	3	1	1	2	3	1	1	-	-	-	-	-	-	-	-	-	-	-	-	23	B
GGW	-	-	2	X	4	-	-	-	2	-	-	-	-	-	3	-	2	2	-	-	-	-	-	-	-	-	3	1	1	-	-	-	-	-	-	-	-	-	20	B
GGG	-	-	-	1	X	-	-	4	2	-	-	-	-	-	1	-	1	-	-	-	1	-	1	-	1	-	-	2	-	1	1	-	-	-	-	-	-	-	16	B
BBP	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	5	1	9	B
BBR	-	-	-	-	-	-	X	-	-	-	-	5	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	8	A
PGP	-	-	-	-	-	-	-	X	-	-	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	5	B
GRW	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	2	B
GBG	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2	A
BRR	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	B
RGB	-	-	-	-	-	1	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	B
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Total	0	0	6	16	6	1	7	4	6	0	0	5	2	2	10	2	5	4	4	3	3	5	3	3	2	1	3	3	1	1	3	2	1	2	0	0	5	2	166	

b)

Dominant \ Subordinate	YYB	RYR	BOG	BRB	BGO	RBR	GBO	YYR	GGY	RRO	BOR	OBP	PBO	RGW	BBY	BYY	GWW	WGG	BPO	GWG	GGP	ROG	YRR	GGW	PPP	YYY	GPG	YBY	BRR	BRO	YGY	GGR	RRY	PGG	WGB	GRR	Total	Age	Ovar
YYB	X	32	4	-	13	-	12	-	-	2	5	5	-	-	-	-	12	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	87	C	II
RYR	-	X	-	1	8	4	4	1	12	-	17	5	2	1	-	-	8	-	2	3	-	1	1	-	-	-	1	-	1	-	-	1	-	-	2	1	76	B	I
BOG	2	5	X	-	-	-	-	-	-	-	12	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	B	IV
BRB	-	-	-	X	-	-	-	4	-	-	2	-	1	-	1	-	-	1	1	1	-	-	1	-	-	1	1	1	2	1	-	-	-	-	-	-	18	A	II
BGO	-	-	-	-	X	-	5	-	-	1	-	3	-	-	-	-	3	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	13	C	I
RBR	-	-	-	1	1	X	-	-	-	-	2	-	-	1	-	-	-	-	-	2	-	1	-	-	-	-	-	-	1	-	1	-	-	-	-	-	10	A	I
GBO	-	-	-	-	2	-	X	-	-	-	1	3	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	C	I
YYR	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	1	-	1	-	1	-	1	2	-	-	1	-	-	-	-	-	1	-	-	-	-	8	B	I
GGY	-	1	-	1	-	2	-	-	X	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	A	III
RRO	-	3	-	-	-	-	-	-	-	X	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	C	I
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OBP	-	2	2	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	C	I
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BBY	-	-	-	-	-	-	-	-	3	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	B	I
BYY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	1	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	3	B	I
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WGG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	A	I
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
Total	2	43	6	4	24	7	21	6	17	3	41	18	3	2	1	1	27	3	4	7	1	5	8	1	2	3	2	2	4	1	1	2	1	1	2	1	277		

Table 3.7 Rank matrices observed in experimental group ABS 2/2 a) before and b) after removal of the dominant egg layer RRR based on the number of agonistic interactions initiated. The reproductive individual is underlined. Age refers to the age at the time when the respective observation started. If callow workers eclosed during observations could not be re-identified after the end of the observations, their ovarian condition was assigned n.a. Further explanations in text.

The term "rank matrix" is used here in a solely descriptive manner to refer to the position of an individual worker in the matrix as determined by the number of agonistic interactions the individual initiated and does not imply any suggestion on the future reproductive potential of that individual. Conventionally the construction of a rank matrix of agonistic interactions allows the determination of hierarchical relationships among pairs of interacting workers. The clear determination of dominance ranks is however impaired by the presence of dominance reversals and missing observations among any given pair of individuals. The definition used here takes into account reversals that were sometimes observed among individuals that scored high in the matrix. For example, in ABS 2/2 YYB dominated BOG 4 times by biting her antennae and dragging her through the nest within 20 minutes but during the next 3 hours BOG performed dragging behavior (additionally curling her gaster) while biting YYBs antennae two times (Tab. 3.7.a)). Summing up these agonistic interactions would have resulted in YYB being more dominant than BOG whereas BOG performed dragging after aggressions from YYB had ceased. Similarly, in VB 1/1 four hours after the old reproductive was removed GRG and RRR engaged

a)

Dominant	Subordinate														Age
	RRG	**GRG**	**GGG**	**GGR**	**GBG**	**BGG**	**RRR**	**BBB**	**BBG**	**GGB**	**GRR**	**RGG**	**RGR**	**Total**	
RRG	**X**	-	6	7	15	-	4	10	8	2	-	3	-	55	B
GRG	-	**X**	1	-	-	30	1	-	2	-	-	-	5	39	C
GGG	3	-	**X**	1	6	-	2	2	-	3	3	3	-	23	B
GGR	6	-	3	**X**	-	-	1	1	1	-	-	4	-	16	B
GBG	3	-	2	1	**X**	-	2	1	-	2	-	-	-	11	B
BGG	-	-	3	-	-	**X**	-	-	-	-	-	-	6	9	C
RRR	-	-	-	-	3	-	**X**	-	-	-	-	-	-	3	B
BBB	-	-	1	-	-	-	-	**X**	-	-	1	-	-	2	B
BBG	-	-	-	-	-	-	1	-	**X**	-	1	-	-	2	B
GGB	-	-	1	-	-	-	-	-	-	**X**	-	-	-	1	C
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
Total	12	0	17	9	24	30	11	14	11	7	5	10	11	161	

b)

Dominant	Subordinate																Age	Ovar
	GRG	**BGB**	**RRR**	**BGG**	**GBB**	**BRR**	**CW**	**RRG**	**BBB**	**GRR**	**GBG**	**GGG**	**RGG**	**GGR**	**RGR**	**Total**		
GRG	**X**	-	24	6	1	-	-	10	8	10	19	9	6	9	12	114	A	IV
BGB	4	**X**	55	-	6	10	-	2	12	9	3	-	2	2	-	105	B	n.a.
RRR	26	3	**X**	1	-	-	-	-	2	1	1	1	2	2	-	39	A	I
BGG	-	-	-	**X**	-	-	-	6	2	-	5	1	2	3	7	26	B	I
GBB	7	5	-	-	**X**	3	-	-	-	-	-	-	-	-	-	15	B	n.a.
BRR	1	9	-	-	4	**X**	-	-	-	-	-	-	-	-	-	14	C	I
CW	-	3	-	-	-	6	**X**	-	-	-	-	1	-	-	-	10	C	n.a.
RRG	-	-	-	2	1	-	-	**X**	-	-	-	-	-	1	-	4	A	I
BBB	-	-	-	1	-	-	-	-	**X**	-	-	-	-	-	-	1	A	I
GRR	-	1	-	-	-	-	-	-	-	**X**	-	-	-	-	-	1	A	I
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
Total	38	21	79	10	12	19	0	18	24	20	28	12	12	17	19	329		

Table 3.8 Rank matrices observed in experimental group VB 1/1 a) before and b) after removal of the dominant egg layer BBG based on the number of agonistic interactions initiated. The reproductive individual is underlined. Age refers to the age at the time when the respective observation started. If callow workers eclosed during observations could not be re-identified after the end of the observations, their ovarian condition was assigned n.a. Further explanations in text.

in rapid aggressive interactions involving short leaps towards the opponent while threatening her with open mandibles. Dominance in these cases could not unambiguously be assigned. Subsequently GRG dominated RRR 24 times but in reverse RRR dominated GRG 26 times (Tab. 3.8.b); compare also matrices in annex for other instances). Therefore ranks are numbered and the usage of "*alpha*", "*beta*" etc. to refer to single individuals implying linear dominance relations is avoided. Conventionally these labels are used to refer to individuals that are arranged in a nearly-linear hierarchy (see studies quoted in section 3.1).

In nine experimental groups only a small proportion of workers (mean 20.8 ± 12.6 percent (mean ± SD), range 3.3 to 37.1 percent) initiated one or more agonistic interaction before the reproductive worker was removed (percentage of group size as measured directly before the old reproductive was removed). Only in group VB 1/1 which was little older than 2 month when the reproductive was removed 69.2 percent of workers engaged in agonistic interactions. The situation in this group however is exceptional in that all individuals were either callows or young workers that generally showed higher rates of agonistic behavior than older workers (Fig. 3.7). On average, five workers were responsible for 90 percent of total agonistic interactions (Fig. 3.8). For most individuals no agonistic interactions could be observed, therefore their dominance relationship remains unknown.

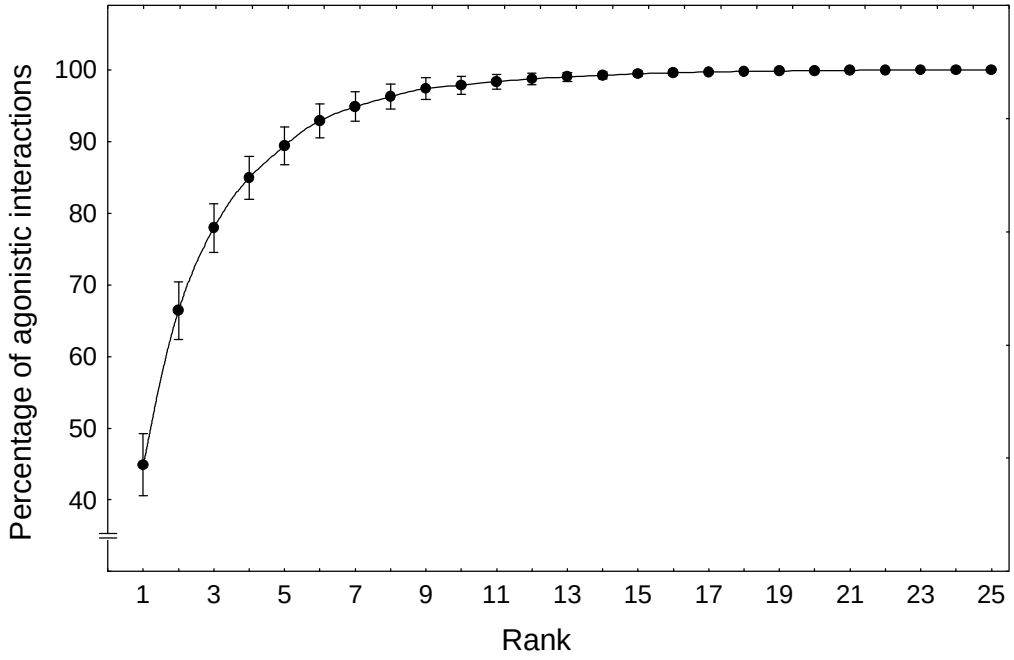

Figure 3.8 Cumulative percentage of 4,367 agonistic interactions initiated by workers summarized over 5 agonistic behaviors (antennal boxing, biting, dragging, leap and immobilization). Increasing ranks reflect a decreasing number of interactions initiated per individual. Error bars indicate standard error.

Including all individuals that engaged in agonistic interactions (initiators and targets) leaves most matrices highly incomplete. The rank matrices in most experimental groups therefore were found to be non-linear (Kendall's coefficient of linearity K = 0.111 ± 0.105 (mean ± SD), range 0.030 to 0.244; χ^2-test, p = ns; improved according to de Vries (1995)) with the exception of ABS 27/1 where the matrix showed significant linearity after the manipulation (K = 0.471, χ^2 = 35.17, p < 0.001). When linearity was tested including only those workers that initiated aggressive behaviors, 18 out of 20 matrices were equally non-linear (Kendall's coefficient of linearity K = 0.179 ± 0.150 (mean ± SD), range 0.001 to

0.536; χ^2-test, p = ns). In both PR 2/1 and PR 4/2 the matrix constructed after the removal of the dominant egg layer could not be tested statistically. Further analysis therefore included all aggressive interactions regardless of whether the recipient itself behaved aggressively or not.

A confounding problem of the statistical analysis is the incomplete information on aggressive interactions among most group members since a large number of possible interactions were not observed (observational zero's). Since this fact leaves the ranking of group members, especially among lower-ranking individuals, uncertain, the possibility to obtain significance in the measure of linearity becomes increasingly unlikely as completeness of information declines (Appleby 1983). Whenever there are unknown relationships the linearity indices are systematically too low (de Vries 1995). Given the duration of observations it is quite unlikely that agonistic interactions have been overlooked in a large number of individuals. Therefore aggressive interactions resulted in rank matrices that are best described as oligarchic with usually a group of few individuals being responsible for the majority of agonistic interactions towards other group members (Fig. 3.8). Within the oligarchic group individual interactions that led to the construction of rank matrices were highly dynamic in most of the observed experimental groups due to reversals in dominance relations. Additionally among these individuals observational zeros were frequent. As a result, even among the group of most aggressive workers, dominance relations were difficult to assign. These dynamics will be highlighted in the following section using both a larger worker group (ABS 2/2) and a small (VB 1/1) as example (for individual profiles of other groups refer to annex 2.1 to 2.8).

Dynamics in rank orders

In the large experimental group ABS 2/2 containing 51 workers at the start of the observations a total of 166 agonistic interactions were observed in 69 hours prior to the removal of the dominant reproductive (RRR) and 277 interactions in 63 hours following the removal reflected in an increase of the hourly rate of agonistic interaction from 2.4 to 4.4 interactions after the removal (χ^2 = 9.22, p < 0.01). Again antennal boxing was the most frequent agonistic behavior accounting for 78.3 percent of total interactions before but only 53.8 percent after the removal of RRR took place (χ^2 = 5.89, p < 0.05). The percentages of most other behaviors increased significantly in the post-removal period (biting from 18.7 to 32.5 percent (χ^2 = 5.87, p < 0.05); dragging from 0 to 4.7 percent (χ^2 = 5.38, p < 0.05); leap from 2.4 to 3.2 percent (χ^2 = 0.24, p = ns); immobilization from 0.6 to 5.8 percent (χ^2 = 7.06, p < 0.01)).

The callow YGO initiated about 25 percent of all pre-removal encounters, followed by four other young workers (BPP, PGG, GGW and GGG) being responsible for a total of 83.1 percent of interactions (Fig. 3.9). The dominant reproductive RRR neither initiated agonistic interactions nor was she victimized before the removal. After removal of RRR both the callow OBP and the designated reproductive worker BOG engaged in prolonged fights lasting several minutes in which dominance

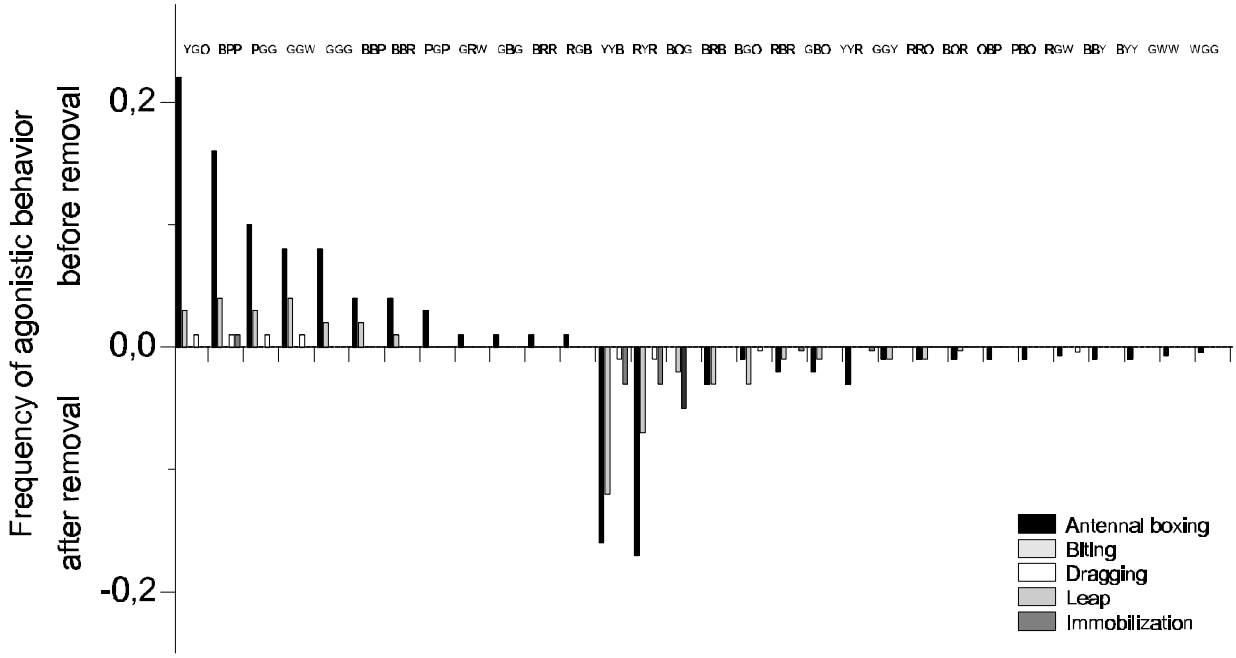

Figure 3.9 Individual aggressive profiles for 30 workers in experimental group ABS 2/2 before and after removal of the reproductive individual RRR. Only individuals that initiated agonistic interactions are included.

status could not be clearly assigned. During these encounters one individual would leap with opened mandibles towards the other and try to bite. It had its gaster curled under the body facing the opponent as described above. The opponent reacted by biting the initiator's legs or antennae, curling its gaster and both individuals would then vigorously chase each other through the nest. After 3.5 hours these encounters settled with BOG finally dominating OBP. Both individuals were born after the first observation period ended. Similarly these intense fights in which dominance could not unambiguously be assigned were occasionally observed in other groups after the old reproductive was removed (e.g. in VB 1/1 and PR 11/1).

In the case of the small experimental group VB 1/1, a total of 161 agonistic interactions were observed in 122 hours prior to the removal of the dominant reproductive and 329 interactions in 63 hours post-removal reflecting a significant increase in aggressive frequency from 1.3 to 5.2 interactions per hour ($\chi^2 = 60.39$, $p < 0.001$). Frequency of individual behaviors however did not change after the removal: Antennal boxing was the most frequent agonistic behavior accounting for 52.7 percent of total interactions before and 60.7 percent after the removal took place. While the frequencies of more aggressive behaviors like biting and leap slightly decreased slightly from 33.3 and 10.3 percent before removal to 24.4 and 7.6 percent post-removal respectively frequency of dragging behavior increased from 3.6 to 7.3 percent (all differences not significant, χ^2-test). No instance of immobilization was observed.

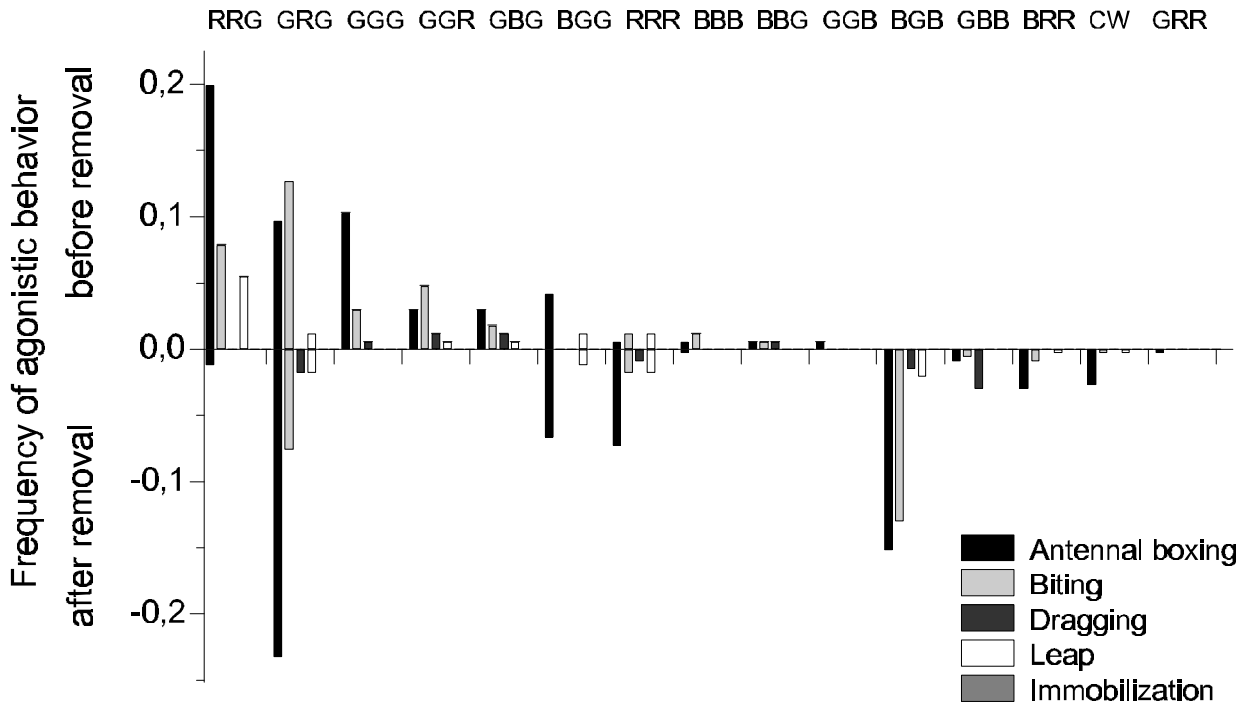

Figure 3.10 Individual aggressive profiles for 15 workers in experimental group VB 1/1 before and after removal of the reproductive individual BBG. Only individuals that initiated agonistic interactions are included.

The young worker RRG initiated about one third of all encounters, followed by callow GRG being responsible for another quarter (Fig. 3.10). These interactions were, however, not evenly distributed throughout the total observation period but frequently several were clustered within few minutes. The dominant reproductive BBG was observed to initiate agonistic interactions only on two occasions (towards RRR and GRR) but was targeted by RRG and GRG 10 times. In all other groups the dominant reproductives did not participate in agonistic interactions at all with the only other exception of group PR 21/1 where PGG was targeted trice by GGO, the individual with the highest rank order. The ovaries of PGG were resorbed but contained few lightly colored yellow bodies and were of similar length (3.52 mm) as those of established reproductive individuals (3.84 ± 0.36 mm (mean ± SD)) and new reproductives (3.24 ± 0.78 mm (mean ± SD)). It is likely that PGG was naturally superceded by GGB in PR 21/1 during the observations. However both individuals were observed to jointly reproductive over a period of at least one month (Tab. 3.5). Therefore it cannot be excluded that PGG and GGB shared reproduction for a longer period of time.

On the contrary the designated reproductive was observed to initiate 21.3 ± 22.2 percent (mean ± SD) of agonistic interactions in six of the pre-removal groups (range 1.3 to 55.7 percent). After the removal of the dominant reproductive BBG in group VB 1/1, rate of agonistic interaction increased from 1.4 to 5.2 interactions / hour. There was also a sharp increase in agonistic interactions shorter than

20 seconds which were not included in the analysis. Four individuals were mainly responsible for this increase: GRG who had already scored high in the pre-removal rank order, BGB who was not observed to participate in agonistic interactions before, and RRR and BGG who both were observed to interact on only a few occasions before. These four workers accounted for a total of 86.3 percent of total interactions. Within 7.5 hours after removal of the old reproductive BBG, RRR and GRG were observed to engage in brief chases lasting less than 20 seconds in most cases. Dominance status of the two opponents was difficult to assign during each encounters which consisted of rapid leaps towards the opponent with mandibles opened. Both sides initiated these approaches. The approached individual reacted by bending its gaster forward between her legs, exposing its dorsal region towards the opponent. A similar behavior is known to occur in other ants (Bourke 1988a) and has been termed 'gaster curling' in *Dinoponera quadriceps* (Monnin and Peeters 1999). A total of 232 of these short interactions were observed within the following 9.5 hours. Similarly BGB and RRR engaged in 64 of such brief chases within 3 hours involving rapid antennation on the opponent's head capsule or dorsal body parts. Overall RRR was observed to equally or less frequently attack both GRG (26 times) and BGB (3 times) than vice versa (24 and 55 times). This pattern results from a single reversal of dominance between RRR and GRG that took place 28 hours after post-removal observations started: While RRR dominated GRG 26 times immediately after BBG was removed from the group by antennal boxing, biting and leap (excluding the short agonistic interactions described above), after 5 hours without observed interactions GRG started to dominate RRR for a total of 24 times until the end of observations. During the period of raised agonism in the group GRG started to develop its ovaries and subsequently became the new reproductive in the group, RRR did not develop its ovaries. A callow who eclosed during the observations initiated its first attack against BGB only 2:15 hour after it eclosed. Other callows behaved more timid on the first day after eclosure.

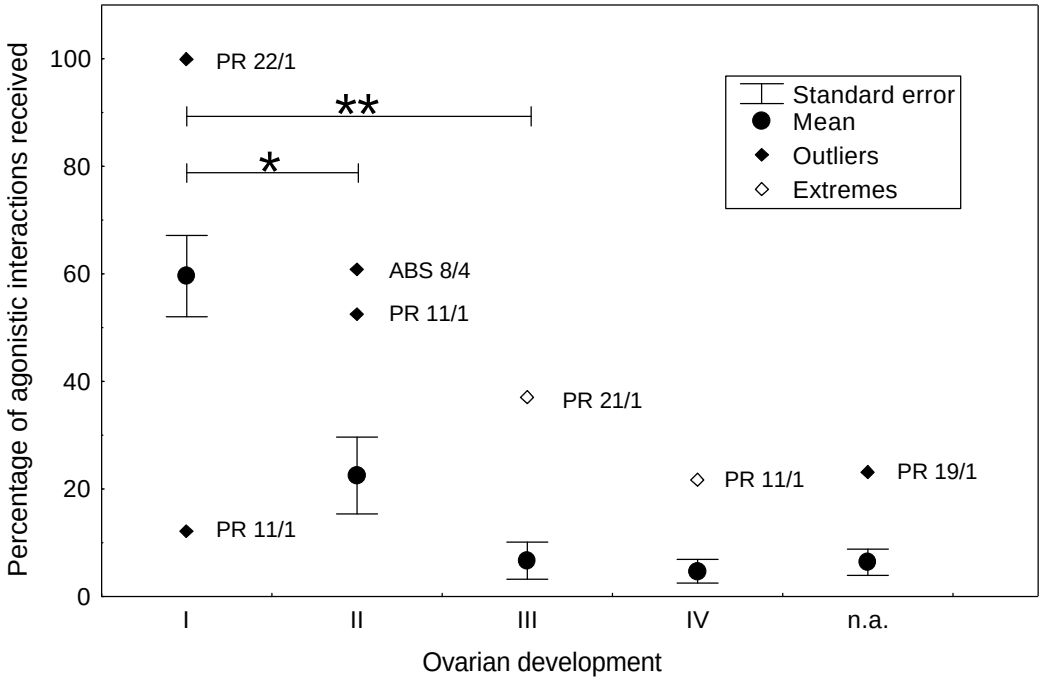

Figure 3.11 Percentage of total aggressive interactions received according to the degree of ovarian development. Inter-group variation in received aggression is considerable in some cases therefore both extreme values and outliers are given for individual experimental groups (n.a. = ovarian development not determined). Overall differences are significant (Kruskal-Wallis-ANOVA, $H_{(4,\ 50)} = 22.73$, $p < 0.001$).

According to theoretical considerations workers that have already activated their ovarian development should pose the greatest threat to any reproductively dominant individual within the colony. Therefore most of the aggression should be directed towards such individuals. In the experimental groups both workers with undeveloped ovaries (type I) and those who had already started developing their ovaries (type II) were targeted significantly more often than both workers with degenerated ovaries and reproductive individuals (Fig. 3.11).

On most occasions aggressions were directed towards individuals with undeveloped ovaries. They were victimized significantly more often than individuals that had activated their ovaries (Mann-Whitney U-test, $U = 13.0$, $p < 0.05$) or those which had degenerated ovaries (Mann-Whitney U-test, $U = 2.0$, $p < 0.01$) although this was not the general case in all groups. In PR 22/1 for example most of the workers had undeveloped ovaries (89.1 percent) and aggression was exclusively directed towards these workers. Some of them may already have undergone physiological changes related to ovarian activation that were perceived by their nestmates triggering the attacks. In two groups most of the aggression was directed towards individuals with developing ovaries: Both ABS 8/4 and PR 11/1

contained a higher proportion of workers with activated ovaries (23.3 and 18.5 percent) as compared to other groups.

Age and rank

The distribution of mean age of all group members varied significantly between different phases of the experiment as is shown in Fig. 3.12 (Kruskal-Wallis ANOVA, $H_{[2,30]} = 6.900$, $p < 0.05$). The first group of columns ("before removal") summarizes the age distribution among all group members present prior to the onset of behavioral observations. The following group depict the age distribution among the individuals present at removal and the third set of columns of individuals at the last observations after the removal of the reproductive individual. Before the removal about half of all individuals within the experimental groups were younger workers, followed by older workers and callows. Both at the time of removal (Mann-Whitney U-test, $U = 19.0$, $p < 0.05$) and after the egg layer was removed (difference not significant) the proportion of older workers was lower than at the start. Among callow workers this effect reversed: Considerably more callows were present both at removal (Mann-Whitney U-test, $U = 19.5$, $p < 0.05$) and after the manipulation (Mann-Whitney U-test, $U = 18.0$, $p < 0.05$) as compared to their initial representation. There is however no apparent difference in the proportion of the three age classes at the removal as compared to the post-removal period (all post-hoc comparisons are Bonferroni-adjusted). Almost all of the callows that eclosed during the observations did actually engage

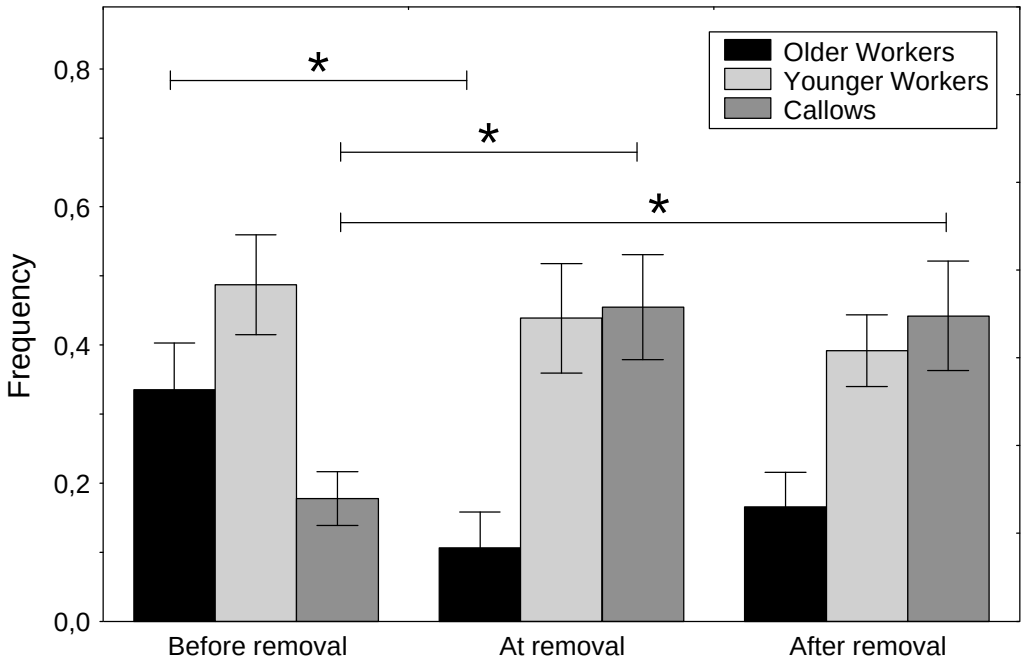

Figure 3.12 Mean age distribution at three different phases of the observations. Overall differences between phases are significantly different. Error bars indicate standard error. For discussion see text.

in agonistic interactions (98.0 ± 7.5 percent (mean ± SD), range 66.7 to 100.0 percent).

Do these callows acquire a high social status within the group? If so they would be expected to initiate most of the agonistic interactions and thereby occupy higher rank positions. To examine whether age does have a negative effect on the position individuals assume in the observed matrices mean frequency of each age class before and after the removal of the egg-layer was plotted for the first eight ranks as determined by the number of agonistic interactions initiated (Fig. 3.13). The resulting picture however does not present a pronounced effect of age on the rank position. As would be expected, the mean frequency of older workers in the higher rank positions 1 to 4 was lower than their mean frequency in the lower ranks positions 5 to 8 (Mann-Whitney U-test, $U = 3.0$, $p < 0.01$). But both for younger workers and callows the reverse tendency was not so pronounced (Mann-Whitney U-test, $U = 24.0$ and $U = 26.0$; p = ns). They tended to vary in their proportion across all rank positions. Although age does seem to play a role in the overall frequency with which agonistic interactions are initiated, it does not appear to be a good predictor of the rank position an individual will find itself in.

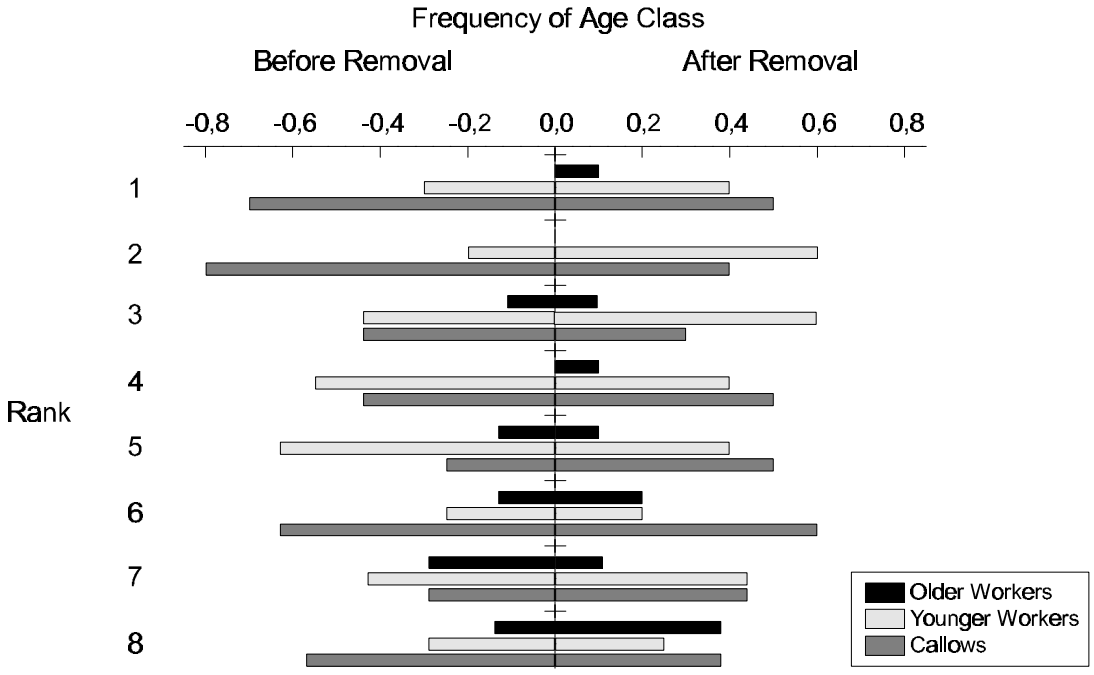

Figure 3.13 Distribution of age classes across the first 8 assigned ranks (representing the number of initiated agonistic interactions) in 10 experimental groups. For discussion see text.

Individuals that were initially reproductive within the experimental groups were usually older individuals having monopolized reproduction unchallenged for a longer period of time (see Tab 3.4 and Tab. 3.5). In all cases, however, when these individuals were experimentally removed reproductive dominance was achieved by callows or younger workers being not older than a maximum of 4 month. Equally callows and younger workers achieved higher rank positions during agonistic interactions than

older workers. Before the removal of the old reproductive callows held 52.9 percent of ranks 1-8 but 60.5 percent of rank 1-4 , after the manipulation however only 43.4 percent of rank 1-8 and 42.5 percent of rank 1-4. Younger workers only held 38.2 percent of rank 1-8 before and 43.4 percent after removal, older workers 8.8 percent of rank 1-8 before and 13.1 percent after removal. Clearly callows and young workers initiated more agonistic interactions than older workers. However their respective number within the group of aggressive individuals after removal was not significantly different from the number expected according to their representation in the whole experimental group (Fisher's exact test, 10 groups tested separately: callows, $p < 0.05$ in ABS 27/1 and PR 2/1 only; younger workers, $p = ns$ in all 10 cases).

Who becomes reproductive?

In eight out of ten experimental groups a single individual succeeded in monopolizing reproduction after the old reproductive was experimentally removed. Only in the case of PR 2/1 and PR 21/1 two workers started to lay eggs after removal. Most interestingly it was also experimental group PR 21/1 in which two individuals have been observed to lay eggs before the manipulation. Comparing the individual history of the workers that monopolized reproduction after the old egg-layer was removed three characteristic groups can be differentiated:

1. If the designated reproductive most frequently initiated aggressive interactions resulting in rank position 1 (YRR in ABS 8/4 and GRO in PR 11/1), she reduced post-removal aggression levels considerably resulting in a low position (YRR dropped to position 10) or did not show any aggression at all (GRO). Both individuals eclosed shortly before the old reproductive was removed and were not older than month at that time. Although both were fully reproductive at the end of observations YRR had only few yellow bodies and in GRO none could be detected suggesting that they have not been reproductive for a longer period. Yet they had consolidated their social rank sufficiently before to become reproductive after the manipulation. GRG in VB 1/1 (rank 2 before removal) behaved differently: The percentage of agonistic interactions she initiated increased from 24.2 to 34.7 percent post-removal reflecting a move from the second most to the most aggressive individual in the group. As explained above the social structure in this group however is less stable. GRG, although 2.5 month old, probably did not establish her social dominance in the group before BBG was removed.
2. If the designated reproductive occupied a lower social rank because she rarely initiated aggressive interactions before the removal, she became more aggressive afterwards. YYG in PR 4/2 initiated 3.1 percent of observed interactions before (rank 6) and 11.7 percent afterwards (rank 3), frequency of aggression of BOB in group PR 22/1 increase from 1.3

percent (rank 6) to 5.0 percent (rank 3). YYB in ABS 27/1 however maintained an aggressive level (4.7 percent, rank 7) comparable to the one before the manipulation (5.3 percent, rank 8). However frequency of agonistic interactions after the removal remained rather low in all cases. Social dominance of these individuals is likely to be maintained by non-aggressive cues.

3. In four groups the designated reproductive worker was not present during the initial observation period but eclosed only shortly before the old reproductive was removed (ABS 2/2, PR 19/1, PR 2/1 and PR 21/1). In this category there are two groups (PR 2/1 and PR 21/1) in which two callows each became reproductive after the manipulation. They did not show any aggression towards one another. Only two of six new reproductives were observed to behave aggressively towards nestmates following the removal of the old reproductive (BOG in ABS 2/2 assumed rank 3 and GRO in PR 21 /1 assumed rank 2). In these cases the regulatory nature of aggressive interactions cannot easily be judged. These callows are able to achieve reproductive dominance without overt aggression.

3.4 Discussion

Virgin workers in experimental groups of *Platythyrea punctata* are able to sustain colony growth through reproduction by thelytokous parthenogenesis in all investigated populations in Florida, Puerto Rico and Barbados. Experimental colonies of virgin workers raised in the laboratory grew to comparable or even larger size as compared to naturally collected colonies, indicating that prolonged thelytoky does not limit colony productivity at least under controlled conditions. Both queens and males are rarely found in field-collected colonies. Queens, while present in some of the colonies collected in Florida, did not regularly reproduce. Worker thelytoky is thus not confined to small, marginal subpopulations but instead occurs over a broad range of the species distribution and therefore constitutes a major mode of reproduction in *P. punctata*.

Worker reproduction by means of arrhenotokous parthenogenesis (but rarely by thelytokous parthenogenesis) is widespread in the higher eusocial Hymenoptera such as bumble bees, stingless bees, honey bees, vespine wasps or ants (Bourke 1988b). Worker reproduction (of males) in queenright colonies is favored by selection on workers, despite queen opposition. An analysis on data assembled on 40 ant species reveals that workers in monogynous species reproduce mostly in queenless conditions, whereas those in polygynous species reproduce in queenless and queenright conditions equally often suggesting that worker reproduction may be associated with monogyny because of the high risk of

orphanage in monogynous colonies (Bourke 1988b). Thelytoky may be an even higher evolved response to such a risk allowing fragmented colonies without reproductives (that in theory might be reduced down to a single individual) to reestablish a mature colony. Additionally, orphaned colonies can produce a new queen by thelytoky and then possibly readopt her as the new queen after she has mated (Bourke and Franks 1995: 356). Currently there is no evidence to sustain this hypothesis in *P. punctata*.

Thelytokous reproduction has been repeatedly argued to constitute an evolutionary dead-end that may trap a certain genotype adapted to a specialized ecological niche as long as that ecological niche persists in space and time (Maynard Smith 1978). This is because parthenogens presumably lack the ability to rapidly generate genotypic diversity in reaction to changing environmental conditions (Itow *et al.* 1984). Several authors nevertheless pointed out that, based on modeling, rates of phenotypic evolution under unisexual and asexual conditions may be of similar magnitude and parthenogens may do as well as obligately bisexual species under similar environmental conditions assuming elevated mutation rates and depressed environmental sensitivity (Lynch and Gabriel 1983; Gabriel and Wagner 1988). The establishment of a parthenogenetic lineage may therefore be a dynamic evolutionary process depending on alterations in life history patterns that determine the successful establishment of an asexual population (Templeton 1982). Environmental conditions which favor the evolution of thelytoky may include situations where a) locating a mate is difficult, e.g. after a severe bottleneck or in marginal, low-density habitats (Stalker 1956), b) there is a high risk of colony fragmentation (Tsuji 1988b) or queen loss (Moritz 1986; Allsopp *et al.* 1997), c) isolated worker groups occasionally found colonies (Lenoir and Cagniant 1986) or d) when higher colonizing abilities are advantageous (Moritz 1991, 1993; Niklasson and Parker 1994). Fission and an elevated risk of colony fragmentation seems to be characteristic for *P. punctata*, at least in the populations studied. Though thelytoky limits genetic recombination, the "general purpose genotype" model states that long-term selection will allow persistence of clones with broad tolerance of abiotic environmental variation (Baker 1965; Lynch 1984). As a further advantage of asexual reproduction, beneficial mutations are more easily passed on to subsequent generations.

Short episodes of sexual reproduction would allow for increased rates of phenotypic evolution and the generation of new clonal lineages in the parthenogen. However, sexuals are rare in *P. punctata*. The rare occurrence of males in *P. punctata* is known both from the field (see Tab. 2.1) and the laboratory, and only two of a total of 824 females from field colonies were found to be inseminated (a dealate queen and a worker), though only in one of them (the worker) developing eggs were present. Both mated individuals were from the same colony (ABS 13) from Florida. The co-occurrence of mated workers and a mated queen in the same nest is a rare phenomenon since gamergates usually do not occur with queen in the same nest (Ward 1983). Both a mated queen and gamergates have been described to reproduce at the same time in a nest of *P. arnoldi* (Villet 1993). One male was found in a

colony from Puerto Rico and Wheeler (1905) reports the occurrence of winged queens in the Bahamas. In the lab, newly eclosed males were never observed to solicit copulations from virgin workers. Whether they mate under natural conditions is unclear. The occasional occurrence of males has been noted in a number of thelytokous Hymenoptera though in most cases their reproductive function remains unclear. In the obligate thelytokous ant *Pristomyrmex pungens* a small number of males (2 to 3 percent) is usually produced during the summer (Itow *et al.* 1984). Production of males in this species can simply be explained as phylogenetic inertia from an ancestral sexual state that has not been counter-selected so far. In lab-reared lines of the thelytokous biotype of *Anaphes diana* Girault out of over 8,000 offspring only 3 males emerged, all of which made no attempt to mate (Aeschlimann 1990). In the pteromalid parasite *Muscidifurax uniraptor* „functionless" males are occasionally produced that do not mate with conspecific females but are cross-fertile with the presumed parental strain *M. raptor* (Cornell 1988).

In three of the four ant species (including *P. punctata*), where thelytoky has been clearly demonstrated, it appears to be the only mode of reproduction at least in some populations. In the queenless myrmicine ant *P. pungens*, 97.6 % of the intranidal workers are reproductively active for a certain period of their life and mated individuals have never been found, suggesting that this species is obligatorily parthenogenetic (Tsuji 1988a). The same appears true at least for the Okinawa population of *Cerapachys biroi*, though ergatoid queens are known from other populations (Tsuji and Yamauchi 1995). In contrast, workers of *Cataglyphis cursor* start reproducing by parthenogenesis only after the death of the queen (Cagniant 1979, Cagniant 1983, Cagniant 1984). As shown above, thelytoky is by far the most important mode of reproduction in the studied populations of *P. punctata*, though insemination has been documented at least in the Florida population.

In contrast to the initial hypothesis on the absence of aggression under thelytokous reproduction, aggression was observed in *P. punctata* both before and after the reproductive individual was removed from virgin worker groups. Following the removal a sharp increase in agonistic interactions such as antennal boxing, biting or dragging the opponent through the nest, leaping towards the opponent or immobilization was frequently observed. These aggressions led to the rearrangement of social status among a small group of workers that actively initiated agonistic interactions. In a number of Hymenopteran species it is known that upon removal of the queen such agonistic interactions between workers significantly increase in frequency. Subsequently, one or more of these high-ranking workers start to reproduce (Heinze *et al.* 1994). Upon queen death or removal workers in polistine paper wasps usually establish a dominance hierarchy through agonistic interactions (Miyana 1991; Spradbery 1991). Similarly this has been described for a number of leptothoracine ants such as *Leptothorax unifasciatus* or *L. gredleri* (Heinze *et al.* 1997; Heinze and Oberstadt 1999). In ponerine ants an increase in agonistic interactions resembling those observed in *P. punctata* is observed when the top-ranking gamergate is removed. In *Pachycondyla sublaevis* the rate of agonistic interactions increased almost 30-

fold upon removal of the gamergate (Higashi *et al.* 1994). In disturbed hierarchies of *Dinoponera quadriceps* (i.e. after natural replacement or artificial removal of the *alpha* female) the proportion of more aggressive dominance interactions such as gaster rubbing and gaster curling increased compared to stable hierarchies (Monnin and Peeters 1999). In thelytokous worker groups of *P. punctata* rate of agonistic interactions increased in 8 out of 10 experimental groups after the old reproductive was experimentally removed. Agonistic interactions after the removal consisted of behaviors within a more aggressive context such as biting or dragging the opponent through the nest. Although aggressive interactions are highly ritualized a shift towards more aggressive behaviors reflects a response to an increased probability to upgrade ones social status. The removal of the old reproductive individual thus creates a situation of temporal social instability within the colony in which individual status can be rearranged through agonistic interactions. The next section will evaluate the significance of aggression for the social organization of *P. punctata*.

Or slightly rephrased: How do these dominance interactions contribute to the regulation of social status? Contrasting to the situation described in many ponerine ants (Oliveira and Hölldobler 1990; Ito and Higashi 1991; Medeiros *et al.* 1992; Ito 1993a, 1993b; Heinze *et al.* 1994; Higashi *et al.* 1994; Sommer *et al.* 1994; Liebig *et al.* 1995; Trunzer 1999), individuals in experimental worker groups of *P. punctata* did not form clear linear hierarchies. However, it is common that not all nestmates engage in dominance interactions and resulting hierarchies do not include all nestmates, e.g. in leptothoracine ants (Cole 1981; Franks and Scovell 1983; Heinze *et al.* 1997; Heinze and Oberstadt 1999). In the absence of linearity in dominance, the resulting social structure in *P. punctata* is characterized as an oligarchy with one or few individuals dominating most others, with reversals of dominance occurring among those. Top-ranking workers initiated a disproportionally high number of agonistic interactions. Calculated dominance matrices are, with one exception, non-linear involving only a fraction (between 3.3 and 37.1 percent, without experimental group VB 1/1) of all workers in the experimental groups (measured at the time of removal of the reproductive). Earlier investigations of agonistic interactions in a single medium-sized colony of *P. punctata* from Florida resulted in a near-linear dominance order, although similarly to this investigation only among few individuals (Heinze and Hölldobler 1995). In this study linearity of dominance however was not tested statistically. Similarly, only a small fraction of workers (7 out of a total of 51 individuals) initiated aggressive interactions. Therefore the absence of linearity can be safely assumed given the restricted number of observations. These results more closely match what is suggested here as an oligarchic social structure. In the absence of more detailed information on the consequences of the observed aggression social dominance relations may best be regarded as non-linear.

There are several possible reasons for the absence of linearity in rank orders some of which also apply to *P. punctata* (Appleby 1983): The complete inconsistency of individual interactions due to

frequent reversals; chance as critical factor determining the outcome of the first encounter, which predetermines subsequent interactions; and small group size or incomplete information among group members. As has been suggested for larger associations, dominance relations in *P. punctata* can therefore be better described as being organized in a set of dominance layers, the first of which contains individuals initiating agonistic interactions, the second containing individuals only being targeted by attacking nestmates and a third layer consisting of individuals not involved in interactions (Reeve and Ratnieks 1993). The recruitment of future reproductives in *P. punctata* is however not confined to individuals within the top social layer.

Many studies have shown that animals successfully dominating one individual several times in a row have a greatly increased probability of successfully attacking a third individual although this effect appears to be rapidly diminishing over time (e.g. in sticklebacks; compare Bakker *et al.* 1989; Bakker and Sevenster 1983). Among most ants that show aggression behavioral domination results in the complete monopolization of reproduction, which leads either to secondary monogyny or functional monogyny (e.g. Buschinger 1968; Heinze *et al.* 1994). Prior social experience seems to be involved in what has been termed the "winning effect" (Chase *et al.* 1994 and citations therein). This effect might also be responsible for a short-term increase in attacking frequency of a dominant individual self-reinforcing its dominant position within the worker group (Bonabeau *et al.* 1996). This is indeed what is observed in *P. punctata*: When an individual dominates another in a large number of agonistic interactions these tend to occur in short sequence. Clearly the presence or absence of the winning effect depends on whether one defines winning as the outcome of single agonistic interactions or integrates it as an overall summary of a series of agonistic interactions (Chase *et al.* 1994).

Interestingly, callows and young workers in *P. punctata* initiated more agonistic interactions than older workers. However, contrasting findings in some other ants (e.g. Ito and Higashi 1991; Higashi *et al.* 1994) age is not a good predictor in the formation of social hierarchies. The proportion of both callows and young workers aggressively interacting was not higher than their overall representation in the colony. Similar results have recently been reported for worker hierarchies in the ant *Leptothorax gredleri* (Heinze and Oberstadt 1999). While in *P. punctata* the dominant reproductive was able to maintain its reproductive status without being challenged for months, aggressions among other workers ceased with increasing age. Once a high social status is achieved it is maintained without the need for physical interactions. In 8 experimental groups of *P. punctata* callows or younger workers replaced the old reproductive upon removal. Replacement did however not correlate with prior social status. Similar age effects have been observed in the queenless ponerine ant *Dinoponera quadriceps* where callows dominated older workers with the exception of the *alpha* worker who generally was unchallenged. Callows subsequently excluded older workers from higher ranks in the hierarchy. In sharp contrast here *beta* usually superceded *alpha* (Monnin and Peeters 1999). In *Pachycondyla sublaevis* it is usually

younger workers that occupy the highest ranks. Callows and other younger workers tended to fill higher positions than did old workers. The dominance order among newly eclosed callows occupying higher ranks was not stable but subject to frequent reversals (Higashi *et al.* 1994). In the queenless *Pachycondyla* sp. from West Java callows and young workers positioned higher in the hierarchies (Ito 1993a). In *Ophthalmopone berthoudi* young workers form the pool from which new gamergates are produced during periods of male activity (Peeters and Crewe 1985). A similar picture emerges in dominance interactions in the primitively eusocial paper wasp *Polistes instabilis* (Hughes and Strassmann 1988). Once callows and young workers established themselves in the social hierarchy, frequency of agonistic interactions declines to the point where older individuals are rarely observed in such interactions. In a number of paper wasp species aggression ceases once a stable hierarchy is formed and individuals maintain a non-confrontational dominance where social status is recognized by other, possibly endocrine, factors (Röseler and Röseler 1989, Röseler 1991).

Evolutionary theory predicts that younger workers may have been selected to occupy higher ranks because thereby they can perform non-risky intranidal tasks while cueing for reproduction. In addition they have a longer life expectancy compared to older individuals and, once they superceded the old reproductive, may monopolize reproduction for several month or even years. By avoiding a frequent turnover in the individual monopolizing reproduction overall colony efficiency could be increased by reducing the disturbing effects of frequent aggression. Staying in the nest as a hopeful reproductive therefore seems to be a successful strategy in thelytokous workers. Social queuing has been recently modeled as one reason why subordinates may remain in the group while giving up on direct reproduction. By staying in the nest they increase their possibility of acquiring reproduction in the future (Kokko and Johnstone 1999). When the dominant reproductive becomes older and less fecund, younger high-ranking workers that established their social status before will start to develop their ovaries probably because the inhibitory ability of the dominant individual may decrease with decreasing fecundity. Social queuing could serve as a regulatory mechanism in ants where workers do not have the choice to leave the nest and found a new colony semiclaustrally due to associated risks such as predation or parasitism (Liebig *et al.* 1998). In *Pachycondyla sublaevis* (Peeters *et al.* 1991) and *Streblognathus aethiopicus* (Ware *et al.* 1990) presumably older gamergates have been unable to suppress ovarian development and oviposition in virgin nestmates that subsequently superseded them. In *P. punctata* social queuing could function to translate a high social status obtained early in life into a high probability to become reproductive later. The proximate facilitating mechanism however is not understood.

Dissections of both field-collected colonies and those that were kept in the laboratory for more than three years demonstrate that in each colony typically only one individual is reproductively active at a given time regardless of colony size, although some colony members may possess ovaries in various

stages of development. Ovaries of these individuals will degenerate before maturation. This supports the notion that there is a strong tendency for a reproductive monopoly by a single reproductive individual in this species. In *P. schultzei* only a single virgin replacement worker (usually the second-ranking) develops her ovaries after removing the single gamergate (Villet 1991b). The mechanism leading to the establishment of a reproductive monopoly in *P. punctata* is however not well understood. There are some gamergate species in which virgin workers have developed ovaries while coexisting with one or more gamergates (summarized in Peeters 1993). However, oocyte development in virgin workers of *Rhytidoponera* sp. 12 is abnormal and these eggs never mature indicated by the absence of yellow bodies in these individuals (Peeters 1987). In general, individuals who activate their ovaries in periods of social instability should pose the greatest threat to dominant reproductives and therefore should receive most of the aggression. Worker policing is a possible mechanism that prevents nestmates from developing their ovaries by antagonism directed towards these individuals (Ratnieks 1988). In *P. punctata* both individuals with undeveloped ovaries and those which already started to develop their ovaries after the removal of the dominant reproductive were more often attacked than workers who had resorbed ovaries. However, contrasting the scenario outlined above, workers with undeveloped ovaries were attacked almost three times as much as those whose ovaries were already activated. Pheromonal cues reflecting subtle physiological changes at the early stages of ovarian activation may therefore be involved in fine-tuning recognition of ovarian status. An increased rate of aggression indiscriminantly targeting a larger number of workers would merely serve as a signal, correlated to the reproductive needs of the colony, for most of the workers to refrain from reproduction. Ovarian development after orphanage could therefore be down regulated in all but one individual by the perception of direct physical aggression in the colony.

But why is *P. punctata* functionally monogynous although multiple individuals would have equal potential for reproduction? In theory several parthenogenetic workers should simultaneously produce diploid offspring thereby increase colony productivity by adding to the workforce of the colony. In this case however a decreasing proportion of workers would contribute to colony labor which might seriously affect overall colony efficiency. Experimental removal or addition of post-reproductive foragers in large colonies of *P. pungens* decreased colony efficiency quite dramatically (Tsuji 1994). The proposed benefits of polygyny in unpredictable habitats (e.g. Hölldobler and Wilson 1977) could further be offset by other costs such as a limitation in resources available for colony growth or by costs related to the proximate mechanism of reproductive regulation itself which would seriously decrease overall colony success. It is well established that individual queen fecundity declines as size of pleometrotic foundress groups increases, a phenomenon termed "reproductivity effect" (Wilson 1971). A similar effect can also be observed in mature polygyne colonies, e.g. in *Plagiolepis pygmaea* (Mercier *et al.* 1985) or in the fire ant *Solenopsis invicta* (Vargo and Fletcher 1989; Vander Meer *et al.*

1992). Several mechanisms including differential availability of food (Mercier *et al.* 1985), brood cannibalism (Tschinkel 1993) and pheromone-mediated mutual inhibition (Vargo 1992) have been discussed. In theory a polygyne situation in *P. punctata* would be advantageous directly after colony fission of a thelytokous worker group to facilitate the rapid growth of the colony fragment. Too little is however known about the ecological characteristics of *P. punctata* and most other ponerine ant species to evaluate the applicability of this hypotheses.

Since only a limited number of individuals were observed in aggressive interactions the question arises whether aggression plays at all a role in the reproductive regulation of *P. punctata* or whether it is merely a phylogenetic left-over from sexual reproduction? Slightly rephrased, does social status have a predictive value for future reproductive status? While it does not seem to be important to achieve a high social status within the colony to successfully supersede the reproductive agonistic interactions obviously play an important role in determining the social status. In *P. punctata* the dominant reproductive is rarely attacked by nestmates and equally does not initiate agonistic interactions. Rank position did not have a clear predictive value on future reproductive success since both socially high-ranking workers (e.g. in groups ABS 8/4, VB 1/1 and PR 11/1) and those that did not interact aggressively with nestmates before (e.g. ABS 2/2, PR 19/1 and PR 2/1) superseded the old reproductive. Since observations after removal were not continuous the possibility remains that agonistic interactions among the new reproductives with lower social status were simply not recorded. Given the extensive amount of observations this is unlikely for such 'non-interacting' workers should have been targeted too on occasion during the observation periods. Aggression clearly is not necessary to achieve the dominant reproductive status within the colony. There are other examples of social structures in ponerine ants in which the principal egg-laying female is not physically attacked by aggressive nestmates or she herself does not take part in agonistic interactions. In *Pachycondyla apicalis* dominant workers are never attacked by the queen or any other colony member (Oliveira and Hölldobler 1990). More extremely physical aggression has never been observed in the queenless *Pachycondyla krugeri* (Wildman and Crewe 1988), *Platythyrea lamellosa* (Villet *et al.* 1990), *P. schultzei* (Villet 1991b) or *Steblognathus aethiopicus* (Ware *et al.* 1990). In all these cases reproductive dominance is likely to be controlled by more subtle, non-aggressive cues such as a volatile short-ranged or contact pheromone that are produced by the dominant reproductive. In the Indonesian queenless ant *Amblyopone* sp. for instance gamergates inhibit ovarian development of virgin workers without physical harassment suggesting that inhibition of ovarian development is caused by pheromones (Ito 1993b). Similarly in *Aphaenogaster cockerelli* workers from a queenright colony fragment attacked workers from a queenless fragment of the same colony who had developed their ovaries upon reunification. Recognition may be mediated by olfactory cues (Hölldobler and Carlin 1989). The dominant reproductive status may be "honestly" signaled by a particular cuticular hydrocarbon profile

characteristic of dominant individuals: In *Dinoponera quadriceps* the hydrocarbon 9-C31 may supply honest information about *alpha's* reproductive status to low-ranking workers (Monnin and Peeters 1999). It has been demonstrated that workers in larger colonies of *Harpegnathos saltator* who developed their ovaries are aggressively inhibited from oviposition by infertile nestmates (Liebig *et al.* 1999b). Here, a hypothetical fertility signal consisting of longer-chained hydrocarbons present on the cuticle is correlated with the physiological state of reproductive queens and gamergates to the colony (Liebig *et al.* 1999a). Recently, a similar mechanism has been discussed in *Diacamma* sp. from Japan (Tsuji *et al.* 1999). Additionally, other cues may contribute to recognition of reproductive status. Juvenile hormone titer in the hemolymph was found to be positively correlated with dominance in *Polistes gallicus* which may be perceived by olfactory cues (Röseler *et al.* 1984). Additionally ovariectomized foundresses could achieve a high social status but were unable to become reproductively dominant (Röseler and Röseler 1989). Similarly social status and reproductive dominance in *P. punctata* are not closely correlated.

In conclusion, the function of agonistic interactions in thelytokous worker groups of *P. punctata* remains unclear given its thelytokous nature of reproduction. In general, dominance interactions among morphologically identical individuals are known to lead to differential reproductive success that can be measured by the degree of reproductive skew (e.g. Reeve and Ratnieks 1993). This has been observed in primitively eusocial bees (Breed and Gamboa 1977), wasps (Reeve 1991; Röseler 1991) and bumble bees (Röseler and van Honk 1990). The theory predicts that a high skew Hymenopteran society will only be evolutionary stable if relatedness among nestmates is high (reviewed in Keller 1993a). Positive empirical evidence accumulates in eusocial insects (Reeve and Ratnieks 1993) but does not always support this prediction: In some high skew societies such as in functionally monogynous species worker relatedness is indeed lower than expected (Heinze 1995). Under the assumption that thelytoky is the only mode of reproduction in *P. punctata*, reproductive competition however should be absent since workers are clonal copies of one another. Because they gain as much through inclusive fitness benefits as they would through direct reproduction, workers should refrain from testing the dominant unless her reproductive capacity declines. Challenging the reproductive worker at any times could carry associated costs that would significantly reduce colony productivity (Cole 1986). Intense aggression potentially carrying such costs occurs among worker 'pseudo-queens' in the thelytokous Cape honeybee *Apis mellifera Capensis* upon queen loss that decreases efficiency of the division of colony labor considerably (Moritz 1989; Greeff 1996b).

Reproductive competition might additionally be exerted through other behavioral mechanisms such as oophagy and the destruction of brood (reviewed in Bourke 1988b). No incidence of oophagy (neither freshly laid eggs nor eggs that have been deposited on the egg pile) was, however, observed in over 1,638 hours of observation including 38 oviposition events. In thelytokous worker colonies of *P.*

punctata egg cannibalism is not expected to occur because of the absence of genetic conflict over reproduction. Yet on various occasions egg-layers were observed to hold the freshly laid egg for several minutes in their mandibles before it was deposited. This behavior may constitute a form of egg-guarding by which laying workers could temporarily protect their own eggs from destruction by nestmates before it blends in with the colony odor. This situation would arise in genetically more heterogeneous colonies that may result from episodes of sexual reproduction. In this regard the mechanism regulating reproduction in queenright colonies that contain mated individuals would be interesting. Under thelytoky however it would be without function.

Are there alternative pressures selecting for aggressive interactions among non-reproductive workers in *P. punctata*? A possibly alternative is related to task allocation. Aggression could well function to facilitate a smooth division of labor both between reproductive and non-reproductive and among non-reproductive nestmates securing optimal task allocation and thereby a maximal colony efficiency. On the proximate side aggression against potential reproductives (owing to thelytoky in *P. punctata* all workers are potential reproductives) would either manipulate them by direct ovarian suppression or could signal to them to refrain from reproduction in the colony's own interest of limiting the number of reproductives. Either mechanism would guarantee functional monogyny in the colony. The underlying assumption that more than one reproductive worker would decrease overall colony efficiency however has not been tested in *P. punctata*. If it is true one would however expect a decrease in colony growth in colonies were two or more reproductives are reproducing. In the two case where two workers were reproducing (PR 2/1 and PR 21/1) this effect was not pronounced. Aggression could provide a mechanism by which cooperation and regulation the division of labor within the colony can be achieved. This system would be maintained by strong colony-level selection for high efficiency in the division of labor especially under adverse environmental conditions. Interestingly, dominance hierarchies in *Pachycondyla sublaevis* have been suggested to both regulate reproduction and polyethism. Like in *P. punctata*, most members of the colony are potential reproductives, i.e. gamergates (Ito and Higashi 1991). In the primitively eusocial polistine wasp *Ropalidia marginata* the queen is reproductively dominant but does not seem to play a role in the regulation of colony activity (Premnath *et al.* 1996). Instead, task allocation seems to be based on a self-regulatory process by the workers themselves (Premnath *et al.* 1995). Recently, a similar flexibility within an age-based task allocation system mediated by social interactions of workers has been modeled (Naug and Gadagkar 1999). Aggressive interactions among non-reproductives in *P. punctata* could equally serve to enhance colony efficiency by regulating social status. The proximate mechanism leading to the determination of reproductive dominance however is not yet clear.

4 Genetic population structure

4.1 Introduction

Hewitt and Butlin (1997) define population structure as partial variation in density and genetic composition of individuals in a given species. Social insects serve as ideal model systems because they form highly structured populations with unusual genetic structure: Closely related individuals live in eusocial colonies that are relatively stable in composition over space and time but may vary in genetical composition due to differential reproduction of colony members; these colonies may be subdivided into semi-isolated subpopulations, or demes, on a larger geographical scale; and finally populations themselves may differ genetically due to differential dispersal and genetic drift between them. Since evolution is the result of progressive changes in the genetic composition of a given population (Hartl and Clark 1989) studying the population genetic structure aims at clarifying the evolutionary history of this species or population. Population genetics investigates how genetic (such as segregation, recombination and mutation), ecological (population size, mating system and migration) and evolutionary factors (natural selection) in concert influence the population structure of a given species (Wright 1951; Amos and Harwood 1998).

The rapid development of highly variable molecular DNA markers within the last 15 years facilitated the study of complex population structures. Polymorphisms at the DNA sequence level can be visualized as changes in the cleavage pattern of DNA fragments treated with restriction endonucleases. These polymorphisms are termed restriction fragment length polymorphisms (RFLP). With the advent of the polymerase chain reaction (PCR) (Mullis 1990) genetic variation could be easily studied at the level of mtDNA sequences. In the 1980's analysis of variable number of tandem repeats (VNTR) loci (or minisatellite DNA) became established known as DNA fingerprinting (Jeffreys *et al.* 1985). Finally during the early 1990's other techniques such as random amplified polymorphic DNA (RAPD), single-stranded conformation polymorphism (SSCP) or amplified fragment length polymorphism (AFLP) have been applied to population genetic analysis.

In order to estimate the frequency of alternative reproductive tactics in *P. punctata* and the population genetic consequences of thelytoky, the genetic variation within and between colonies and populations was investigated using yet another class of polymorphic markers: microsatellites.

Microsatellites are nuclear DNA markers that consist of 2 to about 100 tandem repeats of 1 to 5 nucleotide motifs (such as $(GT)_n$ or $(GC)_n$) which can be abundant in insect genomes (Hamada *et al.* 1982; Thoren *et al.* 1995). Some dinucleotide $(CT)_n$ motifs estimated to occur every 15 kbp in the genome of *Apis mellifera* (Estoup *et al.* 1993). Because of their high mutation rate they generally have a large number of alleles (Weber and Wong 1993). It is likely that slipped-strand mispairing during replication is the major cause of observed allelic length polymorphism. Mutations typically involve the gain or loss of one, or less frequently, a few repeat units. Recently the stepwise mutation model (SMM) has been revised in an attempt to model length variation at microsatellite loci (e.g. Goldstein *et al.* 1995; Slatkin 1995). However a high degree of microsatellite variability may be due to variation in the repeat flanking regions (Grimaldi and Crouau-Roy 1997). Microsatellites are currently widely applied in population genetic studies because they are co-dominantly inherited, highly variable and presumably neutral Mendelian markers (Jarne and Lagoda 1996).

In recent years microsatellites have become available for a wealth of Hymenopteran species in a variety of taxa (Tab. 4.1). Within the Formicidae alone primers have been developed for at least 21 species. They serve as a useful tool in investigations of the relatedness structure of colonies (Paxton *et al.* 1996; Herbers and Mouser 1998), reproductive skew within colonies (Bourke *et al.* 1997; Field *et al.* 1998), the detection of mating frequency (Estoup *et al.* 1994; Oldroyd *et al.* 1995; Thoren *et al.* 1995; Gadau *et al.* 1996; Foitzik *et al.* 1997; Gertsch and Fjerdingstad 1997; Chapuisat 1998; Fjerdingstad *et al.* 1998) and genetic differentiation from the colony to the species level (Estoup *et al.* 1995a, 1995b, 1996; Chapuisat *et al.* 1997; Widmer *et al.* 1998).

Several studies have already shown that microsatellites give similar results to allozymes while studying population genetic structure in ants (Seppä and Gertsch 1996; Chapuisat *et al.* 1997; Ross *et al.* 1997). Though microsatellite marker systems are currently the first choice when studying population-genetic differentiation in social insects, various other DNA markers developed in recent years are of similar analytical power. Mitochondrial DNA (mtDNA) markers are especially useful for inferring phylogenetic relationships between and within taxa (e.g. Crozier *et al.* 1995; Ayala *et al.* 1996) or investigate population structure (Stille and Stille 1992, Tay *et al.* 1997). Another class of markers, random amplified polymorphic DNA (RAPD), are currently used to build genetic linkage maps (Hunt *et al.* 1995, 1998; Page *et al.* 1995), study task allocation (Dreller *et al.* 1995; Dreller 1998; O'Donnell 1998) or determine patriline structure within colonies (Fondrk *et al.* 1993; Hasegawa 1995).

Microsatellites were chosen as genetic marker system to investigate population-genetic differentiation in *P. punctata* because a pilot study using 16 allozyme systems (ACOH, ADH, AK, EST, GPI, HK, IDH, LDH, MDH, MDHP, ME, OBP, PGDH, PGI, PGM, XDH) did not reveal any genetic variability within or among populations and therefore could not be further applied (for detailed

allozyme reference see Harris and Hopkinson (1976) and Hillis and Moritz (1990)). Hymenopteran species are commonly known to possess low levels of electrophoretic variability (Metcalf *et al.* 1975; Pamilo *et al.* 1975; Berkelhamer 1983; Graur 1985; Packer and Owen 1992) generally considered to result from the exposure of alleles in males due to haplodiploidy (Pamilo *et al.* 1978; Pamilo and Crozier 1981) though there are few exceptions (Hung *et al.* 1986). Similarly, in another set of pilot experiments multi-locus fingerprinting using digoxigenin-marked oligonucleotide probes ($(GATA)_4$ and $(GGAT)_4$) following a protocol developed by Gadau *et al.* (1996) did not reveal satisfactory results likely due to the limited amount of DNA that could be obtained from individual ants.

The investigation of the population genetic structure of three natural populations of *P. punctata* was carried out to answer the question whether thelytoky does have a significant effect on genetic variation within and among natural colonies. Since populations of eusocial insects are strictly hierarchically structured (individuals live in colonies, colonies belong to subpopulations that are organized within larger meta-populations), genetic variation can be expected at several different levels of organization. Under the assumption of a high proportion of thelytokous reproduction and depending on the rate of mutations either a clonal population structure or a mosaic of several coexisting clones (possibly identical to nesting units) should be expected. So far, a population genetic survey employing microsatellite markers has not been carried out in any other thelytokous ant species, but would be equally desirable.

Family	Subfamily	Species	# of loci	Repeat type	Reference
Andrenidae		*Andrena jacobi*	4	dinucleotide	Paxton *et al*., 1996
Apidae	Apinae	*Apis andreniformis*	4	?	Oldroyd *et al*., 1997
		Apis mellifera	75	dinucleotide	Estoup *et al*., 1993, 1994
			18	mono-/ dinucleotide	Rowe *et al.*, 1997
			2	?	Ratnieks and Keller, 1998
			15	tri-/ tetranucleotide	Haberl and Tautz, 1999
	Bombinae	*Bombus terrestris*	26	dinucleotide	Estoup *et al*., 1993, 1995, 1996
	Meliponinae	*Melipona bicolor*	33	dinucleotide	Peters, pers. comm.
		Scaptotrigona postica	5	dinucleotide	Paxton *et al*., 1999
Cynipidae		*Diplolepis spinosissimae*	1	dinucleotide	Plantard *et al*., 1998
		Diplolepis rosae	1	dinucleotide	Plantard *et al*., 1998
Formicidae	Dolichoderinae	*Linepithema humile*	19	di-/ trinucleotides	Krieger and Keller, 1999
	Formicinae	*Camponotus consobrinus*	5	dinucleotide	Crozier *et al*., 1999
		Camponotus ephippium B	19	dinucleotide	Macaranas, pers. comm.
		Camponotus herculeanus	3	dinucleotide	Gertsch *et al*., 1995
		Camponotus ligniperdus	3	dinucleotide	Gertsch *et al*., 1995
		Formica lugubris B	5	dinucleotide	Chapuisat, 1996
		Polyrachis sp. R5	5	?	Robson, pers. comm.
	Myrmicinae	*Acromyrmex echinatior*	5	dinucleotide	Ortius *et al*., 1999
		Atta colombica	2	dinucleotide	Fjerdingstad *et al*., 1998
		Crematogaster smithi	5	trinucleotide	Haberl, pers. comm.
		Leptothorax acervorum	4	dinucleotide	Bourke *et al*., 1997
		Leptothorax nylanderi	3	dinucleotide	Foitzik *et al*., 1997
		Leptothorax spinosior	10	dinucleotide	Hamaguchi *et al*., 1993
		Myrmica punctiventris	3	dinucleotide	Herbers and Mouser, 1998
		Myrmica tahoensis	3	dinucleotide	Evans, 1993
		Pogonomyrmex rugosus	2	di-/ trinucleotides	Gadau, pers. comm.
		Solenopsis invicta	8	dinucleotide	Krieger and Keller, 1997
	Ponerinae	*Diacamma cyaneiventre*	10	dinucleotide	Doums, pers. comm.
		Platythyrea punctata	10	dinucleotide	Schilder *et al*., 1999
		Rhytidoponera sp. 12	14	?	Tay, pers. comm.
	Pseudomyrmecinae	*Pseudomyrmex pallidus*	19	di-/ trinucleotides	Peters, 1998
Torymidae		*Megastigmus wachtli*	8	dinucleotide	Carcreff *et al*., 1998
Vespidae	Polistinae	*Parachartergus colobopterus*	5	di-/ trinucleotides	Choudhary *et al*., 1993
			?	di-/ trinucleotides	Strassman *et al*., 1996
		Polistes annularis	7	trinucleotide	Hughes and Queller, 1993, Peters *et al*. 1995
		Polistes bellicosus	18	trinucleotide	Strassman *et al*., 1997
		Polistes japonicus	1	trinucleotide	Zhu, pers. comm.
	Vespinae	*Vespula rufa*	47	dinucleotide	Thoren *et al*., 1995

Table 4.1 Overview of Hymenopteran species for which microsatellites have been developed. In addition to taxonomic information number of microsatellite loci and the repeat type are given (? = information not available).

4.2 Material and methods

4.2.1 Study sites and specimens

A total of 51 colonies of *Platythyrea punctata* were used in the population genetic analysis. This included 21 colonies from Archbold Biological Station, Highlands county ('ABS'), 1 colony from Vero Beach ('VB'), 3 colonies from Ft. Pierce ('FP'), both St. Lucie county, and one from No Name Key ('NNK'), Monroe county (Fig. 2.1), 23 colonies collected in Puerto Rico ('PR'), and 2 colonies from Barbados, West Indies ('BAR') (for details on collecting location see chapter 2). Individual workers from 29 other ant species of the subfamilies Cerapachyinae (*Cerapachys biroi*), Formicinae (*Camponotus castaneus*, *C. rufipes*), Myrmeciinae (*Myrmecia forficata*, *M. gulosa*, *M. nigriceps*, *M. simillima*), and Ponerinae (*Amblyopone* spec., *Diacamma ceylonense*, *D.* spec., *Dinoponera quadriceps*, *Ectatomma ruidum*, *E. tuberculatum*, *Harpegnathos saltator*, *Hypoponera opacior*, *Odontomachus bauri*, *O. bruneus*, *O. clarus*, *O. troglodytes*, *Pachycondyla apicalis*, *P. hottentota*, *P. obscuricornis*, *P. rufipes*, *P. villosa*, *P.* near *oberthueri*, *Platythyrea parallela*, *P. quadridenta*, *P. tricuspidata*, *P.* spec.) both from the new and old world tropics were obtained from various collectors alive or preserved in 96% ethanol.

4.2.2 DNA extraction

Nuclear DNA was extracted from freshly frozen whole individual workers taken from lab-reared colonies using either a phenol / chloroform extraction protocol modified after Heinze *et al.* 1994 and Gadau *et al.* 1996) or following a Chelex® 100 resin (Bio-Rad) extraction protocol modified slightly after Thoren *et al.* 1995 and Altschmied *et al.* 1997. Following the phenol / chloroform extraction protocol individuals were homogenized in 150 µl extraction buffer A (10 mM Tris-HCl pH 7.5, 60 mM NaCl, 10 mM EDTA). After adding 150 µl extraction buffer B (0.2 mM Tris-HCl pH 9.0, 30 mM EDTA, 2 % SDS) and 5 µl Proteinase K (10 µg/µl, Boehringer Mannheim) the homogenate was incubated for 60 to 120 min at 56 °C. DNA was extracted once using 300 µl Phenol (Roth) and once using 300 µl chloroform - isoamyl alcohol (24 : 1). Isolated DNA was precipitated from the aqueous phase by adding 1/10 of the volume 3 M sodium acetate and two volumes of 99 % ethanol overnight. DNA was washed once with 70 % ethanol, vacuum-dried, resuspended in 80 µl bidest. water and stored at -20 °C until use. For Chelex® extraction freshly frozen workers were homogenized in 100 µl aqua

dest. and subsequently 10 µl Proteinase K (10 µg/µl), 7 µl DTT buffer (1 M DTT, 10 mM NaAc pH 5.2) and 100 µl 10 % Chelex® solution added. Homogenates were vortexed briefly and, after incubating for 60 min at 56 °C, briefly vortexed again and incubated for 8 min at 100 °C. Homogenates were briefly vortexed, centrifuged for 3 min and the supernatant was transferred into a new reaction tube avoiding Chelex® carry-over. DNA was stored at -20 °C until use.

4.2.3. Construction of partial genomic library

A partial genomic library was constructed according to protocols by Hughes and Queller 1993) and Estoup & Turgeon (pers. comm., protocol available at http://www.inapg.inra.fr/dsa/microsat/microsat.htm) using pooled genomic DNA from about 30 *P. punctata* workers collected in Florida ('ABS'). 5 micrograms of high-molecular-weight DNA were digested to completion with *Sau*3AI (Pharmacia Biotech) and electrophoresed in a 1 % agarose gel (Gibco BRL, in TBE buffer). DNA fragments in the size range of 400 to 600 bp were excised from the gel and DNA was purified using the NucleoTrap Kit (Macherey & Nagel).

Phagemid pBluescript® SK (+/-) vector derived from pUC19 (Pharmacia Biotech) was linearized with *Bam*HI (Pharmacia Biotech) and subsequently dephoshorylated according to Sambrook *et al.* (1989). Competent *E. coli* cells (DH5α strain, Gibco BRL) were prepared according to Sambrook *et al.* (1989). The ligation reaction was optimized by conducting several test-ligations. Ligation reaction was carried out in a total volume of 10 µl over night at 16°C. A molar ratio of 1:2 of vector-to-target DNA resulted in the maximum number of transformants. 2 µl of the transformation reaction was plated on standard LB-medium agar plates containing 0.01 % IPTG, 0.001 % X-Gal and 0.01 % Ampicillin. Following over-night incubation at 37 °C, clones were transferred onto nitrocellulose filters (Sartorius). After bacterial lysis DNA was bound to the nitrocellulose filter by baking at 80 °C for 2 h.

4.2.4 Microsatellite identification and DNA sequencing

Synthetic oligonucleotides $(AG)_7A$ and $(GT)_7G$ for library screening were kindly provided by M. Haberl, Munich. 16 pmol of a 1:1 oligonucleotide mix was end-labeled with [α^{33}P] dATP (Amersham) using 9 units of T4-kinase (MBI) and purified on a NucTrap® probe column (Stratagene). Hybridization of labeled oligonucleotide to the nitrocellulose filters was carried out in 6 × SSC, 10 × Denhardt's, 0.5 % SDS for 24 h at 45 °C. Filters were washed at 65 °C with 6 × SSC, 0.2 % SDS and exposed to X-ray film (Noras) for 2-3 days at -20 °C. Positive clones were identified, picked from the plate, grown overnight in LB medium (containing 0.01 % Ampicillin) and plated on LB medium agar plates. Mini-

prep DNA was prepared following Sambrook *et al.* (1989), digested using *Eco*RI and *Xba*I (both Pharmacia Biotech) and run on a 1 % agarose gel together with a size marker to determine insert size.

Positive clones were sequenced either using the ^{T7}Sequencing™ Kit (Pharmacia Biotech) or using an ABI PRISM 377 DNA automated sequencer following manufacturer's instructions. The sequences were analyzed using the program CLONE Manager 4.0 (Scientific & Educational Software). Cloned sequences were searched for significant sequence alignments in the GenBank nucleotide database using the NCBI sequence similarity search tool BLAST 2.0.8 (Altschul *et al.* 1990). Clones containing dinucleotide microsatellite loci were subsequently screened for genotypic variability.

4.2.5 PCR analysis

Synthetic PCR primers corresponding to unique flanking sequences of 10 dinucleotide microsatellite loci were designed using the program Oligo 3.3 (MedProbe). Potential primers were chosen according to the following criterions: Avoidance of strong primer dimerization and internal hairpining, melting temperature difference between primers of less than 10°C, G+C base contents in the range of 40-60 % and increase of internal stability towards the 5'-end. 14 additional primer pairs originally developed for the ponerine ant *Rhytidoponera* sp. 12 were kindly provided by T. Tay to test for cross-species amplification in *P. punctata*. 1-5 µl of individual worker DNA, either phenol or Chelex extracted as mentioned above, was used as template in a total of 20 µl reaction volume. Each reaction contained 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.08 % Nonidet P40, 1.5 or 2 mM $MgCl_2$, 0.25 U of *Taq* polymerase (MBI Fermentas), 100 µM dNTPs (10 µM dATP), one microcurie of [α^{32}P] dATP (ICN) and 10 pmol of each primer (MWG-Biotech). Amplifications were carried out in a Genius thermocycler (Techne) using 0.2 ml reaction thin-walled tubes (Biozym Diagnostik) without mineral oil overlay. An initial 5 min denaturation step at 92 °C was followed by 30 cycles of 60 s denaturation at 92 °C, 75 s annealing at the primer-specific annealing temperature (Table 1), 60 s extension at 72 °C and a final 10 min extension step before cooling to 4 °C.

6.5 µl of PCR product was mixed with 2 µl stop solution, heat denatured at 95 °C for 5 min and analyzed by electrophoresis through 6 % denaturizing polyacrylamide gels containing 1 × TBE and 8 M urea (Sambrook *et al.* 1989). Gels were run at 2500 V for 4-6 h. Allele size was determined using the SequaMark™ sequencing size marker (Research Genetics). Following electrophoresis gels were transferred to Whatman paper and exposed to X-ray film (Noras) for 1-3 days at -20°C.

4.2.6 Data analysis

Allele identity was scored by hand using a transluminescent screen. All data were subsequently analyzed using the following computer programs: "GENEPOP 3.1b" (Raymond and Rousset 1995; ftp.cefe.cnrs-mop.fr) to determine and test genotypic differentiation and "POPGENE 1.21" (Yeh *et al.* 1997; http://www.ualberta.ca/~fyeh) to calculate heterozygosities. Expected heterozygosity was calculated following Nei (1978), with correction for small sample size.

4.2.7 Chromosome preparation

Chromosome preparation technique was modified after Imai *et al.* (1988). A total of 31 adult workers each from one colony from Florida (ABS 26), one from Puerto Rico (PR 17) and one from Barbados (BAR 1) were isolated and starved for 2 days before they were fed with a 1:1 mixture of colchicine (0.1 % stock solution) and honey-water (50 %). On the next day ovaries or digestive tracts were dissected out in freshly prepared colchicine-hypotonic solution (0.5 ml of 0.1 % colchicine stock solution, 9.5 ml 1 % hypotonic solution (1g trisodium citrate dihydrate / 100 ml aqua dest.)) on a cavity slide under a stereo microscope and transferred to fresh colchicine-hypotonic solution using a Pasteur pipette. Additionally pupal cerebral tissue was dissected from 31 pupated larvae, 91 worker pupae and 2 male pupae (here also testis tissue was dissected) from eight additional colonies (ABS 2, ABS 19, ABS 21, PR 4, PR 6, PR 15, PR 19 and PR 22) and treated in the same manner. Tissue was incubated for 20 - 60 minutes at room temperature. Using a Pasteur pipette tissue was transferred to a pre-cleaned slide. The slide was inclined to drain of remaining hypotonic solution. On the inclined slide several drops of freshly prepared fixative I (1.5 ml glacial acetic acid, 1.5 ml 99.5 % ethanol, 2 ml aqua dest.) were added. Remains of fixative were removed from end of slide using filter paper. 2 more drops of fixative I were added onto tissue. After 15-30 seconds, the tissue was macerated with dissecting needles. Before fixative I retracted, 2 drops of freshly prepared fixative II (2 ml glacial acetic acid, 2 ml 99.5 % ethanol) were added in the middle of the slide. Remaining fixative I was removed using rolled filter paper. After 2-3 minutes fixative II evaporated and immediately 2 drops of fixative III (pure glacial acetic acid) were added in the center of the spreading cells. Fixative II was removed from both ends of the slide and the slide was left to completely dry for at least 2 hours. Slides were stained using 3% Giemsa solution (filtered) for 10 minutes at room temperature. Slides were rinsed with water and air-dry against a vertical surface. Phase contrast light microscopy (Zeiss Axiophot) was used to examine slides for metaphase plates.

4.3 Results

4.3.1 Microsatellite marker loci

A total of 2,800 recombinant clones were screened for dinucleotide repeats. A total of 27 positive clones was identified and sequenced. Five of a total of 10 microsatellite loci identified from the library screening were found to be polymorphic in *P. punctata* in an initial screening of 5 to 10 workers each. The other five loci showed monomorphic amplification under the reaction conditions tested. Six loci

Locus	Core sequence	Orientation	Sequence of primers	Cloned allele size [bp]	Annealing temperature [°C]	Variability
2001	$(TC)_{12}TT(TC)_2TATC$	Up	5'-GCG TTA CCT TAC TCT CCC-3'	127	50	no
		Down	5'-GTT TCT TAT AGG CGG CGG-3'			
2701	$(AG)_{30}$	Up	5'-GAA TGA AAG GCA CGG ACC-3'	nd	52	no
		Down	5'-GCG ACT TTT CAT GCG GAT-3'			
2801	$(CT)_{31}C(CT)_4$	Up	5'-CGC TTC CCA TCC CTG TGT-3'	389	54	yes
		Down	5'-CGG TTT CCT CTC CTT CCC-3'			
2902	$(CT)_{11}CGCTCG(CT)_7TTCCTT(CT)_3TTCCTT(CT)_2$	Up	5'-GAC ATC GGG CGT CTC GTA-3'	191	52	yes
		Down	5'-TCA GAA GCG AGT CGA TGA-3'			
3302	$(GA)_{28}$	Up	5'-GAA GAG CGA GGA AGG CAG-3'	240	54	no
		Down	5'-GCG TCT TGG GAC CAT CTC-3'			
3303	$(GT)_{11}AAAA(GT)_5$	Up	5'-TCA GTA AAA GCA GGA ACC GT-3'	215	53.5	yes
		Down	5'-GGG CGA TTT ATT CGG TTA TT-3'			
3401	$(GT)_4(GC)_9$	Up	5'-TCT CAT ACT TTC GTC AAT CA-3'	130	44	no
		Down	5'-CAC GCA TCC ACA CG-3'			
3404	$(GT)_2CG(GT)_2TTAT(GT)_3GCGA(GT)_{13}$	Up	5'-GCC AGT CCG AAA CAT CCC-3'	171	56	no
		Down	5'-GCG TGC CAG TCA GGC TAT-3'			
3506	$(GA)_4AA(GA)_{13}GT(GA)_3$	Up	5'-GGA TAA GAT TGG CGG TCG-3'	209	54	yes
		Down	5'-TCT GCC GAT GAA AAC CTC-3'			
4101	$(CCTT)_5CCTCTT(CT)_2TT(CT)_2CC(CT)_{11}TT(CT)_3$	Up	5'-CTT TGT ACG CCT TGG ACG G-3'	202	55	yes
		Down	5'-GCG GGT GAG AAA AGG GAA T-3'			

Table 4.2 Characteristics of 10 microsatellite DNA sequences (locus name, core sequence of repeat, primers used, cloned allele size, annealing temperature used and variability) developed from a partial genomic library of *P. punctata* (nd = not completely determined).

belonged to the imperfect microsatellite type, two repeat motifs each were either of the perfect or the compound type. Core sequences, primer sequences, allele size of cloned sequence, optimized annealing temperature and variability for all 10 loci are summarized in Tab 4.2. Mean repeat number of the longest run of uninterrupted repeats for all loci was 16.9 ± SD 8.9. Repeat motifs ranged from 9 to 31 uninterrupted repeats. There was no general trend for polymorphic loci in *P. punctata* to consist of longer or uninterrupted repeat motifs than monomorphic loci as suggested in the literature (reviewed e.g. in Jarne and Lagoda 1996).

A nucleotide sequence comparison on nucleotide sequences stored in GenBank (NCBI) did not produce any significant sequence alignments larger than 25 bps. Neither were any of the cloned sequences similar to nucleotide sequences published for any Hymenopteran species. All microsatellite sequences are accessible through the NCBI Nucleotide Sequence Database at http://www.ncbi.nlm.nih.gov/BLAST/ (accession numbers AJ006381 - AJ006390).

From a total of 14 microsatellite primers originally developed by T. Tay for the ponerine ant *Rhytidoponera* sp. 12, seven (R3[5], R13[73], R13[336], R13[525], R14[107], R14[249] and R14[336]) yielded amplification products in a small sample of *P. punctata* workers (n = 5) drawn from all of the available populations using reaction conditions suggested by T. Tay (pers. comm.). Due to the apparent lack of both intra- and interpopulation variability in the PCR products none of the primers could however be successfully included in the population genetic study of *P. punctata*.

A total of 314 workers (from 25 colonies from various locations in mainland Florida, one from the Florida Keys, 23 colonies from Puerto Rico and 2 from Barbados) were analyzed at one or more of the five polymorphic loci. For each of all 51 colonies at least two workers were genotyped at every locus (Tab. 4.3). Of the resulting total of 799 individual PCR reactions, 103 were run twice to test for PCR reproducibility, with the results of the second reaction in all cases confirming the original amplification pattern. In four colonies, larger numbers of workers (n = 19 to 52) were genotyped at

			2801		2902		3506		4101		3303	
Location	Site	*n* colonies	Genotype	(*n*)	Genotype	(*n*)	Genotype	(*n*)	Genotype	(*n*)	Genotype	(*n*)
Mainland Florida	ABS + FP + VB	21 + 3 + 1	AA	(57)	AC	(80)	AA	(57)	BB	(54)	AB	(53)
Florida Keys	NNK	1	CD	(27)	AC	(6)	BB	(23)	BB	(10)	AB	(2)
Barbados	BAR	2	AA	(12)	AC	(6)	BB	(6)	AB	(34)	AB	(4)
Puerto Rico	PR	23	AB	(58)	AB	(59)	BB	(100)	AC	(60)	BB	(48)
									AA	(43)		

Table 4.3 Genotypes of 314 workers analyzed in 51 colonies of *P. punctata* at five microsatellite loci from four populations (ABS, Archbold Biological Station; FP, Fort Pierce; VB, Vero Beach; NNK, No Name Key; BAR, Barbados; PR, Puerto Rico). For each colony a minimum of two individuals was genotyped, frequently three or four. In several colonies more individuals were genotyped at locus 2801 (FP 4 = 5; NNK 1 = 27; BAR 1 = 5; BAR 2 = 7; PR 20 = 7), 2902 (FP 4 = 19; VB 1 = 5; NNK 1 = 6; PR 20 = 7), 3506 (NNK 1 = 23; PR20 = 52) and 4101 (NNK 1 = 10; BAR 1 = 9; BAR 2 = 25; PR 20 = 50; PR 25 = 6).

some loci to investigate the genetic homogeneity in the colony structure (FP 4 at locus 2902; NNK 1 at 2801 and 3506; BAR 2 at 4101; PR 20 at 3506 and 4101). A typical autoradiograph of 15 workers genotyped at locus 4101 is shown in Fig. 4.1. Due to its high sensitivity, short exposure times and easy documentability on X-ray films radioactive visualization using [α^{32}P] dATP for internal labeling was

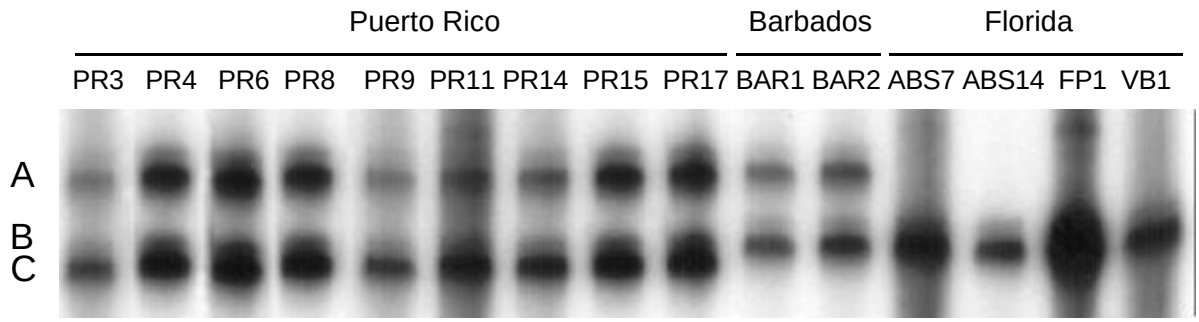

Figure 4.1 Autoradiograph showing microsatellite genotypes of 15 workers of *P. punctata* from Puerto Rico (PR), Barbados (BAR) and Florida (ABS = Archbold Biological Station, FP = Fort Pierce, VB = Vero Beach) at locus 4101. Letters A to C specify the different alleles. DNA was amplified using [α^{32}P] dATP for internal labeling, separated on a 6 % denaturizing polyacrylamide gel for 6 hours and exposed for 24 hours.

used throughout the experiment. Preliminary work suggests that with slight changes of PCR conditions [α^{32}P] dATP can easily be substituted with [α^{33}P] dATP to significantly reduce the risk of exposure to radiation.

All loci showed rather low intraspecific variation, expressed both in the number of alleles (two to four at the most polymorphic locus 2801) and observed heterozygosity (Tab. 2). With rare exceptions, all individuals from the same population shared the same homozygous or heterozygous genotype. Within populations, the frequency of the most common allele therefore ranged from 0.500 to 1.000, and the observed heterozygosities per locus varied from 0.000 to 1.000. The mean observed and expected heterozygosities summed over all loci and populations were 0.509 ± SD 0.356 and 0.523 ± SD 0.105, respectively. In none of 11 possible locus-population comparisons, a significant heterozygote deficiency was detected (Genepop U-test, ns). Similarly, pooling across all five loci, neither population showed a heterozygote deficiency (Fisher's exact test, ns). No evidence for raised ploidy levels were found in any of the genotyped individuals.

4.3.2 Intra-population survey

Even at loci where two alleles were present in a population, only one or two genotypes were found in each population: All investigated individuals from the 23 colonies from Puerto Rico were heterozygous AB at the loci 2801 (n = 58 individuals) and 2902 (n = 59) and homozygous BB at 3506 (n = 100) and 3303 (n = 48), whereas all individuals from mainland Florida (25 colonies from ABS, FP, VB) had the genotypes AA at 2801 (n = 57), AC at 2902 (n = 80), AA at 3506 (n = 57), BB at 4101 (n = 54), and AB at 3303 (n = 53) (Tab. 4.3).

Only in colony PR 20, 43 of 50 individuals were homozygous AA at locus 4101, whereas 7 nestmates and 53 investigated individuals from 22 other colonies from Puerto Rico were heterozygous AC. This pattern likely results from the occurrence of a mutational event in the flanking sequence of the repeat region in the present reproductive worker leading to the occurrence of a null allele of type AX at this locus. The alternative explanation of allele C gaining 6 motives (12bp) in a single mutational step seems rather unlikely (Crozier *et al.* 1999).

4.3.3 Inter-Population survey

The fixation of all individuals from a population for a single heterozygous or homozygous genotype obviously has consequences in inter-population comparisons: populations of *P. punctata* resemble clones and strongly differ in their genotypic (clonal) distribution. Simultaneous comparisons of the genotypic distributions across the four populations at each locus revealed them to be significantly different (log-likelihood based Fisher's exact test, $p = 0$ for each). Of the 30 pair-wise comparisons of genotypic differentiation for all pairs of populations for all loci, 19 yielded significant differences ($p < 0.01$; p-values for multiple comparisons are Bonferroni-adjusted). The remaining 11 comparisons were meaningless due to identical genotypes present in each pair. Colonies collected at three locations in mainland Florida ('ABS', 'FP' and 'VB') shared identical genotypes at all five loci but differed at loci 2801 (CD; n = 27) and 3506 (BB, n = 23) from a colony collected from a remote location on No Name Key ('NNK'), situated on the Florida Keys island chain about 190 km apart from the mainland Florida colonies (Tab 4.3). Genotypes of colonies from Puerto Rico differed at all loci from the genotype of colonies from mainland Florida. Other pairs of populations shared genotypes at one or more loci.

4.3.4 Cross-species amplification

The results of the cross-species amplification are summarized in Tab. 4.4. All 10 primer pairs showed similar low levels of cross-species amplification under the conditions used. In general, most primers worked only for a few species, namely the palaearctic cerapachyine *Cerapachys biroi* and the Australian myrmeciines *Myrmecia forficata*, *M. gulosa*, *M. nigriceps* and *M. simillima*, and the neotropical ponerine *Pachycondyla villosa*. Most primers surprisingly failed to amplify DNA in 4 oriental species of the genus *Platythyrea* (*P. parallela, P. quadridenta, P. tricuspidata and P.* spec.). No amplification was detected in the subfamilies Formicinae (*Camponotus castaneus* and *C. rufipes*, both from the neotropics) and the ponerine tribe Ectatommini (*Ectatomma ruidum* and *E. tuberculatum*,

both from the neotropics). Additional experimentation, varying annealing temperatures or $MgCl_2$ concentrations over a wider range, may still improve cross-species amplification in some of the species.

Although intercolonial variability in most of these species was not further investigated, at least in *Pachycondyla villosa* considerable between-colony variability was detected. Here, all of 6 additionally genotyped individuals from 6 different colonies of *P. villosa* yielded positive amplifications for all 10 loci. The data suggest that even among closely related species within the same subfamily flanking regions of microsatellite loci may not be sufficiently conserved to permit amplification.

Subfamily	Species	Microsatellite locus										
		2001	2701	2801	2902	3302	3303	3401	3404	3506	4101	
Subfamily	Species	50°C	50°C	50°C	50°C	51°C	52°C	44°C	52°C	52°C	52°C	Origin
Cerapachyinae												
	Cerapachys biroi	●	●	●	●	●	●	●	●	●		Okinawa, Japan[15]
Formicinae												
	Camponotus castaneus											Florida, USA[13]
	Camponotus rufipes											Misiones, Argentina[12]
Myrmeciinae												
	Myrmecia forficata	●	●	●	●	●		●	●	●	●	Eden Hills, Australia[2]
	Myrmecia gulosa	●	●	●	●	●	●	●	●	●	●	Marra Marra, Australia[2]
	Myrmecia nigriceps	●	●	●	●	●		●	●	●	●	Marra Marra, Australia[2]
	Myrmecia simillima	●	●	●	●	●	●	●	●	●	●	Marra Marra, Australia[2]
Ponerinae												
	Amblyopone spec.				●							Jog Falls, India[8]
	Diacamma ceylonense											Bangalore, India[4]
	Diacamma spec.											Chizen, Japan[15]
	Dinoponera quadriceps									●		Bahia, Brazil[10]
	Ectatomma ruidum											Barro Colorado Island, Panama[11]
	Ectatomma tuberculatum											Amazon, Brazil[9]
	Harpegnathos saltator											Jog Falls, India[8]
	Hypoponera opacior		●	●	●	●			●			Luquillo, Puerto Rico[13]
	Odontomachus bauri											Gigante, Panama[2]
	Odontomachus bruneus											Florida, USA[5]
	Odontomachus clarus											Florida, USA[5]
	Odontomachus troglodytes											Comoé NP, Iovry Coast[1]
	Pachycondyla apicalis											Bahia, Brazil[14]
	Pachycondyla hottentota											Springbok, South Africa[7]
	Pachycondyla obscuricornis											Bahia, Brazil[14]
	Pachycondyla rufipes											Jog Falls, India[8]
	Pachycondyla villosa	●	●	●	●	●	●	●	●	●	●	Bahia, Brazil[14]
	Pachycondyla near *oberthueri**											Bahia, Brazil[14]
	Platythyrea parallela		●		●	●					●	Bogor Botanic Gardens, Java[6]
	Platythyrea quadridenta				●							Ulu Gombak, West-Malaysia[6]
	Platythyrea tricuspidata				●						●	Ulu Gombak, West-Malaysia[6]
	Platythyrea spec.	●			●						●	Bogor Botanic Gardens, Java[6]

Table 4.4 Cross-species application of ten microsatellite loci. Two individuals each were tested unless otherwise noted (* - one worker tested). Annealing temperatures used are given for every locus. Filled circles (●) indicate amplification in both individuals, open circles ( ) amplification in only one of the individuals genotyped. Superscripts at the locations of origin indicate collectors of specimens: [1] - U. Braun; [2] - V. Dietemann; [3] - B. Ehmer; [4] - R. Gadagkar; [5] - B. Hölldobler; [6] - F. Ito; [7] - U. Kryger; [8] - J. Liebig; [9] - P. Oliveira; [10] - C. Peeters; [11] - S. Prett; [12] - F. Roces; [13] - K. Schilder; [14]- B. Trunzer and [15] - K. Tsuji.

4.3.5 Karyotype

17 metaphase chromosome spreads from 2 preparations of individual pupal cerebral tissue were photographed and the number of chromosomes counted. 84 chromosomes were counted in 12 metaphase spreads, 86 chromosomes in 3 spreads and 82 chromosomes and 74 chromosomes in one spread respectively (Fig. 4.2) resulting in a mean chromosome number of 83.6 ± 2.7. Therefore 2n = 84 chromosomes represent the correct diploid number of chromosomes in *P. punctata* with higher or lower observed numbers assumed to reflect methodological artifacts. Due to their high number and relative small size further description of chromosome morphology or ploidy level was not possible. *P. punctata* is among the species with the highest observed chromosome numbers in ants. Within the tribe Platythyreini equally high chromosome numbers are only reported for *P. tricuspidata* (2n = 96), but lower number were reported for *P. quadridenta* (2n = 18) and *Probolomyrmex* sp. (2n = 28) (Goni *et al.* 1982, Imai *et al.* 1984).

Figure 4.2 Diploid metaphase karyotype of *P. punctata*. Chromosome number is 2n = 84.

4.4 Discussion

The genetic population structure of the ponerine ant *Platythyrea punctata* was investigated using 10 dinucleotide microsatellite loci in four populations from mainland Florida, the Florida Keys, Barbados, and Puerto Rico. All five polymorphic loci had interrupted dinucleotide repeat motifs with 11 to 31 uninterrupted repeats. Interrupted microsatellites are known to be more appropriate for investigating population differentiation between distant populations because they are generally characterized by a higher variance in repeat number and consequently lower size homoplasy (Estoup *et al.* 1995c).

Genotypic variability is almost completely absent within populations and drastically reduced among populations of *P. punctata*. All loci showed fixation of alleles in the homozygotic or heterozygotic state for the majority of populations investigated. Therefore populations resemble single clones. This pattern is reflective of the predominance of asexual, thelytokous reproduction leading to a clonal mosaic over a large geographic scale. Even in those populations, where two alleles were present at a single microsatellite locus, individuals were fixed for only one or two of the three possible genotypes. Additionally, populations shared a common allele each of the five loci indicating a small genetic distance. In general, such a paucity of alleles indicates that the clonal lineages arose rather recently and have not been evolving independently for millions of years. Using allozyme electrophoresis a similarly low number of alleles has been found in the thelytokous ichneumonid wasp *Mesochorus nigripes* (Hung *et al.* 1988). Isolation in small habitat patches and repeated genetic bottlenecks may be responsible for the maintenance of low genotypic variation in *P. punctata*. The alternative explanation for the apparent fixation of heterozygous genotypes, duplication of microsatellite loci and fixation at different alleles, is rather unlikely as it must have occurred simultaneously in at least 4 loci. However, on the basis of the available data, one cannot rule out the possibility of reiterated polyphyletic appearance of thelytoky in different populations (Pongratz *et al.* 1998).

Several investigations on parthenogenetic species have shown clonal diversity to vary over a wide geographical range. In *Daphnia pulex* for example some clones are widely distributed while others occupy very restricted areas (Wilson and Hebert 1992). Clonal variation has also been investigated in a number of cyclical parthenogenetic aphid species using RAPD markers. Genetic diversity was found to be extremely reduced which was attributed to both a high migratory capacity of clones and to frequent extinction and recolonization events (Martinez-Torres *et al.* 1997). In the aphid *Ceratovacuna nekoashi*, on the other hand, members of one gall constitute a clonal population confined to an extremely small area (Fukatsu and Ishikawa 1994). Similarly, using allozyme electrophoresis, local populations of the parthenogenetic freshwater ostracods *Candonocypris novaezelandiae* and *Cypridopsis* sp. had highly clonal structures with low levels of genotypic diversity (Chaplin and Ayre 1997; Little and Hebert 1997). In two other species of freshwater ostracods however clonal diversity

was extensive which might result from rare bouts of recombination following a number of colonization events (Little and Hebert 1997).

Occasional incidences of sexual reproduction in parthenogenetic species would allow for an increase in genetic variability. In *P. punctata*, sexual reproduction by fertilized eggs appears to be very uncommon in the studied populations. Though two of a total of 824 individuals collected from the field have been found inseminated, it is not clear whether sperm is used for fertilization of eggs or whether mated workers and queens reproduce by thelytoky (Schilder *et al.* 1999a). Males are known from Florida and Puerto Rico but occur in rare frequency both in the field and in the lab (Schilder *et al.* 1999a) suggesting severe spanandry in all populations. Males have been found to be rare in a number of asexual Hymenopterans, including the obligate thelytokous ant *Pristomyrmex pungens* (Tsuji 1988a), the parasitic wasp *Dinocampus coccinellae* (Geoghegan *et al.* 1998), the endoparasitic *Encarsia formosa* (Zchori-Fein *et al.* 1992) and *Taeniogonales venatoria* (Weinstein and Austin 1996). Rare genotypes as found in one colony from Puerto Rico are likely to result from mutational events during parthenogenesis instead of incidences of sexual reproduction. Even when functional males are rare in the population, they would nevertheless diversify the genotypic structure substantially (e.g., Plantard *et al.* 1998). Likewise in the apomictic greenbug *Schizaphis graminum* it has been suggested that even sporadic periods of sexual reproduction would be sufficient to generate considerable genetic variability (Shufran *et al.* 1997). In *P. punctata* we may currently witness the transition from sexuality to thelytoky over a broad range of the species distribution with remnants of sexuality such as production of sexuals and mated individuals still present due to phylogenetic inertia (Schilder *et al.* 1999a). This evolutionary process is certainly further evolved than in the case of the Cape honey bee where asexual reproduction occurs only in certain populations in South Africa with sexual reproduction by queens still dominating (Greeff 1997; Hepburn *et al.* 1998; see also general discussion).

Thelytoky might in several ways lead to decreased genotypic variability. Rapid genetic drift in isolated parthenogenetic populations may have been responsible for the loss of rare genotypes, a process that may have been accelerated by a high mortality due to harsh climatic conditions in the periphery of the species range and / or frequent re-colonization events following extinction. Most of the colonies obtained from Florida originate from locations at the northernmost distribution limit of the species (Deyrup *et al.* 1989), suggesting a strong selection pressure due to adverse environmental conditions. Minimal winter temperature in Central Florida can drop below the freezing point for several days in a row. According to some authors, parthenogenesis is of particular selective advantage in isolated or marginal populations, where it facilitates rapid population growth in periods of unfavorable conditions or after repeated population crashes (Tomlinson 1966; Cuellar 1977; Glesener 1978). There is circumstantial evidence for *P. punctata* that following the extraordinarily harsh winter of 1995 / 96 the Florida mainland populations at Archbold Biological Station (‘ABS’) and from the East Coast (‘VB’

and 'FP') decreased close to extinction (Schilder, unpubl. observation). Thelytokous reproduction after a genetic bottleneck event (be it caused by repeated population crashes or the original founder effect) would then delay the introduction of novel alleles into the population, assuming that mutational events are rare.

Yet caution has to be used in evoking genetic bottlenecks as the only explanation for low genotypic variability in a species (Amos and Harwood 1998). To explain the large geographic range of the same genotypes in geographically widely spaced populations in mainland Florida, one would have to assume a strong colonization capacity or a reproductive advantage of this genotype. Nothing is known on the dispersal of *P. punctata.* Queens are unlikely to be good dispersers because of the lack of ocelli and strong wing muscles; colonies with gamergates (mated, reproductively active workers) and / or thelytokous workers are likely to reproduce by budding, which also renders them rather poor long-distance dispersers. On the other hand, *P. punctata* is widespread in the Caribbean islands and occurs even on the rather isolated island of Barbados (Wheeler 1905, 1908, 1923). This contradiction therefore cannot readily be resolved.

Low microsatellite variability may also be due to low mutation rates or small size number of repeats (Hedrick and Parker 1997 and references therein). Novel alleles will arise or present alleles will be lost in a thelytokous population due to the occurrence of non-homoplasic mutations increasing individual heterozygosity. Estimation of mutation rates for microsatellites range in the order of 10^{-6} to 10^{-2} mutations per generation in humans, mice, *Drosophila* and honey bees (Dallas 1992; Weber and Wong 1993; Estoup 1995). A high genotypic homogeneity within a large sample of individuals argues for a low rate of mutations in *P. punctata*. The observed loss of one allele at locus 4101 in 43 homozygous individuals from colony PR 20 could be the result of such a rare mutation in the flanking region of the repeat leading to the occurrence of a null allele.

Evolution of parthenogenetic reproduction from sexual ancestors is believed to have occurred repeatedly by hybridization of sexual species (Crow and Kimura 1965; Moritz 1993; Griffiths and Butlin 1995; Pongratz *et al.* 1998). Hybridization events often lead to polyploidy (Bell 1982). Karyological investigations so far did not reveal any evidence for polyploidy in *P. punctata*. In addition average observed heterozygosities for polyploid organisms are expected to be higher than for their closely related diploid taxa. Mean observed heterozygosity in *P. punctata* (0.509 ± SD 0.356) however is well within the range for mean expected microsatellite heterozygosities measured in other eusocial Hymenoptera (0.291 to 0.872, Estoup *et al.* 1995a; 0.283 to 0.729, Hedrick and Parker 1997). In contrast, comparable values of mean heterozygosity in parthenogenetic species - currently available only from allozyme studies - indicate that parthenogens tend to have slightly higher mean heterozygosity than their sexual ancestors (Dessauer and Cole 1989; Honeycutt and Wilkinson 1989; Moritz 1993;

MacCulloch *et al.* 1995; Murphy *et al.* 1997; Fu *et al.* 1998). This probably indicates that parthenogenesis in *P. punctata* did not arise during a hybridization event.

The cytological mechanism underlying thelytokous parthenogenesis in *P. punctata* remains unclear. Only in some thelytokous Hymenoptera, such as the Cape honey bee *Apis mellifera capensis* (Tucker 1958; Verma and Ruttner 1983) and the parasitic wasp *Aphytis mytilaspidis* (Rössler and DeBach 1973) the genetic mechanisms have clearly been demonstrated. Parthenogenesis in *P. punctata* could either be apomictic, i.e., meiosis does not occur (Vielle Calzada *et al.* 1996), or could follow an automictic mechanism with subsequent fusion of meiotic products similar to that in Cape honey bees, where during meiosis the egg fuses with a polar body to restore diploidy (Tucker 1958; Verma and Ruttner 1983; Moritz and Haberl 1994). General automictic parthenogenesis will lead, through the fusion of meiotic products, to complete genetic homozygosity (see chapter 5). *Drosophila mangabeiri*, *Solenobia lichenella* and *Devorgilla canescens* are among the few exceptions of automictic insects that retain heterozygosity either by close linkage of loci to the kinetochore or by chromosomal rearrangements, namely inversions and translocations (Carson 1967). With the possible exception of few individuals in colony PR 20 however no homozygous individuals appeared in populations of *P. punctata* that were clonally fixed for a heterozygous genotype. Apomixis instead retains heterozygosity in the offspring of a thelytokous mother because in the absence of meiosis and recombination through crossing over heterozygotes will not produce any homozygous offspring. Both in apomixis and in automixis the resulting offspring is genetical identical to the parent assuming no crossing over between homologous chromosomes in the latter. On the basis of the available data no firm conclusion can be drawn on the mechanism of thelytoky in *P. punctata*. Unfortunately none of the other Hymenopteran thelytokous parthenogens has been studied using microsatellite markers. The various cytological mechanisms causing parthenogenesis will be dealt with in the next chapter in more detail.

In nine species of parasitic wasps and several other Hymenopterans thelytoky is caused by infection with the rickettsia-like proteobacterium *Wolbachia* (Zchori-Fein *et al.* 1992; Stouthamer *et al.* 1993, Stouthamer and Werren 1993) by a process called "gamete duplication" that is likely to result in complete homozygosity (Stouthamer and Kazmer 1994; Plantard *et al.* 1998). An investigation of 6 ant species including *P. punctata* and the Cape honeybee, all known to reproduce thelytokously, with *Wolbachia*-specific 16S rDNA PCR primers did however not reveal an infection in samples of any of the populations studied (Wenseleers and Billen 2000). Similarly, treatment with several antibiotics did not "cure" thelytoky. These results are surprising since infection with the bacterium *Wolbachia* generally appears to be widely spread within the Formicidae (Wenseleers *et al.* 1998). In conclusion thelytokous parthenogenesis in *P. punctata* is likely to be based on a genetic mechanism that might be similar to that in the Cape honey bee.

Primers developed specifically for *P. punctata* gave amplification products in a number of 29 ant species comprising four different subfamilies in which cross-amplification was studied. Similar cross-amplification has been reported from other Hymenoptera (e.g. in honey bees (Estoup *et al.* 1995a); in bumble bees (Estoup *et al.* 1995b); in several ant species (Gertsch *et al.* 1995; Chapuisat 1996; Herbers and Mouser 1998; Peters 1998); in epiponine wasps (Strassmann *et al.* 1996); in polistine wasps (Strassmann *et al.* 1997b) and in seed chalcids (Carcreff *et al.* 1998)). However, even within the genus *Platythyrea* (4 species), most primers failed to amplify. Microsatellites are known to be situated in rapidly evolving non-coding regions and therefore cross-species amplification is generally restricted to closely related species (Queller *et al.* 1993). Our data confirm the notion that across ant genera but similarly even within the same genus flanking sequences of microsatellites are often not sufficiently conserved to allow amplification but that successful cross-priming may allow investigators to circumvent the laborious process of microsatellite development in some cases.

The karyological investigation revealed *P. punctata* to be among the species with the highest chromosome numbers observed in ants. Almost no other ant species has a comparably high diploid chromosome numbers with the average ranging much lower (2n range: 6 - 56; Crozier 1970, 1975; Goni *et al.* 1982; Imai *et al.* 1984). Ants feature the whole range of chromosome numbers known in the order. The myrmeciine ant *Myrmecia croslandi* (formerly *M. pilosula*) is exceptional in that it has the lowest chromosome number known in higher organisms (2n = 2, Imai and Taylor 1989). Only within the tribe Platythyreini the Malaysian *P. tricuspidata* has an equally high chromosome number (2n = 96, Imai *et al.* 1984). Since no karyotype information is available for other Plathythyreine species it remains however unclear whether this situation is characteristic for the genus. The available evidence suggests that chromosomal rearrangements by Robertsonian changes (i.e. reduction or increase of chromosome number by fission or fusion of two acrocentric into one metacentric chromosome) rather than polyploidy have been responsible for changes in chromosome number although this is difficult to judge in ancient lineages (Crozier 1975). In general, higher-numbered karyotypes are characterized by smaller chromosomes and higher proportion of acrocentrics. This is consistent with a chromosome evolution by Robertsonian changes. There is however dispute about the evolution of Hymenopteran chromosome numbers: While some authors believe high numbers to be primitive for ants and Robertsonian changes to be responsible for chromosomal evolution (Crozier 1975) others regard low numbers to be primitive with higher numbers being derived by centric fissions, pericentric inversions and translocations (Imai 1969; Hoshiba and Imai 1993). Since chromosomal evolution in ants is known to occur with higher speed relative to morphological change ("bursts of chromosome evolution") both scenarios remain possible (Crozier 1975; Imai *et al.* 1994). Parthenogens that arise after hybridization of closely related sexual species are likely to be polyploid (see the following chapter for details) and Hymenopteran genetics might permit polyploidy to evolve more readily than in other bisexual animals

(Crozier 1975). Polyploidy in *P. punctata* however is unlikely to be causally related to thelytoky since a polyploid genotype should be detectable as multiple banding patterns in individual microsatellite genotypes due to the presence of more than two alleles at a given locus (e.g., Samadi *et al.* 1998). Since no multiple bands were observed at 10 microsatellite co-varying between clonal populations it is reasonable to conclude that polyploidy is absent in *P. punctata*. Given the lack of comparative data within the genus a high number of small chromosomes in *P. punctata* likely reflects an evolutionary history of smaller or larger chromosomal rearrangements.

Several interesting questions regarding the evolution of thelytokous parthenogenesis in *P. punctata* remain to be answered. These include the importance of parthenogenesis in other, more central populations of the species distribution in Central and South America, the mechanism by which genotypic variation is generated in this species and the cytological processes leading to thelytoky. The extensive geographic range of distribution of *P. punctata* coupled with the predominance of thelytokous parthenogenesis suggests that thelytoky in this species has been very successful. Clonal populations may therefore be well adapted to local conditions. To evaluate this hypothesis, detailed comparisons of the ecological determinants in several populations and populations of closely related sexual taxa in South America will be necessary.

5 Evolution of thelytoky among the Hymenoptera

5.1 Introduction

The evolution of parthenogenetically reproducing taxa from sexual ancestors and their persistence through time are two of the great puzzles of evolutionary biology (Crow and Kimura 1965; Slobodchikoff and Daly 1971; Cuellar 1977; Bell 1982; Templeton 1982; Uyenoyama 1984, Uyenoyama 1985; Suomalainen *et al.* 1987; Grebelnyi 1996). Though sexual reproduction is thought to be advantageous under most ecological conditions, reproduction by parthenogenesis (i.e., the production of individuals from unfertilized gametes) may be highly advantageous in the early stages of its evolution and only later turn into an evolutionary crisis for any parthenogenetic taxon (see section 5.4). Parthenogens theoretically reproduce at a higher rate than sexual species resulting in the classical two-fold advantage of asexual reproduction (Maynard Smith 1971). According to Maynard Smith's (1978) famous ‘cost of sex’ argument, they propagate their genes more efficiently because they are twice as closely related to their offspring than sexually reproducing individuals (Williams 1975), and they may have a higher colonizing ability due to single individuals being capable of forming a new population (Tomlinson 1966). Parthenogens may also be in a better situation to build up mutually coadapted gene pools (Crow and Kimura 1965) or show lower susceptibility to genetic bottlenecks because of selective advantages following the establishment of the unisexual lineage (Templeton 1982). To examine the origin of parthenogenesis, therefore those species, in which sexual reproduction and parthenogenesis co-occur, are of special interest in evolutionary biology (section 5.5).

5.2 Thelytokous parthenogenesis in the Hymenoptera

The ability of parthenogenetic reproduction is universal among the haplodiploid Hymenoptera (bees, wasps, ants etc.) and distinguishes them from the majority of other diploid insects. In most species all females can produce only male offspring from unfertilized eggs by means of arrhenotokous parthenogenesis (White 1973). Thelytokous parthenogenesis (the production of diploid females from unfertilized eggs), on the other hand, does not appear to be a very common phenomenon in this order

(reviewed in Slobodchikoff and Daly 1971). In fact, it is generally rare in the animal kingdom with hardly more than 1,500 thelytokous animal species (White 1984). Because of its evolutionary transience only few ancient asexuals are currently known (e.g. Hurst *et al.* 1992; Judson and Normark 1996; Taylor *et al.* 1999). Thelytoky within the Hymenoptera is patchily distributed and appears to be restricted to taxa at the distant tips of phylogenies (section 5.7). This is consistent with the hypothesis of several independent evolutionary origins of thelytoky from arrhenotokous ancestors (White 1973; Bull 1983). Evidence accumulating through the description of new thelytokous species is suggesting that it may not be as rare in the Hymenopterans as previously thought. In a review on sex determination in the parasitic Hymenoptera about 270 cases of thelytoky have been described (Luck *et al.* 1992; Quicke 1997) excluding more than 2,000 species within the Cynipoidea that reproduce by cyclic thelytoky (Hebert 1987). In the parasitic superfamilies alone, Clausen (1962) lists 30 genera in 8 families that contain thelytokous species. Up to 24 percent of the known parasitic Encyrtidae, 16 percent of Aphelinidae and 19 percent of Braconidae are thelytokous (Luck *et al.* 1992). In the aphelinid genus *Aphytis* alone 30 percent of all species are thelytokous, within the Signiphoridae another 40 percent (DeBach 1969). According to a review of published literature 25 families comprising 70 genera are identified here to reproduce thelytokously either in their entire range or in some populations (Tab. 5.1). This summary is build upon and enlarges earlier reviews of thelytokous parthenogenesis in the Hymenopteran although it does not list most of the known parthenogens in the Cynipoidea (Clausen 1962; Slobodchikoff and Daly 1971; Crozier 1975; Bell 1982; Suomalainen *et al.* 1987; Luck *et al.* 1992). This list however must remain incomplete for new cases are permanently described and because it omits a number of species in various groups in which thelytoky occurs at very high frequency but where detailed references are lacking, such as the Aphelinidae with 38 described cases, the Chalcidoidea (n = 121), the Ichneumonoidea (n = 32) and the Tenthredinoidea (n = 90) (Luck *et al.* 1992). With a growing interest in alternative reproductive tactics this list is expected to grow continuously in the future.

Within the family Formicidae, obligate or facultative thelytokous parthenogenesis has been unambiguously demonstrated only in four phylogenetically very distant species, namely *Pristomyrmex pungens*, Myrmicinae (Tsuji 1988a), *Cerapachys biroi*, Cerapachyinae, (Tsuji and Yamauchi 1995), *Cataglyphis cursor*, Formicinae, (Cagniant 1979, Cagniant 1983, Cagniant 1984), and *Platythyrea punctata*, Ponerinae, (Heinze and Hölldobler 1995, Schilder *et al.* 1999a). In *P. pungens* and *C. biroi,* all female offspring is thought to develop from unfertilized worker eggs, whereas in *C. cursor* sexual reproduction by mated queens appears to be the predominant mode of the production of diploid offspring. In eight other species thelytoky has been described but in most cases more detailed investigations are necessary to confirm these reports (Tab. 5.1).

Superfamily	Family / Species	Mechanism (if known)	Reference
Tenthredinoidea			
	Diprionidae		
	Diprion polytomum	automixis (TF)	Smith 1941
	Tenthredinidae		Benson 1950
	Empria spp.	automixis	Doncaster 1906
	Fenusa		Pieronek 1973
	Nematus ribesii	automixis	Doncaster 1907
	Perga affinis		Carne 1962
	Pristophora erichsonii	automixis	Heron 1955
	Pristophora pallipes	automixis (TF)	Comrie 1938
	Pristophora rufipes	automixis (TF)	Comrie 1938
	Pristophora tener	automixis	Comrie 1938
	Pristophora spp.	automixis	Smith 1955
	Strongylogaster macula	apomixis	Peacock and Sanderson 1939
Cephoidea			
	Cephidae		
	Cephus cinctus		Farstad 1938; Smith 1938
Trigonaloidea			
	Trigonalidae		
	Poecilogonalos fasciata		Tsuneki 1991
	Taeniogonalos venatoria		Carne 1969; Weinstein and Austin, 1996
Ichneumonoidea			
	Aphidiidae		
	Aphidius colemani complex		Tardieux and Rabasse 1988
	Braconidae		
	Apanteles sp. [2]		Laing and Heraty 1981
	Bracon hebetor	automixis	Speicher 1934; Speicher and Speicher 1938
	Dinocampus coccinellae		Balduf 1926; Geoghegan et al. 1998
	Meteorus cypris		Li 1984
	Meteorus nigripes		Hedlund 1984; Day and Hedlund 1988; Hung *et al.* 1986, 1
	Meteorus pulchricornis		Fuester et al. 1993; Li 1984
	Microctonus colesi [2]		Dysart and Day 1976
	Pygostelus falcatus		Loan 1961
	Ichneumonidae		
	Gelis tenellus [2]		Allen 1962
	Mesochorus nigripes		Hung *et al.* 1988; Day and Hedlund 1988
	Tersilochus sp. [2]		Clancy 1969
	Trathala flavoorbitalis	obligate thelytoky	Sendanayake and Edirisinghe 1992
	Venturia canescens	automixis (CF)	Speicher 1937; Speicher *et al.* 1965; Slobodchikoff 1983
Chalcidoidea			
	Aphelinidae		
	Aphytis mytilaspidis	automixis (TF)	Rössler and DeBach 1973
	Aphytis chilensis		DeBach 1969
	Aphytis chrysomphali		DeBach 1969
	Aphytis lingnanensis	BA	Zchori-Fein *et al.* 1994, 1998
	Aphytis yanonensis	BA	Zchori-Fein *et al.* 1998
	Azotus perspeciosus		Pedata and Viggiani 1991

Table 5.1 Summary of the known case studies of thelytokous parthenogenesis in the Hymenoptera. This table significantly extends older reviews by Clausen 1962; Slobodchikoff and Daly 1971; Crozier 1975; Bell 1982; Suomalainen *et al.* 1987 and Luck *et al.* 1992. Where available the cytogenetic mechanism of thelytoky is given (GD: gamete duplication; CF: central fusion; TF: terminal fusion; BA: bacterial).

Superfamily	Family / Species	Mechanism (if known)	Reference
Chalcidoidea			
	Encyrtidae		
	Apoanagyrus diversicornis	BA	Pijls *et al.* 1996
	Encyrtus fulginosus		Flanders 1943
	Habrolepis rouxi		Flanders 1946
	Ooencyrtus submetallicus	BA ?	Wilson and Woolcock 1960
	Ooencyrtus fecundus	BA	Laraichi 1978
	Pauridia peregrina		Flanders 1965
	Plagiomerus diaspidis	BA ?	Gordh and Lacey 1976
	Trechnites psyllae		Hagen 1970; Slobodchikoff and Daly 1971
	Tropidophyrne melvillei		Doutt and Smith 1950
	Eulophidae		
	Encarsia formosa	BA	van Meer *et al.* 1995
	Encarsia perniciosi		Stouthamer and Luck 1988
	Pediobius foveolatus		Sheng and Wang 1992
	Prospaltella perniciosi		Flanders 1953
	Tetrastichus asparagi [2]		Burks 1943
	Eurytomidae		
	Tetramesa grandis		cited in Luck *et al.* 1992
	Leucospidae		
	Leucopsis	geographical parthenogenesis	Berland 1934
	Mymaridae		
	Anaphes diana		Aeschlimann 1990
	Anaphes sp. [2]		Aeschlimann 1986
	Pteromalidae		
	Muscadifurax uniraptor	BA, GD	Legner 1969, 1987a, 1987b; Stouthamer *et al.* 1993
	Spalangia endius		Bandara and Walter 1993
	Signiphoridae		
	Signiphora boringuensis	BA ?	DeBach 1969; Quezada *et al.* 1973
	Thysanus spp.		DeBach 1969
	Trichogrammatidae		
	Trichogramma cordubensis	BA, GD[3]	Vargas and Cabello 1986; Cabello and Vargas 1985
	Trichogramma embryophagum	GD[3]	Birova 1970; Pintureau *et al.* 1980
	Trichogramma evanescens	GD[3]	Pintureau and Babault 1981
	Trichogramma kaykai	BA, GD[3]	Schilthuizen *et al.* 1998
	Trichogramma oleae	BA, GD[3]	Louis et al. 1993
	Trichogramma maidis	GD[3]	Pintureau and Babault 1981
	Trichogramma minutum	GD[3]	Wang 1994
	Trichogramma pretiosum	GD[3]	Stouthamer *et al.* 1990
	Trichogramma semifumatum	GD[3]	Bowen and Stern 1966; Stern and Bowen 1968
	Trichogramma sp.	BA, GD[3]	Stouthamer and Werren 1993
Cynipoidea			
	Cynipidae		
	Andricus mukaigawae	cyclical parthenogenesis	Abe 1986
	Andricus targionii	obligate thelytoky	Abe 1986
	Diplolepis bicolor [1]	BA	Plantard *et al.* 1999
	Diplolepis californica [1]	BA	Plantard *et al.* 1999
	Diplolepis eglanteriae [1]	BA	Plantard *et al.* 1999
	Diplolepis mayri [1]	BA	Plantard *et al.* 1999
	Diplolepis nodulosa [1]	BA	Plantard *et al.* 1999

Table 5.1 continued.

Superfamily	Family / Species	Mechanism (if known)	Reference
Cynipoidea			
	Cynipidae		
	Diplolepis polita [1]	BA	Plantard *et al.* 1999
	Diplolepis radicum [1]	BA	Plantard *et al.* 1999
	Diplolepis rosae	automixis (BA, GD)	Stille 1985; Stille and Dävring, 1980; van Meer *et al.* 1995
	Diplolepis spinosa [1]	BA	Plantard *et al.* 1999
	Diplolepis spinosissimae	BA, GD	Plantard *et al.* 1999
	Liposthenes glechomae	BA	Folliot 1964; Plantard *et al.* 1999
	Neuroterus lenticularis	apomixis	Doncaster 1910
	Phanacis hypochaeridis	BA	Folliot 1964; Plantard *et al.* 1999
Proctotrupoidea			
	Diapriidae		
	Ecitovagus gibbus n. sp.		Masner 1977
	Pelecinidae		
	Pelecinus polyturator	geographical parthenogenesis	Brues 1928
	Platygastridae		
	Amitus fuscipennis		Viggiani 1991
	Platygaster virgo		Day 1971
	Scelionidea		
	Telenomus nakagawai		Hokyo and Kiritani 1966
Chrysidoidea			
	Bethylidae		
	Scleroderma immigrans		Keeler 1929a, 1929b
	Dryinidae		
	Gonatopus lunatus		Guglielmino and Virla 1998
	Pseudogonatopus chilensis		Virla 1995
Apoidea			
	Apidae		
	Apis mellifera	automixis (CF)	Tucker 1958; Anderson 1963; Verma and Ruttner 1983
	Ceratina acantha		Daly 1971
	Ceratina dallatorreana	automixis	Daly 1966
	Nomada japonica	obligate thelytoky	Maeta et al. 1987
Formicoidea			
	Formicidae		
	Aphenogaster lamellidens		Haskins and Enzmann 1945
	Aphenogaster rudis		Haskins and Enzmann 1945
	Atta cephalotes		Tanner 1892
	Cataglyphis cursor		Cagniant 1979, 1982, 1983, 1984
	Cerapachys biroi		Tsuji and Yamauchi 1995
	Crematogaster scutellaris		Soulié 1960
	Formica polyctena	automixis	Otto 1960
	Lasius niger		Bier 1952
	Messor capitatus		Grasso *et al.* 1998
	Oecophylla longinoda	apomixis (in workers)	Ledoux 1949, 1950
	Platythyrea punctata		Heinze and Hölldobler 1995; Schilder *et al.* 1999
	Pristomyrmex pungens		Tsuji 1988a

[1] Formal demonstration of thelytoky in this species (e.g. through rearing experiments) still missing.
[2] No males have been described but may be present in unstudied populations. In addition sample size may be low.
[3] The genetic mechanism of thelytoky has been described by Stouthamer and Kazmer (1994).

Table 5.1 continued.

Although thelytoky among the Hymenoptera is not a common phenomenon is has been argued that they might quite easily abandon arrhenotoky in favor of thelytoky (Cornell 1988). This hypothesis is supported by the sporadic appearance of thelytokous parthenogenesis in several parasitoid families (Crozier 1975). High inbreeding as frequently found among the Hymenoptera seems to be an additional pre-adaptation facilitating the evolution of thelytoky since the more highly inbred a population, the greater the removal of accumulated recessive lethal alleles that would abort any parthenogenetic lineage that appears (Cuellar 1977). Thelytoky further removes the problem to precisely adjust female-biased sex ratios (Cornell 1988). Haplodiploidy may also provide a mechanistic pre-adaptation to the evolution of thelytoky: Unfertilized eggs are usually viable and develop into males. Therefore there is a strong potential for the tychoparthenogenetical pathway (i.e., the occasional or accidental parthenogenetic development in unfertilized eggs (Vrijenhoek 1998)) to thelytoky.

5.3 Mechanisms of thelytokous parthenogenesis

Four main causes of thelytoky have so far been identified in the Hymenoptera: cytogenetic, hybridization, extrachromosomal agents and gynogenesis (Luck *et al.* 1992). The cytogenetic mechanisms underlying thelytoky, its possible origin by hybridization and the role of endosymbiontic microorganisms will be investigated in this section. Gynogenesis (i.e. sperm-dependent parthenogenesis, in which sperm activates embryogenesis but fusion with oocyte does not take place (Vrijenhoek 1998)) is believed to be rare within the Hymenoptera (but see Tardieux and Rabasse (1988)) and will not be discussed further.

5.3.1 Genetic origin of thelytoky

A number of cytological mechanisms of thelytokous parthenogenesis have been described in insects some of which are also acting in the Hymenoptera (Asher 1970; Lamb and Willey 1987; Suomalainen *et al.* 1987). They are traditionally grouped into two major cytological mechanisms depending on whether the maternal ploidy level is retained or restored. While asexual reproduction without chromosome reduction or fusion of gametes is referred to as *apomixis*, reproduction involving chromosomal reduction but no fusion of gametes and the restoration of the original ploidy level by a different mechanism is called *automixis* (e.g. Vrijenhoek 1998). The term *clone* here refers to a set of genetically uniform descendents. In the following other terms and definitions (e.g. Nur 1979; Lamb and Willey 1987) will be subsumed under the terms mentioned. Until now none of the genes responsible for the cytogenetical changes occurring under thelytoky has been identified. In a preliminary study in the

Cape honeybee *Apis mellifera Capensis*, thelytoky has been attributed to the action of a simple recessive Mendelian gene, although this needs to be confirmed (Ruttner 1988). Given the multitude of observed meiotic distortions that all lead to a thelytokous phenotype, parthenogenetic species may differ to a great extend in the genomic cause of thelytoky.

Under apomixis true meiosis is dramatically altered: Resulting from the omission of chromosome pairing and subsequent reduction division, offspring are identical genetic copies of their mothers (Fig. 5.1.a). Both the ploidy level and the heterozygosity are therefore preserved. Genetic variation could however be created through mutations, polyploidy, somatic crossing over or transposition (Suomalainen *et al.* 1987). Some authors believe that apomixis, due to the significant

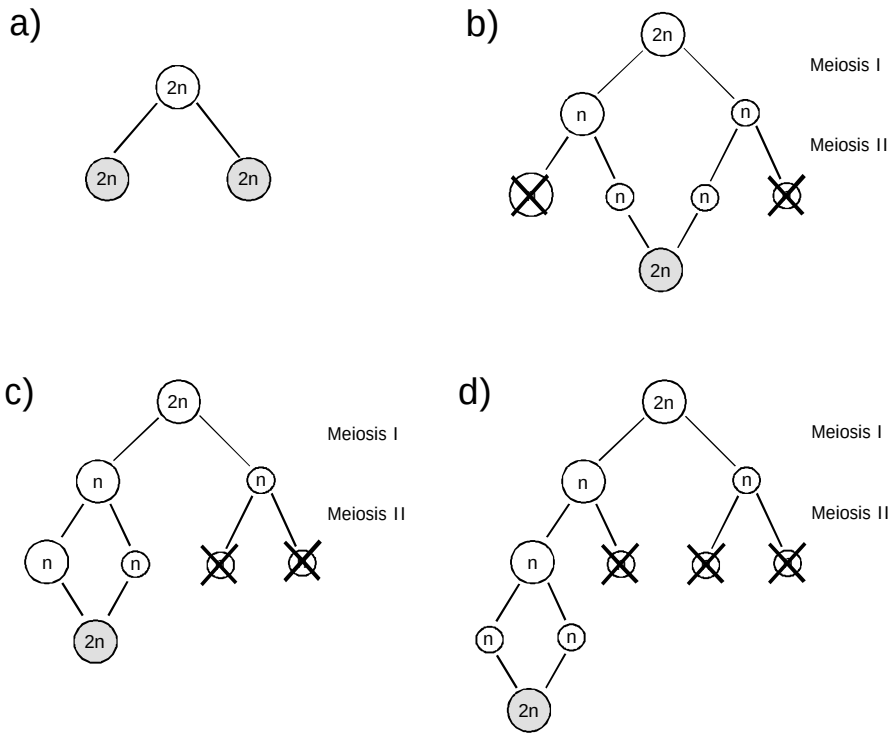

Figure 5.1 Some of the cytological mechanisms causing thelytoky in the Hymenoptera: a) apomixis (mitotic parthenogenesis), b) automixis with central fusion, c) automixis with terminal fusion, d) automixis with gamete duplication (modified after Suomalainen *et al.* 1987).

changes in meiosis, was evolutionary preceded by an automictic transitional period (see Suomalainen *et al.* 1987). Apomixis is by far the most common form of parthenogenesis in insects (Suomalainen 1950; White 1973; Bell 1982), but occurs only sporadically in the Hymenoptera (Cook 1993). Currently there are only three documented examples of apomictic species: the sawfly *Strongylogaster maculata* (Peacock and Sanderson 1939), the gall wasp *Neuroterus lenticularis* (Doncaster 1910) and the weaver ant *Oecophylla longinoda*, where only workers produce thelytokous offspring, while queens are arrhenotokous (Ledoux 1949, 1950). In *N. lenticularis* meiosis I is inhibited and subsequently the

products of meiosis II become the first cleavage nuclei. Cytokinesis then gives rise to heterozygotes or mosaic zygotes made up of homozygous cells depending on the occurrence of recombination (Suomalainen 1950; Asher 1970; Suomalainen *et al.* 1987).

The majority of Hymenopteran parthenogens for which the cytological mechanism has been investigated however are automictic (Cook 1993) thus preserving some form of meiosis in which chromosome pairing and crossing-over may occur. Automixis leading to the restoration of maternal ploidy can occur in at least seven cytological ways (reviewed in Asher 1970; Lamb and Willey 1987; Suomalainen *et al.* 1987), of which only those known to occur in Hymenopterans will be considered here.

Most forms of automixis immediately lead to complete homozygosity or at least increase observed homozygosity. Automictic parthenogenesis following central fusion is quite common and has been described in the honeybee *Apis mellifera* (Tucker 1958) including its African thelytokous race *A. m. Capensis* (Verma and Ruttner 1983), and in the ichneumonid sawfly *Venturia canescens* (Speicher *et al.* 1965; Beukeboom and Pijnacker, in prep.). Under central fusion the polar nuclei of different secondary oocytes fuse after normal meiosis II (Asher 1970). Meiosis I is either abnormal or completely inhibited leading to an increased homozygosity (Fig. 5.1.b). Only in the exceptional case of *Drosophila mangaberai*, heterozygosity is maintained even under central fusion due to the fusion of heterokaryotypic nuclei (Carson 1967).

Terminal fusion is the second mechanism known to occur in several Hymenopterans. While meiosis I is not distorted, meiosis II is inhibited and subsequently the second polar body fuses with the egg nucleus (Fig. 5.1.c). Clearly this mechanism will lead to immediate homozygosity. More detailed knowledge on terminal fusion again is restricted to three examples: In the aphelinid wasp *Aphytis mytilaspides* (Rössler and DeBach 1973), and in the sawflies *Diprion polytomum* (Smith 1941), *Pristophora rufipes* and *P. pallipes* (Comrie 1938). In the fruitfly *Drosophila parthenogenetica* it has been argued on theoretical grounds that selection can sustain certain levels of heterozygosity even under terminal fusion if crossing over occurs (Asher 1970). A combination of these mechanisms is possible but has not yet been observed in the Hymenoptera. Rare males are fertile, inseminate females and may generate heterozygosity within the population (Suomalainen *et al.* 1987).

Modeling the genetic population structure resulting from these different reproductive mechanisms has shown that populations reproducing by central fusion will approach complete homozygosity more slowly than those employing terminal fusion or a mixture of both (Asher 1970). In the absence of selection homozygosity will usually be attained in 10-15 generations. However, premeiotic doubling of chromosomes followed by normal meiosis can maintain heterozygosity in certain cases (Carson 1967), e.g. when the locus is absolutely linked to the kinetochore, by chromosomal rearrangements (inversions and translocations) and by selection (Asher 1970; Suomalainen *et al.* 1987).

Maintenance of heterozygosity has been observed in the grasshopper *Warramaba virgo* (White *et al.* 1963) and might also occur in *Drosophila mangabeirai* and *Venturia canescens* (Suomalainen *et al.* 1987; Beukeboom and Pijnacker, in prep.). According to a cytogenetic model, central fusion can maintain heterozygosity at a lower cost to the population and should have a selective advantage over other mechanisms such as terminal fusion (Asher 1970).

A thorough cytological analysis of thelytoky in the Hymenoptera is needed to enable researchers to draw conclusions from an observed zygoidy pattern on the underlying mechanism in any one species. Methodological problems partially explain why the mechanism of parthenogenesis in many Hymenopteran species has not been studied in greater detail. As has been shown, thelytoky in many cases leads to complete homozygosity but there are several opportunities to maintain genetic variation even within asexual populations and thereby presumably increase their evolutionary potential. Among the Formicidae most of the cases of thelytoky still await investigations along these lines.

5.3.2 Bacteria as causing agents

Most examples of thelytoky studied to date are associated with a bacterial infection of the genus *Wolbachia* acting as a cytoplasmatic sex-ratio distorter (Tab. 5.1). *Wolbachia* are endosymbiontic proteobacteria that infect reproductive and somatic tissues of arthropods (reviewed in Stouthamer 1997; Dobson *et al.* 1999). They are vertically transmitted through the egg cytoplasm and known to be able to change the reproductive behavior of their hosts. *Wolbachia* is associated with a variety of changes in the reproductive mode such as cytoplasmatic incompatibility (reviewed by Hoffmann and Turelli 1997), feminization of genetic males in isopods (reviewed by Rigaud *et al.* 1997) and parthenogenesis (Stouthamer *et al.* 1990; Stouthamer *et al.* 1993). There is strong evidence for horizontal transmission between different host taxa which may be important for the colonization of potential hosts (O'Neill *et al.* 1992; Werren *et al.* 1995b; Schilthuizen and Stouthamer 1997).

Infection is quite widespread within insects. In a survey of 157 Panamanian arthropods 16.9 percent of the species were infected, including 6 Hymenopterans (Werren *et al.* 1995a), and in an investigation of 50 Indo-Australian ant species 50 percent turned out to be positive (Wenseleers *et al.* 1998). However in most of these species the effect of *Wolbachia* infection is unknown. Since parthenogenesis induction within the Hymenoptera is assumed to have evolved several times independently in *Wolbachia*-strains (e.g. in the genera *Leptopilina*, *Trichogramma*, *Encarsia*, *Muscidifurax* and *Aphytis*), biochemical changes necessary might be relatively simple and easily give rise to new parthenogenetic populations (Werren *et al.* 1995b).

Cytological investigations revealed a mechanism called gamete duplication to be responsible for thelytoky in at least 10 species of the parasitic wasp *Trichogramma* (Stouthamer and Kazmer 1994),

the cynipid gall wasps *Diplolepis rosae* and *D. spinosissimae* (Stille 1985; Plantard *et al.* 1998) and the sawfly *Muscidifurax uniraptor* (Stouthamer *et al.* 1993). In *Trichogramma* wasps and possibly also in the other cases of microbe-associated thelytoky meiosis is undisturbed. Rather, post-meiotic diploidization of oocytes is achieved by a segregation failure of the two sets of chromosomes in the first mitotic anaphase (Stouthamer and Kazmer 1994; Riparbelli *et al.* 1998). The two genetically identical mitotical products fuse after the first mitotic division or at some later stage (Fig. 5.1.d). This mechanism immediately leads to complete homozygosity (Stouthamer and Kazmer 1994). Assuming a single-locus compensatory sex determination (sl-CSD) common to most Hymenopterans (i.e., females are heterozygous at the sex locus), the postmeiotic restoration of diploidy in haploid eggs will lead to immediate homozygosity at the sex locus resulting in the deleterious production of diploid males (Cook 1993). In the Hymenoptera, diploid males are usually sterile and less viable (Crozier 1977; Cook and Crozier 1995). Although gamete duplication occurs, the majority of progeny in *D. rosae* are females. Occasional diploid males have one chromosome set completely heterochromatinized, i.e. they are functionally haploid (Stille and Dävring 1980). How the bacteria interfere with sex determination is not known. In *M. uniraptor* infection causes endomitotic divisions that lead to complete homozygosity (Legner 1985). Curiously bacterial induction of thelytoky has not been found outside of the parasitic superfamilies Calcidoidea and Cynipoidea (Stouthamer 1997). Possibly this is because these are the only two Hymenopteran taxa in which gamete duplication does not lead to the production of diploid (sterile) males (Cook 1993). The chalcidoid and cynipoid wasps therefore most likely have a sex determining mechanism different from sl-CSD although its exact nature is still controversial (Cook 1993; Dobson and Tanouye 1998). Interference with the sl-CSD could provide a strong counter-selection for the acquisition of thelytoky through *Wolbachia* infection in other Hymenoptera. Indeed, although a large number of species are infected within the Formicidae (Werren *et al.* 1995a; Wenseleers *et al.* 1998), all thelytokous ant species are *Wolbachia*-negative excluding parthenogenesis induction as a possible role for *Wolbachia* in ants (Wenseleers and Billen 2000).

5.3.3 Hybrid origin

Most commonly known in plants, hybridization is another mechanism that is responsible for parthenogenesis in a wide range of Hymenopteran species, e.g. in parasitic wasps (Nagarkatti 1970; Nagarkatti and Fazaluddin 1973; Pintureau and Babault 1981; Legner 1987a; Tardieux and Rabasse 1988; Quicke 1997).

Hybridization may lead to polyploidy which in turn causes immediate reproductive isolation from closely related bisexual species (Bell 1982). In most cases it appears that diploid thelytoky evolved first and was followed by the establishment of polyploidy, such as in the tetraploid *Solenobia*

triquetrella (Suomalainen *et al.* 1987). The evolutionary pathways from the ancestral diploid forms may however vary, e.g. to tetraploid forms by a failure to extrude the first polar body during meiosis or to triploid forms by the fusion of three polar nuclei or by the fertilization of a diploid egg (Suomalainen *et al.* 1987). Hybridization in parthenogens can fix heterosis (superior performance of heterozygotes relative to homozygotes) and thereby confer broad ecological tolerance to its bearers (Vrijenhoek 1998). In other words, parthenogenesis can freeze highly adapted gene combinations in hybrids that bisexual recombination would otherwise distort (Suomalainen *et al.* 1987). Contrarily, in several parthenogenetic hybrids it has been shown that hybrid fitness is less than those of either sexual ancestor (compare section 5.7). Within the Hymenoptera hybridization leading to polyploidy is rare and known in only few cases (Suomalainen 1962; Bell 1982).

5.4 The ecology of thelytoky

Despite the two-fold costs of sex with respect to reproductive growth, asexual lineages have not at all replaced their sexual relatives, a problem that has previously been referred to as the 'cost of sex', the 'cost of meiosis', the 'cost of single motherhood' or the 'cost of lazy males' (Maynard Smith 1971; Williams 1975; Judson 1995; Hamilton, pers. comm.). Generally the predominance of sex has been explained by the creation of genetic variability to increase chances of offspring survival under increased intraspecific competition (Williams 1975) or changing environmental selection pressures (Maynard Smith 1978). A famous illustration is the comparison of a parthenogenetic female to a man who buys 100 lottery tickets and finds that they all have the same number (Williams 1975).

Despite the preponderance of sex, a number of ecological scenarios have been proposed to explain selective advantages originating from asexual reproduction. According to biogeographical surveys parthenogenetic reproduction is more frequent at the margin of a species distribution, at more extreme latitudes, at higher altitudes and in more disturbed habitats (Bell 1982; Peck *et al.* 1998). In most cases it remains, however, unclear whether this pattern is due to an enhanced colonizing ability of asexual lineages (no need to find males at low density occurrence), a higher tolerance of adverse ecological conditions or the inability to successfully compete with sexual lineages in the center of their distribution (Bell 1982). Since growth of the founding population is not limited by initial sparseness, the critical population size is small, often corresponding to a single individual (Gerritson 1980). There are only few ecological case studies in the Hymenoptera. In the thelytokous sawfly *Venturia canescens* a high clonal variability in wing morphology is correlated with a high colonizing ability of new environments (Slobodchikoff 1983). The deletion of the sexual generation in bivoltine populations of the gall wasp *Andricus mukaigawae* is attributed to be an adaptation to cold northern climates (Abe 1986).

This explanation may be confounded by a general structural degeneracy of males at higher latitudes (Bell 1982). On the contrary, in other parthenogenetic Hymenopterans unfavorable environmental conditions may induce the production of males (cited in Stille and Dävring 1980).

The existence of clonal diversity in addition to the introduction of novel genotypes through mutations implies that natural selection can act among clonal lineages. Even in obligate parthenogenetic species this can lead to a form of 'parasexuality' that might explain why ancient asexuals were able to persist despite their genetic impoverishment: Within a mixture of several clonal lineages, single clones may change in frequency in response to selective pressures, e.g. fluctuations in parasite presence, much like sexual populations fluctuate in a limit cycle model (Hamilton, pers. comm.). Narrowly adapted clones might do extremely well at times, but are at a higher risk to go extinct than broadly adapted ones (Forbes *et al.* 1997). It has been argued that such selection would then result in broadly adapted genotypes having wider ecological tolerances as compared to their sexual ancestors, an idea also known as the ‘general-purpose genotype hypothesis’ (Templeton 1982; Lynch 1984). The empirical evidence for this is equivocal (Forbes *et al.* 1997). A widespread geographical distribution alone is not sufficient evidence for the general-purpose genotype hypothesis since a single clone could equally occupy a small but universally available niche (Vrijenhoek 1998). The evolutionary success of general purpose parthenogens should critically depend on the spatial and temporal scale of environmental variability: General-purpose genotypes are expected to have high fitness in response to environmental variation that has played a key role in their evolutionary history, but to a lesser amount in novel selective regimes (Forbes *et al.* 1997). Further studies are needed on this subject.

5.5 When to have sex?

According to recent models an asexual lineages that engages in rare sex can obtain many of the advantages of sexual reproduction without paying the twofold cost of sex (Green and Noakes 1995; Hurst and Peck 1996). Sexual reproduction in asexual lineages however does not receive much empirical support. A recent study on the parasitoid wasp genus *Lysiphlebus* provides some molecular evidence. Although several asexual species within the genus consist of well established mitochondrial lineages, the lack of persistence of nuclear genomes without recombination supports the notion that cryptic and rare sex may be more common in asexual lineages than previously thought. Rare males are observed in the wasp *Lysiphlebus* and may account for raised levels of heterozygosity in the species (Belshaw *et al.* 1999). In other thelytokous species, males often appear to be functionless aberrations due to phylogenetic inertia. They may however be evolutionary significant in providing for occasional outcrossing between parthenogenetic lineages and thereby maintain genetic variability within these

lineages (Crozier 1975). In the thelytokous form of the parasitic wasp *Aphytis mytilaspidus* sperm can successfully fertilize one of the female polar nuclei and prevent the normal fusion of second-division non-sister nuclei that thelytokously restore diploidy (Rössler and DeBach 1973). Similarly sperm retained the ability to restore diploidy in some *Wolbachia*-induced asexual parasitoid wasps (Stouthamer and Kazmer 1994). So far this mechanism of gene exchange under thelytoky has not been looked at in other species but may be quite widespread (Crozier 1975; Rispe and Pierre 1998). In *Eucypris virens* the occasional hybridization of parthenogenetic clones and bisexual populations may explain the recruitment of new clonal genotypes (Rossi *et al.* 1998). Interestingly, also parthenogenetic, non-marine ostracods with sexual congeners show a higher clonal diversity than those from all-female genera (Turgeon and Hebert 1994). However fertilization by males may induce triploidy in female offspring thereby disrupting functional parthenogenetic lineages. In this species females are thus faced with the problem of avoiding willing males (Krieber and Rose 1986). In conclusion it still appears quite likely that occasional incidences of sexual reproduction are common even among older asexual lineages but have remained largely undetected. The assumption raised on theoretical grounds that parthenogenesis is evolutionary irreversible (Bull and Charnov 1985) therefore seems not completely true.

5.6 Hopeful monsters or transposon mutations?

Thelytokous reproduction constitutes an extreme case of sex ratio distortion biasing offspring sex completely towards females (Werren and Beukeboom 1998). Thelytoky in the Hymenoptera, as far as we know, is either caused by nuclear genes or by cytoplasmatic agents such as *Wolbachia*. Whereas thelytoky induced by *Wolbachia* infection is a clear example of the action of a selfish cytoplasmatic element, the potential selfishness of nuclear genes that induce parthenogenesis has not been addressed formally. Within the last decade there have been extensive discussions about the adaptive value of selfish genetic elements frequently found in eukaryotic DNA (e.g. Doolittle and Sapienza 1980; Orgel and Crick 1980; Robinson 1981; Hickey 1982). Additionally various cytoplasmatic factors have been identified that act to increase the frequency of female offspring (reviewed by Hurst 1993). This section investigates the theoretical question of whether nuclear genes coding for thelytoky in the Hymenoptera could be regarded as selfish genetic elements, that are able to spread in the population despite of major deleterious effects they may convey for their hosts (Hickey 1982; Charlesworth *et al.* 1994; Werren and Beukeboom 1998).

How might thelytokous phenotypes evolve? Since the introduction of the concept of the adaptive landscape by Wright (1932) evolution has been regarded as the walk (genetic drift) from one peak of

fitness towards another, higher optimum (Wright's shifting balance theory of evolution). Shifts from one optimum to the other could only occur in single macromutational steps combining all the complex phenotypical changes needed to form, for example, a viable parthenogen from bisexual ancestors. The notion of macromutational change is exemplified by the picture of the 'hopeful monster', e.g. major changes in patterns of gene expression leading to the sudden appearance of drastically changed phenotypes (Goldschmidt 1940). The theme dates back to Elizabethan England (although then viewed from a male-centered perspective) when Shakespeare entertained Othello's monstrous sexual fantasies of parthenogenesis, through which the males seek to escape the deforming power of the female, producing nothing but monstrous births, e.g. the deformed slave Caliban in "The Tempest" (Johnson 1996). The macromutational concept however has been subject to controversy (Fisher 1930; Dawkins 1988). Lately, it has received renewed attention in relation to results obtained by quantitative trait locus (QTL) analyses (e.g. Orr and Coyne 1992; Mitchell-Olds 1995). Evolution of a thelytokous phenotype from a bisexual ancestor (the creation of a hopeful monster) could essentially be following the model of macromutational change.

Recently investigations on the phylogenetic distribution of mobile, repetitive genetic elements, so-called transposons (i.e. nuclear DNA sequences capable of inserting copies of themselves into new genomic locations), revealed that infection is often species-specific, occurring closely to the origin of the infected phylum, and may similarly be associated with macroevolutionary change (reviewed in McFadden and Knowles 1997). Multiple insertions of transposons at different sites, often causing complex structural and regulatory changes, have been proposed to underly rapid evolutionary change reminiscent of the macromutational concept introduced above (Syvanen 1984). More recently transposons were proposed to initiate speciation by the promotion of irreversible deleterious mutations that facilitate the escape from evolutionary stasis (McFadden and Knowles 1997). According to McFadden and Knowles' genetic model, most of these mutations will be lethal but some will allow the organism to leave an optimum and evolve towards another adaptive peak by the subsequent accumulation of micromutational changes. The appearance of a thelytokous phenotype could be the result of such a leap. The concept of transposon mutations differs from the macromutational model since these mutants will not occupy another adaptive peak right away (McFadden and Knowles 1997).

Traditionally, thelytokous species are expected to enjoy a two-fold benefit: compared to a bisexual female a thelytokous female propagates her genome twice as efficiently (Bell 1982). Within a mixed population, however, genes coding for thelytoky will not be able to spread because they will not colonize new genomes. The population will become completely asexual only when a thelytokous clone gets to fixation. Hickey (1982) points out that there will be no systematic tendency towards an increase in the frequency of genomes containing selfish thelytokous elements. In every bisexual individual

thelytoky would have to arise by mutation. As a consequence, a nuclear gene coding for thelytoky per se is not expected to behave selfishly.

Now imagine a hypothetical transposable element that, by some unidentified action, incorporated a gene coding for thelytoky. Extrachromosomal elements such as microorganisms found in a number of thelytokous species (see section 5.3.2) could then provide for the efficient horizontal spread of such transposable elements from thelytokous to bisexual forms. Unfortunately only limited data on horizontal gene transfer in arthropods is currently available. In the isopod *Armadillidium vulgare*, for example, sex determination has been investigated in greater detail (Rigaud and Juchault 1993). In addition to *Wolbachia* populations of *A. vulgare* contain a feminizing factor (*f*), masculinizing autosomal genes and suppressors of feminizing factors at varying frequencies. *f* has been proposed to be a bacterial phage that occasionally exchanges feminizing genomic elements between *Wolbachia* and the isopod genome. In a thelytokous species, this mechanism could allow for the horizontal spread of transposable elements containing a gene coding for thelytoky. Horizontal transfer of *Wolbachia* bacteria is known to occur even between phylogenetically distant insect species possibly mediated by insect parasitoids (Heath *et al.* 1999). In any case the successful integration of a transposable element coding for thelytoky into a bisexual genome will be a rare event depending on the efficiency of transposition and the fitness of these organisms. If genes coding for thelytoky indeed behave similar to transposable elements, we would expect to find copies of them accumulated in a number of bisexual species even when the insertion of the thelytokous element failed to produce a thelytokous phenotype. Given the vagueness of the argument and the lack of empirical support thelytoky is quite unlikely to have evolved in a transposon-like fashion. Only the identification and characterization of the genomic information responsible for thelytoky can determine whether these genes behave similar to selfish genetic elements.

5.7 Why is thelytoky rare?

Why is it that despite its obvious advantages thelytoky has not spread more rapidly through bisexually reproducing Hymenopteran lineages? The answer may be found in the costs and constraints thelytoky involves which may restrict its rapid evolution and persistence over time. Repeatedly, developmental constraints have been claimed to prevent the frequent evolution of thelytoky (Templeton 1982; Kondrashov 1988; Lively and Johnson 1994; Hurst and Peck 1996). Depending on the cytological mechanism a higher mutational load may preserve deleterious alleles for longer time than in bisexual species (Manning and Jenkins 1980). Additionally, fitness of obligate asexual species cannot be increased by regular outbreeding counteracting an inbreeding depression (Crozier 1975). Parthenogenetic species may additionally experience disadvantages in their reproductive potential. It has

been estimated that many parthenogenetic insect species produce only about 60 percent of the offspring of sexuals, possibly due to problems in embryonic formation (Lamb and Whiley 1979). For most thelytokous species detailed comparisons of lifetime fertility and survival with closely related bisexual species in their natural environments are lacking. Egg production and egg viability in the thelytokous beetle *Ptinella errabunda*, for example, are reduced compared to the related bisexual *E. aptera*, possibly to achieve a higher colonizing ability (Taylor 1981). In the Hymenoptera there are only three case studies that have tried to address reproductive success of closely related sexual and asexual lineages. The thelytokous sawfly *Diprion polynotum* produced only about one-third the amount of offspring produced by a females from an arrhenotokous line (although it is not clear whether they belonged to the same species) (Smith 1941). Arrhenotokous forms of *Trichogramma deion* and *T. pretiosum*, derived from thelytokous lines by antibiotic treatment, produced significantly more offspring (Stouthamer 1990). Only in *Aphytis mytilaspidis* the thelytokous line was more productive than the arrhenotokous line (Rössler and DeBach 1973). Results were however confounded by differences in host plant preferences.

5.8 Conclusions

Traditionally, thelytoky is regarded as a rare phenomenon in the Hymenoptera. This belief could however be biased by sparse empirical evidence that has up to now overlooked many cases of thelytoky in various Hymenopteran subfamilies, especially if it is geographically confined or occurs only facultatively. High levels of inbreeding found repeatedly in the Hymenoptera could have provided an evolutionary background that acted as pre-adaptation for the evolution of thelytoky in a large number of species by exposing deleterious genetic alleles to elimination. Thelytokous phenotypes can potentially arise by a variety of cytogenetical changes affecting the normal pathway of meiosis, most of which are found in the Hymenoptera. In addition to a genetic origin in several taxa thelytoky can be caused by the infection with cytoplasmatic, sex-ratio distorting elements such as *Wolbachia*. As has been done for the evolution of genes coding for sex (Hickey 1982), it is tempting to speculate that thelytoky evolved in a way reminiscent to selfish transposable elements. Too little is currently known about the genes coding for thelytoky, its cytogenetic constraints and the ecological correlates that provide the selective background to favor asexual over bisexual reproduction to evaluate this hypothesis. Detailed studies to investigate all these factors are needed to further elucidate the significance of thelytoky in the Hymenoptera.

6 General discussion

Reproductive division of labor is a key concept in the evolution of eusociality. The existence of a sterile (i.e. non-laying) worker caste is one of the three fundamental principles of eusociality (Wilson 1971). Thelytokous eusocial Hymenoptera however seem to strikingly violate the prerequisite of worker sterility in that uninseminated workers, in addition to their capability to produce haploid males, are capable of diploid female production (e.g. Slobodchikoff and Daly 1971). This chapter will evaluate the consequences of thelytoky for the maintenance and stability of eusociality in general by comparing the insights obtained into the reproductive organization of *Platythyrea punctata* with current evidence in other thelytokous Hymenopterans.

Reserve social evolution - or irreversible evolution?

In order to explain the great diversity of existing social systems eight important and novel organizing principles in evolutionary biology have been identified (Maynard Smith and Szathmáry 1995). Major evolutionary transitions include, among others, the transition from asexual to sexual propagation, from unicellular to multicellular organization and from solitary to social life. Although the (forward) evolution of eusociality has been discussed in great detail (compare chapter 1) the transition back to a less complex social structure received little attention (Gadagkar 1997). Frequently it is a priori assumed that the transition to eusociality is an evolutionary dead end (with the subsequent evolution of social parasitism as a possible exception) (Wilson 1971; Wcislo and Danforth 1997). The concept of evolutionary irreversibility is traditionally known as "Dollo's Law" stating that morphological structures, as well as behavioral and other traits, once lost during evolution are usually not regained in their original state (reviewed in Gould 1970). Highly eusocial clades seemingly confirm this hypothesis: In termites, ants, paper wasps, and the highly social honey bees, stingless bees and bumble bees, so far no reversal to solitary nesting has been described (reviewed in Wcislo and Danforth 1997). However, it has been pointed out that irreversibility develops gradually allowing for partial reversals (Maynard Smith and Szathmáry 1995). Through the re-emergence of selfish behavior individuals living in social groups may revert to simpler forms of sociality or even back to solitary life. Eusocial forms could acquire at least some intermediate states in the hypothetical social reversal. This ‘reverse social evolution’ in social insects may include three steps (Gadagkar 1997): 1) Workers revolting against the hegemony of the queen and challenging her as the sole reproductive. 2) Workers stop rearing queens but

take on the function of egg-layers in the colony, i.e. assuming the role of functional queens. 3) Facultative reversal to a completely solitary life.

Good examples of the initial step in reverse social evolution, facultative worker thelytoky in the absence of the queen, include the Cape honey bee, *Apis m. capensis*, and the ant *Cataglyphis cursor*. In the Cape honey bee about 12 percent of the workers in a colony produce female offspring which is mostly reared to workers and few queens. Unlike workers in other honey bee races here workers posses several queen-like pre-adaptations that might have facilitated the evolution of thelytoky: Workers have an increased reproductive rate compared to the closely related *A. m. scutellata*, due to a higher number of ovarioles. They retain a well-developed spermatheca, are capable to produce queen-like pheromones including 9-ODA, show a high reproductive dominance, and are able to inhibit the production of queen cells (Verma and Ruttner 1983; Hepburn and Crewe 1990; Hepburn and Crewe 1991; Hepburn *et al.* 1991; Hepburn 1994; Greeff 1997). In the ant *Cataglyphis cursor* thelytokous workers in orphaned colonies lay diploid eggs that develop both into workers and queens, possibly as an adaptation to the risk of queen loss (Cagniant 1979, 1982, 1983).

Ponerine ants are regarded as possessing both primitive and derived characters. The remaining potential of most workers to mate and reproduce as well as the absence of a pronounced size dimorphism in females are preconditions for the re-emergence of a social system more primitive than eusociality. This is especially obvious in those ponerine ants that have lost their queen caste completely and solely rely on gamergates for reproduction (Peeters 1993). Therefore they are regarded as a clear example of an intermediate second stage in reverse social evolution (Gadagkar 1997).

Transitions from the eusocial state to solitary life, as suggested in the third step of reverse social evolution, are sometimes observed in primitively eusocial species, e.g. in the halictine bees (Michener 1990; Wcislo and Danforth 1997). Using allozyme data eusociality in the genus *Lasioglossum* (*Evylaeus*) has been hypothesized as the ancestral state with one (the solitary *L*. (*E*.) *fulvicorne*), and possibly another one (a solitary population of *L*. (*E*.) *calceatum*), reversal to solitary life (Packer 1991). Equally, in a phylogenetic analysis of 15 species within the genus *Halictus* at least two lineages may have reverted to solitary life from a common social ancestor (Richards 1994). Further examples of reversal to solitary nesting in allodapine bees are reviewed in Wcislo and Danforth (1997). However these possible reversals to solitary life are not undisputed and could equally be explained as different reproductive tactics maintained by selection on intraspecific variation in social behavior. In facultatively social species such as xylocopine carpenter bees and some halictine bees, fluctuations in environmental conditions, i.e. geographic and climatic, may favor either solitary or social behavior (Packer 1990; Stark 1992).

But how can the general absence of secondarily solitary taxa in highly eusocial Hymenoptera be explained? In the highly eusocial Hymenoptera several mechanisms likely function to prevent this

complete social reversal to solitary life (Gadagkar 1997): Workers might not be able to escape queen control (either pheromonal or by physical interactions), the phylogenetic loss of the spermatheca in many species impairs them from mating, morphological specializations in sterile and reproductive castes cause complete loss of reproductive potential in some individuals, caste polyethism might effect the behavioral repertoire and the need for social homeostasis may prevent solitary life-styles. Therefore reversed social evolution is expected to evolve only in those species in which these preventing mechanisms may be diminished or absent. Most of the more advanced social insect species probably evolved beyond a "point of no return", i.e., they lost elements of behavior or morphology that are very difficult to re-attain in future evolution (Wilson 1971).

Thelytoky - an opportunity for reversed eusociality?

Given the diversity of preventing mechanisms mentioned above a complete reversal of eusociality to solitary life remains rather unlikely. Several cases however proof that evolutionary transitions can indeed sometimes be subject to reversal. The (secondary) evolution of asexual reproduction is a good example. While certain taxa such as mammals or gymnosperms, probably because of phylogenetic idiosyncrasies, never evolved parthenogenetic reproduction, apomictic or automictic parthenogens are present in several other taxonomical groups including the Hymenoptera (reviewed in chapter 5). The evolution of thelytoky usually is accompanied by the loss of males and by profound changes in the cytology of meiosis. The restoration of sexual reproduction in an obligate thelytokous species would therefore require both, the re-establishment of males and the restoration of a functional meiosis in females (Bull and Charnov 1985). Once evolved, thelytoky may therefore be difficult to revert. Here, thelytoky will be considered as a step towards reverse social evolution. Table 6.1 summarizes various characteristics that are important for the discussion of reverse social evolution in *P. punctata* and two other thelytokous Hymenopterans.

Functional monogyny is a reproductive strategy very sensitive to the loss of the sole reproductive (e.g. most honeybees are strictly monogynous). Thelytokous parthenogenesis is a possible adaptation to the threat of orphanage allowing worker groups to sustain a colony and possibly rear replacement queens (Moritz 1986). In *A. m. capensis* risk of queen loss during mating flights may be particularly high due to strong and changing winds in the Cape region (Moritz 1986; Allsopp *et al.* 1997). Upon queen loss, orphaned colonies may requeen from the brood of the previous queen, an egg-laying thelytokous worker or it may remain as a laying worker colony (Hepburn 1994). In *P. punctata* a highly seasonal habitat with cold winters might have favored the evolution of thelytoky at least in the periphery of its range. Advantages in the propagation of thelytokous colony fragments by fission is one

possibility to account for the evolution of thelytoky in *P. punctata*. More detailed investigations on the ecological correlates of thelytoky are needed to fully evaluate the significance of this hypothesis.

Greef (1996a), modeling arrhenotokous versus thelytokous reproduction, dismissed a higher risk of orphanage as the underlying reason for thelytoky in *A. m. capensis* and suggested life history peculiarities to be responsible. Due to greater kin-value asymmetries (the product of relatedness, reproductive value and expected mating success as defined in Ratnieks and Reeve 1992) under thelytokous conditions, workers in queenless colonies of *A. m. capensis* are expected to show higher levels of conflict between different subfamilies as compared to arrhenotokous races. However, if relatedness increases within the colony the amount of fighting observed after queen loss should be reduced (Greeff 1996b).

Kin-value asymmetries in *P. punctata* are virtually absent in the populations studied due to clonal propagation. Given occasional sexual outbreeding introduced genetic variation, competition

	Apis mellifera capensis [1]	*Platythyrea punctata* [2]	*Pristomyrmex pungens* [3]
Colony size	several 1000	10 - 170	several 1000
Spermatheca	yes	yes	no[4]
Sexuals	present	produced infrequently	absent
Reproduction	queens, thelytokous workers after loss of queen	thelytokous workers, gamergates, queens (?)	most (all) intranidal workers
Thelytoky	facultative	dominant in some populations	obligate
Aggression	intense among thelytokous workers	yes	no?
Genetic structure	highly variable, clonal in thelytokous workers	clonal	variable?
Possible evolution	high risk of queen loss	seasonal habitat	nomadic life style, risk of colony fragmentation
Social organization	eusocial	eusocial	intermediate subsocial
Social reversal	I	I-II	II

[1] - Anderson 1963, Moritz 1986, Hepburn 1994, Moritz and Haberl 1994
[2] - Heinze and Hölldobler 1994, Schilder *et al.* 1999a, 1999b
[3] - Itow *et al.* 1984, Tsuji 1988, 1992, 1994
[4] - large workers retain a functionless spermatheca (Itow *et al.* 1984)

Table 6.1 Comparison of the social organization and life history characteristics of three thelytokous Hymenopterans that show a tendency towards reverse social evolution. Stage of social reversal is classified according to Gadagkar (1997). See text for further details.

between genetically distinct clonal lineages would account for aggressive interactions among thelytokous workers as long as they do not reduce the colony's total reproductive potential. Whereas in *P. punctata* nestmates still compete aggressively for reproduction, there is hardly any evidence for the widespread existence of sexual reproduction (chapter 3), and populations have a clonal structure with a single thelytokous worker monopolizing reproduction (see chapter 4).

Under thelytoky both aggression and production of sexuals are expected to be without function. The question remains why *P. punctata* still exhibits these traits? Assuming, the production of sexuals does not impose a high cost on individual colony fitness which would provide strong counterselection, phylogenetic inertia and the viscosity of the biological system may have, until now, prevented the loss of these traits in *P. punctata*. The strength of counterselection will depend on the costs accruing by the expression of sexual traits: If they are not costly to the individual or to the colony, i.e. in terms of energy needed for their expression, they will be expressed for a considerable time despite the fact that they might have lost their biological relevance long ago. The queen caste has secondarily been lost in a number of platythyreine and other ponerine ants where mated workers fill their reproductive function (Villet 1992a, 1992b; Peeters 1993). Their social organization is similar to the one of many primitively social halictine bees (Michener 1990).

Thelytoky in the obligate queenless ant *Pristomyrmex pungens* might serve as a potential model for future evolution pathways in *P. punctata*. It is probably the most advanced form of reversed social evolution currently known within the eusocial Hymenoptera (Itow *et al.* 1984; Tsuji 1988a, 1992, 1994). *P. pungens* has lost its queen caste completely and all workers within the colony reproduce at some point in their life. Risk of queen loss and subsequent suspension of queen production might have let to obligate thelytoky (Itow *et al.* 1984). According to some authors (Itow *et al.* 1984; Tsuji 1988a), the species can no longer be described as eusocial because all workers reproduce at one stage of their lifetime (when they are young), and none of the workers is permanently sterile, thus violating the original definition of eusociality. Consequently, *P. pungens* was regarded as a communal nester (Tsuji 1990). This clearly depends on the definition of eusociality used, and subsequently provoked some discussion. Furey (1992) strongly rejected Tsuji's interpretation and stated that each of the three criteria of eusociality is met by *P. pungens*. Subsequently, Tsuji (1992) admitted that communal brood care is clearly present in *P. pungens* but by definition absent in communal nester (Michener 1974) and therefore re-categorized the species as being intermediate subsocial II (Wilson 1971). It appears as if a stabilizing and positive directional selection on the proportion of foragers within a nest is responsible for the maintenance of cooperative behavior in this species over evolutionary time (Tsuji 1995). There is an ongoing discussion on whether the definition of a sterile worker caste must be based on the concept of lifetime sterility (Tsuji 1992; Gadagkar 1994). There are many examples of primitively eusocial wasps

where sterility does not necessarily last for the whole life (Gadagkar 1994). Using such a rigid definition would exclude these species from eusociality.

The absence of reproductive division of labor in *P. pungens* is reminiscent of the social organization of *Ropalidia rufoplagiata*. This polistine wasp is unique among the primitively eusocial wasps in lacking a permanent reproductive caste (Sinha *et al.* 1993). Contrasting the situation found in *P. pungens*, most of the older workers (about 70 percent) are egg-layers while foragers are mainly younger workers. This mixed reproductive strategy within an individual's lifetime could have been advantageous in the early stages of the evolution of eusociality (Gadagkar 1991) but possibly secondarily re-emerged in *P. pungens*. Since queens are completely absent, thelytoky is obligate and reproductive division of labor is temporal with workers performing both reproductive and non-reproductive tasks at some point in their lives, *P. pungens* may have already reached the second step in reverse social evolution (Gadagkar 1997).

What can be concluded for the degree of reverse social evolution in *P. punctata*? Although queens and males are still present in *P. punctata* they are either produced at very low frequency or completely absent. Instead the reproductive role is filled by thelytokous workers replacing morphological queens. In contrast to *P. pungens* (Itow *et al.* 1984), sexual reproduction by workers is still possible since the retain a functional spermatheca. Thelytoky can thus function as a conditional reproductive strategy. Reproductive division of labor in *P. punctata* is still present since most of the workers in thelytokous colonies will not reproduce during their lifetime. Compared to the Cape honeybee and other facultative thelytokous ants, *P. punctata* has left the first step of reverse social evolution behind and can almost be considered as an intermediate stage in reversed social evolution similar to other queenless ponerine ants. As long as the reproductive division of labor is retained, *P. punctata* clearly remains a eusocial species. Without more detailed knowledge on the ecology, geographical distribution of thelytoky and relevance of sexual reproduction in this species one cannot judge whether the queen caste will be completely lost within its close evolutionary future. Considering the internal phylogeny of ants, queen loss in the ponerines clearly is a derived condition that occurred several times (Baroni Urbani *et al.* 1992), at least once in the old world Platythyreini where three queenless species are known (Villet 1992a). In *P. punctata*, outbreeding by sexual reproduction may still persist under certain conditions. *P. punctata* possibly provides one of the rare cases where the successful establishment of thelytoky has set the evolutionary stage for the loss of the queen caste and of reproductive competition.

A hypothetical queenright ancestor species of *P. pungens* might have looked much like *P. punctata* now. If this hypothetical species would have been monandrous (i.e. strong selection for queen-worker conflict over male production) and had a certain level of worker reproduction that did not effect overall colony productivity, the evolution of thelytoky might have been the stepping stone for the

subsequent loss of queens in the ancestral *P. pungens*. In addition, the reproductive system of *P. punctata* might reflect different ecological and sociometric constraints as compared to *P. pungens*. Colony size is a confounding difference between the two species, with *P. pungens* colonies averaging 45,000 workers (Tsuji, pers. comm.). Economical efficiency in the division of labor or resource availability might explain why *P. punctata* is monogynous while in *P. pungens* almost every (intranidal) worker reproduces. *P. punctata* might be selected to maintain a much higher behavioral flexibility due to its smaller colonies, which is especially pronounced after the reduction of colony size following colony fission. In addition the reproductive potential of myrmicine workers in genera is lower than that of a ponerine ant worker, e.g. in *P. pungens* only 0.14 eggs per day (Tsuji 1994). Even under resource limitation, egg-laying by multiple workers could be possible in *P. pungens*. In the absence of conflict of interest over reproduction, i.e. in a clonal society, each colony member is expected to agree to a reproductive system that maximizes colony fitness as a whole. While in *P. punctata* workers are highly related clones, unfortunately no sound genetic evidence on the colony structure in *P. pungens* is available (Tsuji 1994).

As the ability of copulation for gamergates in queenless ponerine ants, thelytoky provides another possible pre-adaptation for the potential evolution of obligate queenlessness and for the evolutionary loss of highly eusocial behavior. Since its evolution is largely independent of phylogenetic constraints this may similarly apply to ants and many other eusocial insects. Thelytokous social Hymenopterans in particular provide valuable insights in the mechanisms that maintain or reverse eusocial evolution, both at the primitively and highly eusocial level.

7 Summary

Highly eusocial insect societies, such as all known ants, are typically characterized by a reproductive division of labor between queens, who are inseminated and reproduce, and virgin workers, who engage in foraging, nest maintenance and brood care. In most species workers have little reproductive options left: They usually produce haploid males by arrhenotokous parthenogenesis, both in the queenright and queenless condition. In the phylogenetically primitive subfamily Ponerinae reproductive caste dimorphism is much less pronounced: Ovarian morphology is rather similar in queens and workers, which additionally retain a spermatheca. In many ponerine species workers mate and may have completely replaced the queen caste. This similarity in reproductive potential provides for the evolution of diverse reproductive systems. In addition, it increases the opportunity for reproductive conflicts among nestmates substantially. Only in a handful of ant species, including *Platythyrea punctata*, workers are also able to rear diploid female offspring from unfertilized eggs by thelytokous parthenogenesis.

The small ponerine ant *P. punctata* (Smith) is the only New World member of the genus reaching as far north as the southern USA, with its center of distribution in Central America and the West Indies. *P. punctata* occurs in a range of forest habitats including subtropical hardwood forests as well as tropical rain forests. In addition to queens, gamergates and thelytokous workers co-occur in the same species. This remarkable complexity of reproductive strategies makes *P. punctata* unique within ants and provides an ideal model system for the investigation of reproductive conflicts within the female caste.

Colonies are usually found in rotten branches on the forest floor but may also be present in higher strata. Colonies contained on average 60 workers, with a maximum colony size of 148 workers. Queens were present in only ten percent of the colonies collected from Florida, but completely absent both from the populations studied in Barbados and Puerto Rico. Males were generally rare. In addition, morphological intermediates between workers and queens (so-called intercastes) were found in 16 colonies collected in Florida. Their thorax morphology varied from an almost worker-like to an almost queen-like thorax structure. Queen and intercaste size, however, did not differ from those of workers.

Although workers taken from colonies directly after collection from the field engaged in aggressive interactions, nestmate discrimination ceased in the laboratory suggesting that recognition cues used are derived from the environment.

Only one of six queens dissected was found to be inseminated but not fertile. Instead, in most queenless colonies, a single uninseminated worker monopolized reproduction by means of thelytokous parthenogenesis. A single mated, reproductive worker (gamergate) was found dominating reproduction in the presence of an inseminated alate queen only in one of the Florida colonies.

The regulation of reproduction was closely examined in ten experimental groups of virgin laboratory-reared workers, in which one worker typically dominated reproduction by thelytoky despite the presence of several individuals with elongated, developing ovaries. In each group only one worker was observed to oviposit. Conflict over reproduction was intense consisting of ritualized physical aggression between some nestmates including antennal boxing, biting, dragging, leap and immobilization behaviors. The average frequency of interactions was low. Aggressive interactions allowed to construct non-linear matrices of social rank. On average, only five workers were responsible for 90 percent of total agonistic interactions. In 80 percent of the groups the rate of agonistic interactions increased after the experimental removal of the reproductive worker. While antennal boxing and biting were the most frequent forms of agonistic behaviors both before and after the removal, biting and dragging increased significantly after the removal indicating that agonistic interactions increased in intensity. Once a worker obtains a high social status it is maintained without the need for physical aggression. The replacement of reproductives by another worker did however not closely correlate with the new reproductive's prior social status.

Age, however, had a profound influence on the individual rate of agonistic interactions that workers initiated. Especially younger adults (up to two month of age) and callows were responsible for the increase in observed aggression after the supersedure of the old reproductive. These individuals have a higher chance to become reproductive since older, foraging workers may not be able to develop their ovaries. Aggressions among older workers ceased with increasing age. Workers that already started to develop their ovaries should pose the greatest threat to any reproductive individual. Indeed, dissection of all experimental groups revealed that aggression was significantly more often directed towards both individuals with undeveloped and developing ovaries as compared to workers that had degenerated ovaries. In all experimental groups reproductive dominance was achieved by callows or younger workers not older than four month. Age is a better predictor of reproductive dominance than social status as inferred from physical interactions.

Since no overt conflict between genetical identical individuals is expected, in *P. punctata* the function of agonistic interactions in all-worker colonies, given the predominance of thelytokous

parthenogenesis, remains unclear. Physical aggression could alternatively function to facilitate a smooth division of non-reproductive labor thereby increasing overall colony efficiency.

Asexuality is often thought to constitute an evolutionary dead end as compared to sexual reproduction because genetic recombination is limited or nonexistent in parthenogenetic populations. Microsatellite markers were developed to investigate the consequences of thelytokous reproduction on the genetic structure of four natural populations of *P. punctata*. In the analysis of 314 workers taken from 51 colonies, low intraspecific levels of variation at all loci, expressed both as the number of alleles detected and heterozygosities observed, were detected. Surprisingly, there was almost no differentiation within populations. Populations rather had a clonal structure, with all individuals from all colonies usually sharing the same genotype. This low level of genotypic diversity reflects the predominance of thelytoky under natural conditions in four populations of *P. punctata*.

In addition, the specificity of ten dinucleotide microsatellite loci developed for *P. punctata* was investigated in 29 ant species comprising four different subfamilies by cross-species amplification. Positive amplification was only obtained in a limited number of species indicating that sequences flanking the hypervariable region are often not sufficiently conserved to allow amplification, even within the same genus. The karyotype of *P. punctata* (2n = 84) is one of the highest chromosome numbers reported in ants so far. A first investigation did not show any indication of polyploidy, a phenomenon which has been reported to be associated with the occurrence of parthenogenesis.

Thelytokous parthenogenesis does not appear to be a very common phenomenon in the Hymenoptera. It is patchily distributed and restricted to taxa at the distant tips of phylogenies. Within the Formicidae, thelytoky has been demonstrated only in four phylogenetically very distant species, including *P. punctata*. Despite its advantages, severe costs and constraints may have restricted its rapid evolution and persistence over time. The mechanisms of thelytokous parthenogenesis and its ecological correlates are reviewed for the known cases in the Hymenoptera. Investigating the occurrence of sexual reproduction in asexual lineages indicates that thelytokous parthenogenesis may not be irreversible. In *P. punctata* the occasional production of sexuals in some of the colonies may provide opportunity for outbreeding and genetic recombination. Thelytoky can thus function as a conditional reproductive strategy.

Thelytoky in *P. punctata* possibly evolved as an adaptation to the risk of colony orphanage or the foundation of new colonies by fission. The current adaptive value of physical aggression and the production of sexuals in clonal populations, where relatedness asymmetries are virtually absent, however is less clear. Quite contrary, thelytoky could thereby serve as the stepping stone for the subsequent loss of the queen caste in *P. punctata*. Although *P. punctata* clearly fulfills all three conditions of eusociality, the evolution of thelytoky is interpreted as a first step in a secondary reverse social evolution towards a social system more primitive than eusociality.

8 Zusammenfassung

Hoch eusoziale Insektenstaaten, einschließlich aller bekannten Ameisenarten, sind durch eine reproduktive Arbeitsteilung zwischen Königinnen, die begattet und reproduktiv aktiv sind, und Arbeiterinnen, die die Aufgaben des Fouragierens, der Nestkonstruktion und der Brutpflege übernehmen, gekennzeichnet. In den meisten Arten bleiben den Arbeiterinnen wenig reproduktive Optionen: Normalerweise zeugen sie haploide Männchen mittels arrhenotoker Parthenogenese, sowohl in königinnenlosen als auch in Kolonien mit Königinnen. In der phylogenetisch ursprünglichen Unterfamilie Ponerinae ist der Dimorphismus der reproduktiven Kasten weniger ausgeprägt: Die Morphologie der Ovarien von Königinnen und Arbeiterinnen, die noch über eine Spermatheka verfügen, ist vergleichsweise ähnlich. In vielen Arten der Ponerinen paaren sich die Arbeiterinnen und haben die Königin-Kaste komplett ersetzt. Die Ähnlichkeit im reproduktiven Potential ermöglichte die Evolution diverser reproduktiver Systeme. Zusätzlich erhöhte sie die Wahrscheinlichkeit für das Auftreten von reproduktiven Konflikten erheblich. Nur in wenigen Ameisenarten, *Platythyrea punctata* eingeschlossen, sind Arbeiterinnen zusätzlich in der Lage, aus unbefruchteten haploiden Eiern durch thelytoke Parthenogenese diploide weibliche Nachkommen zu produzieren.

Die ponerine Ameise *P. punctata* (Smith) ist der einzige Vertreter der Gattung in den Neotropen. Sie ist bis in den Süden der USA verbreitet ist, der Verbreitungsschwerpunkt liegt in Zentralamerika und dem karibischen Raum. *P. punctata* kommt in einer Vielzahl von Habitaten, die von subtropischen Hartholz-Wälder bis zu tropischen Regenwäldern reichen, vor. Zusätzlich zu Königinnen treten sowohl Gamergaten als auch thelytoke Arbeiterinnen in der selben Art auf. Diese bemerkenswerte Komplexität von reproduktiven Systemen macht *P. punctata* innerhalb der Ameisen einzigartig und

bietet ein ideales Modellsystem zum Studium von reproduktiven Konflikten innerhalb der weiblichen Kaste.

Die Kolonien nisten für gewöhnlich in verrottenden Ästen auf dem Waldboden, siedeln wahrscheinlich aber auch in höheren Straten der Vegetation. Die Kolonien enthalten im Durchschnitt 60 Arbeiterinnen, die maximale Koloniegröße beträgt 148 Arbeiterinnen. Königinnen wurden in zehn Prozent der in Florida gesammelten Kolonien gefunden, fehlten jedoch völlig in den auf Barbados und Puerto Rico untersuchten Populationen. Männchen waren generell selten. Zusätzlich wurden in 16 Kolonien, die alle in Florida gesammelt wurden, sogenannte Interkasten, also morphologisch zwischen Königinnen und Arbeiterinnen intermediäre Tiere, gefunden. Die Morphologie des Thorax variierte von einer arbeiterinnenähnlichen bis zu einer fast königinnenähnlichen Struktur. Die Größe von Königinnen und von Interkasten unterschied sich jedoch nicht von der von Arbeiterinnen. Obwohl bei Arbeiterinnen, die aus direkt im Feld gesammelten Kolonien entnommen wurden, aggressive Interaktionen beobachtet wurden, lies diese Nestgenossinnenerkennung im Labor nach. Merkmale, die der Erkennung dienen, sind daher wahrscheinlich aus der Umwelt abgeleitet.

Nur eine der sechs sezierten Königinnen war begattet, jedoch nicht reproduktiv tätig. Stattdessen monopolisierte in den meisten königinnenlosen Kolonien eine einzige, nicht-begattete Arbeiterin die Reproduktion mittels thelytoker Parthenogenese. Eine einzige begattete und reproduktiv aktive Arbeiterin (eine Gamergate) wurde in einer der Kolonien aus Florida, die außerdem eine begattete, geflügelte Königin enthielt, gefunden.

Die Regulation der Reproduktion wurde im Detail in zehn experimentellen Gruppen, die aus im Labor geschlüpften, virginen Arbeiterinnen bestanden, untersucht. In diesen dominierte eine Arbeiterin in der Regel die Reproduktion durch Thelytokie, obwohl mehrere Arbeiterinnen elongierte, sich entwickelnde Ovarien besaßen. In jeder Gruppe legte nur eine Arbeiterin Eier. Konflikte um die Reproduktion waren intensiv und bestanden aus ritualisierter, physischer Aggression, wie heftigem Antennieren, Beißen, Zehren, Vorschnellen und Immobilisierung, zwischen einigen Nestgenossinnen. Die durchschnittliche Frequenz dieser Interaktionen war niedrig. Die aggressiven Interaktionen erlaubten die Konstruktion von nichtlinearen sozialen Rang-Matrizen. Im Durchschnitt waren nur fünf Arbeiterinnen für 90 Prozent der gesamten agonistischen Interaktionen verantwortlich. In 80 Prozent der Gruppen erhöhte sich die Rate agonistischer Interaktionen nach der experimentellen Entfernung der reproduktiven Arbeiterin. Während heftiges Antennieren und Beißen sowohl vor als auch nach der Entfernung die häufigsten Formen agonistischer Verhaltensweisen waren, erhöhte sich die Rate von Beißen und Zehren signifikant nach der Entfernung. Dies ist ein Anzeichen dafür, dass die Intensität der agonistischen Auseinandersetzungen zunahm. Sobald eine Arbeiterin einen hohen sozialen Rank eingenommen hat, wird dieser ohne weitere aggressive Interaktionen beigehalten. Der Ersatz des

reproduktiven Tieres durch eine andere Arbeiterin korreliert jedoch nicht mit deren vorherigem sozialen Status.

Das Alter hatte jedoch einen bedeutenden Einfluss auf die individuelle Rate agonistischer Interaktionen, die Arbeiterinnen initiierten. Besonders junge Arbeiterinnen, nicht älter als zwei Monate, und "callows" waren für den, nach der Ablösung der alten, reproduktiven Arbeiterin beobachteten, Anstieg der Aggression verantwortlich. Diese Arbeiterinnen haben eine größere Chance, selbst reproduktiv zu werden, da ältere, fouragierende Arbeiterinnen ihre Ovarien eventuell nicht mehr entwickeln können. Die Aggressionen zwischen älteren Arbeiterinnen nahmen mit zunehmendem Alter ab. Arbeiterinnen, deren Ovarien sich bereits in Entwicklung befinden, stellen die größte Bedrohung für jedes reproduktive Tier dar. Die Sektion aller experimentellen Gruppen ergab, dass Aggression, verglichen mit Tieren mit resorbierten Ovarien, signifikant häufiger gegen Arbeiterinnen gerichtet war, die unentwickelte oder sich bereits entwickelnde Ovarien besaßen. In allen Gruppen wurde die Reproduktion von callows oder jungen Arbeiterinnen, die nicht älter als vier Monate waren, übernommen. Das Alter hat daher eine größere Vorhersagekraft für die reproduktive Dominanz als der soziale, durch physische Interaktionen regulierte, Status. Da zwischen genetisch identischen Nestgenossinen, die durch Thelytokie entstehen, kein offener Konflikt zu erwarten ist, bleibt die Funktion von agonistischen Interaktionen in Nur-Arbeiterinnen-Kolonien von *P. punctata* unklar. So könnte physische Aggression auch zur Schaffung einer reibungslosen nicht-reproduktiven Arbeitsteilung, und damit zur Erhöhung der Kolonieeffizienz, dienen.

Asexuelle Fortpflanzung, im Vergleich zu sexueller Fortpflanzung, wird oft als evolutionäre Sackgasse gesehen, weil in parthenogenetischen Populationen genetische Rekombination limitiert oder nicht-existent ist. Mikrosatelliten-Marker wurden verwendet, um die Konsequenzen thelytoker Fortpflanzung für die genetische Populationsstruktur von vier natürlichen Populationen von *P. punctata* zu untersuchen. In der Analyse von 314 Arbeiterinnen aus 51 Kolonien wurde an allen Loci nur eine geringe intraspezifische Variabilität, sowohl anhand der Anzahl der Allele als auch der beobachteten Heterozygozitäten, entdeckt. Überraschenderweise gab es innerhalb der Populationen fast keine genetischen Unterschiede. Die einzelnen Populationen wiesen eine klonale Struktur auf, in der alle Arbeiterinnen den selben Genotyp besaßen. Der geringe Grad an genotypischer Variabilität spiegelt die Vorherrschaft thelytoker Reproduktion bei *P. punctata* unter natürlichen Bedingungen wieder.

Zusätzlich wurde die Spezifität von zehn, für *P. punctata* entwickelte Dinukleotid-Mikrosatelliten in 29 Ameisenarten aus vier verschiedenen Unterfamilien durch Kreuzamplifikation untersucht. Positive Amplifikation ergab sich nur in wenigen Arten. Selbst innerhalb derselben Gattung sind die, die hypervariablen Regionen flankierenden Sequenzen, nicht ausreichend konserviert, um Amplifikation zuzulassen. Der Karyotyp von *P. punctata* (2n = 84) zeigt eine der höchsten

Chromosomenanzahlen, die bislang bei Ameisen bekannt sind. Eine erste Untersuchung ergab keine Hinweise auf Polyploidie, die oft mit der Entstehung von Parthenogenese verbunden ist.

Thelytoke Parthenogenese ist innerhalb der Hymenopteren kein sehr häufiges Phänomen. Sie tritt nur verstreut auf und ist auf Taxa an den äußersten Verzweigungen der Stammbäume beschränkt. Innerhalb der Formicidae ist Thelytokie zweifelsfrei nur in vier Arten, *P. punctata* eingeschlossen, beschrieben. Ungeachtet der Vorteile können evolutionäre Kosten und Zwänge die schnelle Evolution und zeitliche Persistenz von Thelytokie verhindern. Die Mechanismen thelytoker Parthenogenese und ihre ökologischen Hintergründe werden für die bisher bekannten Fälle innerhalb der Hymenopteren diskutiert. Das Auftreten von sexueller Reproduktion in asexuellen Linien deutet darauf hin, dass Thelytokie nicht irreversible ist. In *P. punctata* kann das gelegentliche Auftreten von Geschlechtstieren dazu dienen, Auszucht und genetische Rekombination zuzulassen. Thelytokie kann daher als eine konditionelle reproduktive Strategie verstanden werden.

Thelytoke Fortpflanzung bei *P. punctata* evolvierte möglicherweise als eine Anpassung an ein hohes Risiko, die Königin zu verlieren oder als Anpassung an die Verbreitung durch Koloniespaltung. Der derzeitige adaptive Wert physischer Aggression und der Geschlechtstierproduktion in klonalen Populationen, die praktisch keine Verwandtschaftsasymmetrien aufweisen, ist dagegen weniger klar. Ganz im Gegenteil, Thelytokie kann als weiterer Schritt auf dem Weg zum entgültigen Verlust der Königinnen Kaste bei *P. punctata* dienen. Obwohl *P. punctata* alle drei Kriterien für Eusozialität erfüllt, wird die Evolution von thelytoker Parthenogenese als erster Schritt in einer sekundären, reversen sozialen Evolution hin zu einem einfacheren sozialen System, als es Eusozialität darstellt, interpretiert.

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10 Abbreviations

ACOH	Aconitate Hydratase
ADH	Alcohol Dehydrogenate
AK	Adenylate Kinase
BLAST	Basic Local Alignment Search Tool
DTT	Dithiothreitol
EDTA	Ethylendiaminetetraacetic Acid
EST	Esterase
GPI	Glucose 6 phosphate Isomerase
HK	Hexokinase
IDH	Isocitrate Dehydrogenase
IPTG	Isopropyl-Thiogalactopyranoside
LDH	Lactate Dehydrogenase
MDH	Malate Dehydrogenase
MDHP	Malate Dehydrogenase ($NADP^+$)
ME	Malic Enzyme
NCBI	National Center for Biotechnology Information
Nonidet	Non-Ionic Detergent
PGDH	Phosphogluconate Dehydrogenase
PGI	Phosphoglucose Isomerase
PGM	Phosphoglucose Mutase
SDS	Sodium Dodecyl Sulfate
SSC	Sodium Sodiumcitrate
TBE	TRIS - Boric Acid - EDTA
TRIS	Trishydroxymethylaminomethane Hydrochloride
XDH	Xanthine Dehydrogenase

Abbreviations of units follow the standard SI-system

11 Annex

a)

Subordinate

Dominant	GRG	RYY	GRR	RGR	BYR	YYY	RRR	YYB	RGB	YBB	CW1	RYR	YRR	YRY	YBG	GYG	RBR	YBY	BBR	YBR	RRY	GBR	GYB	RBB	BRR	GGR	YRB	GRB	YGY	GGY	GPB	YYR	RGY	GYY	RYB	YYG	RBY	YRG	YGR	GYR	RRB	BGB	BRY	BBG	GBB	BYY	CW2	Total	Age
GRG	X	-	17	3	-	-	11	1	8	-	-	-	-	-	-	-	2	-	-	-	-	3	-	3	2	11	1	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	66	C
RYY	-	X	-	-	-	20	-	-	-	-	-	5	24	-	-	-	-	1	-	-	1	-	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	54	C
GRR	4	-	X	-	-	-	-	-	9	-	-	-	-	-	-	-	5	-	-	-	-	1	-	11	5	13	2	3	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	54	C
RGR	3	-	9	X	-	-	7	-	6	-	-	-	-	-	3	-	1	-	1	-	-	-	3	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35	C
BYR	-	-	-	-	X	-	-	-	-	-	-	-	-	3	-	8	-	-	-	1	-	-	-	-	-	-	1	-	1	-	-	1	3	5	2	1	1	1	1	1	-	-	-	-	-	-	-	30	B
YYY	-	-	-	-	-	X	-	-	-	-	-	8	-	3	-	3	-	1	-	-	4	-	-	-	-	-	2	-	1	-	-	-	2	1	-	1	1	-	2	1	-	-	-	-	1	-	31	C
RRR	1	-	6	-	-	-	X	8	1	-	-	-	-	-	-	-	3	-	-	-	-	1	-	-	2	4	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	C
YYB	4	-	6	1	-	-	-	X	2	-	-	-	-	-	-	-	3	-	-	-	-	1	-	3	1	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	26	C
RGB	2	-	2	-	-	-	4	-	X	-	-	-	-	-	-	-	2	-	-	-	-	3	3	2	1	4	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	C
YBB	-	17	-	-	-	1	-	-	-	X	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	C
CW1	-	1	-	-	-	2	-	-	-	3	X	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	24	C
RYR	-	-	-	-	3	1	-	-	-	-	-	X	-	3	-	-	-	2	-	-	3	-	-	-	-	-	-	-	1	1	-	-	3	3	-	-	-	-	-	-	-	1	-	-	-	-	-	21	C
YRR	-	-	-	-	-	-	-	-	-	-	-	2	X	3	-	2	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	1	-	3	-	1	-	-	-	-	-	-	1	-	1	-	-	-	15	C
YRY	-	-	-	-	1	-	-	-	-	-	-	-	-	X	-	3	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	1	-	2	1	-	-	-	-	1	-	1	-	-	-	13	C
YBG	1	-	-	3	-	-	-	-	2	-	-	-	-	-	X	-	2	-	1	-	-	-	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	C
GYG	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	9	B
RBR	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	C
YBY	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	X	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	5	B
BBR	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	1	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	C
YBR	2	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	5	B
RRY	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	C
GBR	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	C
GYB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	X	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	C
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
Total	18	18	40	7	8	24	22	19	29	3	0	16	30	13	3	16	18	5	2	2	10	10	7	21	13	36	16	10	5	2	1	5	8	15	3	5	3	1	3	3	1	2	2	1	1	1	17	495	

b)

Subordinate

Dominant	YBG	RGB	GRG	RGR	GRR	RRR	YYB	BBR	RBB	GBR	GYB	GGR	RBR	GRB	BRR	Total	Age	Ovar
YBG	**X**	6	4	11	3	12	-	2	-	3	4	3	6	-	-	54	C	I
RGB	-	**X**	7	3	7	5	-	2	5	4	8	8	2	1	3	55	B	I
GRG	-	8	**X**	1	3	6	-	2	4	1	1	9	3	4	1	43	C	I
RGR	-	5	3	**X**	-	4	-	3	2	-	-	-	1	2	4	24	C	n.a.
GRR	-	2	3	-	**X**	-	-	-	1	-	-	4	6	3	5	24	C	I
RRR	-	2	2	1	6	**X**	-	-	-	1	1	4	3	1	1	22	C	II
YYB	-	-	1	1	3	1	**X**	1	2	-	1	1	-	-	1	12	B	IV
BBR	-	-	2	1	1	-	1	**X**	-	3	-	1	1	-	-	10	C	I
RBB	-	-	-	-	-		1	-	**X**	-	-	2	-	1	3	7	C	III
GBR	-	-	1	1	-	-	-	1	1	**X**	-	1	-	-	-	5	C	I
GYB	-	-	-	-	-	-	-	-	-	-	**X**	1	-	-	-	1	C	I
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
Total	0	23	23	19	23	28	2	11	15	12	15	34	22	12	18	257		

Annex 1.1 Rank orders observed in experimental group ABS 27/1 a) before and b) after removal of the dominant egg layer GGB based on the number of agonistic interactions initiated. The reproductive individual is underlined. Age refers to the age at the time when the respective observation started. If callow workers eclosed during observations could not be re-identified after the end of the observations, their ovarian condition was assigned n.a.

a)

Dominant \ Subordinate	YRR	GRO	GRB	RGO	BBG	CW1	BBO	GOG	RRO	RBO	BRR	YYG	ROB	YGO	PPP	GGY	GGW	BGR	OBB	BYB	BRG	GBB	OGG	ORG	CW2	RGR	Total	Age
YRR	X	-	1	-	-	7	-	-	-	-	-	24	19	23	-	1	19	-	-	-	-	-	-	-	-	-	94	C
GRO	-	X	-	8	-	-	-	-	1	-	-	-	-	-	-	-	-	6	50	-	-	-	-	-	-	-	65	C
GRB	-	-	X	-	4	-	4	1	-	-	-	-	-	-	-	3	-	-	-	8	2	1	1	-	-	-	24	B
RGO	-	-	-	X	-	-	-	-	4	-	-	-	2	-	1	-	-	6	1	-	-	-	-	1	-	-	15	C
BBG	-	-	-	-	X	-	6	-	-	-	-	-	-	-	-	1	-	1	-	1	2	-	3	-	-	-	14	B
CW1	-	-	-	-	-	X	2	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	6	-	10	C
BBO	-	-	-	-	2	-	X	-	-	-	-	-	-	-	-	1	-	-	-	3	1	-	-	-	-	-	7	C
GOG	-	-	-	-	1	-	-	X	-	-	-	-	-	-	-	1	-	-	-	1	-	-	4	-	-	-	7	C
RRO	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	3	B
RBO	-	-	-	-	-	1	1	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	B
BRR	-	-	-	-	-	-	-	-	-	1	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	B
YYG	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	2	-	-	-	-	-	-	-	-	-	2	C
ROB	-	-	-	-	1	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	1	C
YGO	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	1	-	-	-	-	-	-	-	-	-	-	1	C
PPP	-	-	-	-	-	-	1	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	1	B
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
Total	0	0	1	8	8	8	14	1	5	1	1	24	21	23	2	8	21	16	51	13	5	1	8	1	6	1	248	

b)

Dominant \ Subordinate	YYY	YYG	GRB	GOG	ROB	YGO	CW	GGW	BBG	YRR	BGR	GRO	BBO	BYB	GBB	BRG	BGB	GGY	OGG	PPP	OBB	Total	Age	Ovar
YYY	X	26	1	-	12	3	-	-	1	-	1	1	-	-	-	-	-	-	-	-	-	45	C	II
YYG	6	X	1	-	5	19	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	45	C	II
GRB	-	-	X	11	-	-	-	-	7	-	-	-	7	3	2	4	1	1	1	-	-	37	B	I
GOG	-	-	-	X	-	-	-	-	1	-	-	-	7	2	-	1	4	1	2	1	1	20	B	I
ROB	-	7	-	-	X	9	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	18	C	I
YGO	5	-	-	-	1	X	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	15	C	II
CW	7	3	1	-	3	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	C	n.a.
GGW	-	1	4	-	-	1	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	6	C	II
BBG	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	1	-	-	-	-	-	1	A	II
YRR	-	-	-	-	-	-	-	1	-	X	-	-	-	-	-	-	-	-	-	-	-	1	B	IV
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
Total	18	37	7	11	21	32	0	25	9	0	2	1	14	5	2	6	5	2	3	1	1	202		

Annex 1.2 Rank orders observed in experimental group ABS 8/4 a) before and b) after removal of the dominant egg layer GGR based on the number of agonistic interactions initiated. The reproductive individual is underlined. Age refers to the age at the time when the respective observation started. If callow workers eclosed during observations could not be re-identified after the end of the observations, their ovarian condition was assigned n.a.

a)

Dominant	Subordinate																		Age
	GRO	**GGR**	**BGB**	**GBB**	**BRO**	**GRG**	**BBB**	**RBB**	**RBR**	**RGB**	**RGO**	**RBG**	**BBR**	**GGB**	**GRB**	**GBR**	**BRO**	**GGG**	**Total**	
GRO	X	20	-	-	-	-	-	-	-	1	13	-	-	-	-	-	-	-	34	C
GGR	-	X	1	2	-	-	-	-	-	2	1	2	1	1	1	-	-	-	11	C
BGB	-	-	X	8	-	-	-	-	-	-	-	-	-	-	-	1	-	-	9	C
GBB	-	-	-	X	-	-	-	-	-	-	-	1	-	-	-	1	-	-	2	C
BRO	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	1	-	1	C
GRG	-	-	-	-	-	X	1	-	-	-	-	-	-	-	-	-	-	-	1	A
BBB	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	1	1	A
RBB	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	1	-	1	A
RBR	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	1	1	A
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
Total	0	20	1	10	0	0	1	0	0	3	14	3	1	1	1	2	2	2	61	

b)

Dominant	Subordinate													Age	Ovar
	GGR	**CW**	**ROB**	**RGO**	**GBB**	**BGR**	**BGB**	**GRO**	**RBG**	**BGO**	**GBR**	**BBR**	**Total**		
GGR	X	20	-	54	5	-	6	41	-	1	13	-	140	C	III
CW	-	X	2	8	-	-	-	-	-	-	-	6	16	C	n.a.
ROB	-	-	X	2	-	-	2	1	-	2	1	2	10	B	I
RGO	-	-	-	X	5	-	4	1	-	-	-	-	10	C	II
GBB	7	-	-	-	X	-	-	-	-	-	-	-	7	B	II
BGR	-	-	-	-	-	X	6	-	-	-	-	-	6	B	I
BGB	-	-	-	-	4	-	X	-	-	-	-	-	4	B	II
GRO	-	-	-	-	3	-	-	X	-	-	-	-	3	B	IV
RBG	-	-	-	-	-	-	2	-	X	-	-	-	2	B	I
Others	-	-	-	-	-	-	-	-	-	-	-	-	0		
Total	7	20	2	64	17	0	20	43	0	3	14	8	198		

Annex 1.3 Rank orders observed in experimental group PR 11/1 a) before and b) after removal of the dominant egg layer RRR based on the number of agonistic interactions initiated. The reproductive individual is underlined. Age refers to the age at the time when the respective observation started. If callow workers eclosed during observations could not be re-identified after the end of the observations, their ovarian condition was assigned n.a.

a)

Dominant	Subordinate														Age
	BGG	**CW1**	**GBB**	**RRB**	**RRG**	**BRR**	**BBG**	**BGB**	**BRG**	**BRB**	**CW2**	**RBB**	**RGG**	**Total**	
BGG	**X**	1	15	-	-	-	6	2	1	-	-	-	-	25	B
CW1	3	**X**	3	-	-	-	-	-	-	-	1	-	-	7	C
GBB	-	1	**X**	-	-	-	-	1	-	1	-	-	-	3	A
RRB	-	-	-	**X**	-	-	-	-	-	-	-	1	-	1	B
RRG	-	-	-	-	**X**	1	-	-	-	-	-	-	-	1	A
BRR	-	-	-	-	-	**X**	-	-	-	-	-	-	1	1	B
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
Total	3	2	18	0	0	1	6	3	1	1	1	1	1	38	

b)

Dominant	Subordinate																Age	Ovar
	GGY	**GOB**	**GOG**	**GOR**	**CW**	**OGG**	**YYY**	**RRB**	**GBB**	**BGB**	**GRR**	**BBG**	**BRG**	**BBR**	**YYG**	**Total**		
GGY	**X**	-	33	-	-	-	10	-	3	1	13	-	-	-	-	60	B	I
GOB	15	**X**	2	-	-	33	5	-	-	-	2	1	-	-	-	58	C	I
GOG	-	-	**X**	-	-	-	18	-	1	1	17	-	2	1	-	40	C	I
GOR	2	6	-	**X**	-	8	-	-	-	-	-	-	-	-	-	16	C	II
CW	-	6	1	-	**X**	3	1	-	-	-	-	-	-	-	-	11	C	n.a.
OGG	-	-	-	-	-	**X**	1	-	-	-	1	-	-	-	1	3	C	I
YYY	-	-	-	-	-	-	**X**	-	-	-	-	1	-	-	-	1	B	I
RRB	-	-	-	-	-	-	1	**X**	-	-	-	-	-	-	-	1	A	I
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
Total	17	12	36	0	0	44	36	0	4	2	33	2	2	1	1	190		

Annex 1.4 Rank orders observed in experimental group PR 19/1 a) before and b) after removal of the dominant egg layer GGR based on the number of agonistic interactions initiated. The reproductive individual is underlined. Age refers to the age at the time when the respective observation started. If callow workers eclosed during observations could not be re-identified after the end of the observations, their ovarian condition was assigned n.a.

a)

Dominant	Subordinate															Age
	GBR	**CW**	**RBG**	**RBR**	**GBG**	**BGG**	**RBB**	**BGB**	**RRR**	**GGG**	**BRB**	**RRB**	**RGB**	**RGR**	**Total**	
GBR	**X**	-	30	-	3	2	1	1	1	-	-	-	-	-	38	B
CW	-	**X**	-	3	-	-	-	-	-	2	1	3	-	-	9	C
RBG	-	-	**X**	-	-	-	-	-	-	-	-	-	7	-	7	B
RBR	-	-	-	**X**	-	-	-	-	-	-	-	-	-	5	5	B
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
Total	0	0	30	3	3	2	1	1	1	2	1	3	7	5	59	

b)

Dominant	Subordinate																						Age	Ovar
	BGR	**GYY**	**YYY**	**YYR**	**YRR**	**CW**	**GYG**	**BRG**	**YGY**	**GBO**	**RYY**	**GOG**	**YRY**	**GGY**	**YRG**	**GRO**	**RGO**	**YGG**	**RGY**	**RGB**	**RYG**	**YGR**	**Total**		
BGR	**X**	74	1	15	-	-	-	4	4	1	2	1	2	1	1	-	-	-	-	-	-	-	106	C	I
GYY	-	**X**	1	6	2	-	-	3	7	-	1	-	2	1	1	-	-	-	-	-	-	-	24	C	II
YYY	-	-	**X**	-	6	-	1	-	-	1	-	-	-	-	4	6	2	1	1	2	-	-	24	B	I
YYR	-	-	2	**X**	2	-	-	5	-	1	2	-	2	1	1	1	1	-	-	-	-	-	18	C	I
YRR	-	-	1	-	**X**	-	-	-	-	3	1	2	1	1	-	2	1	-	-	-	1	-	13	B	I
CW	2	2	1	1	1	**X**	-	-	-	-	-	1	1	1	1	-	-	1	-	-	-	1	13	C	I
GYG	-	-	-	1	-	-	**X**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	B	I
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
Total	2	76	6	23	11	0	1	12	11	6	6	4	8	5	8	9	4	2	1	2	1	1	199		

Annex 1.5 Rank orders observed in experimental group PR 2/1 a) before and b) after removal of the dominant egg layer GGR based on the number of agonistic interactions initiated. The reproductive individual is underlined. Age refers to the age at the time when the respective observation started. If callow workers eclosed during observations could not be re-identified after the end of the observations, their ovarian condition was assigned n.a.

a)

Dominant	Subordinate								Age
	GGO	**RRB**	**GPG**	**PGG**	**GRR**	**GBB**	**GRB**	**Total**	
GGO	**X**	-	1	3	4	1	-	9	C
RRB	-	**X**	-	-	-	-	1	1	B
Others	-	-	-	-	-	-	-	0	
Total	0	0	1	3	4	1	1	10	

b)

Dominant	Subordinate																		Age	Ovar
	GBO	**GRO**	**RGO**	**BOG**	**GRR**	**BGR**	**RBR**	**ROG**	**RBG**	**CW**	**BRR**	**RRR**	**GGO**	**RGB**	**GBG**	**BBR**	**RRB**	**Total**		
GBO	**X**	-	40	7	-	-	-	17	-	-	-	-	-	-	-	-	-	64	B	III
GRO	-	**X**	8	20	-	-	-	16	-	-	-	-	-	-	-	-	-	44	B	IV
RGO	1	-	**X**	-	-	-	-	7	-	-	-	-	-	-	-	-	-	8	B	III
BOG	-	-	-	**X**	-	-	-	3	-	-	-	1	1	-	-	-	-	5	B	I
GRR	-	-	-	-	**X**	-	-	-	-	-	-	-	-	1	1	-	-	2	A	III
BGR	-	-	-	-	-	**X**	-	-	-	-	-	-	-	-	-	2	-	2	A	II
RBR	-	-	-	-	-	-	**X**	-	1	-	-	1	-	-	-	-	-	2	A	I
ROG	-	-	-	-	-	-	-	**X**	-	-	-	-	-	1	-	-	-	1	B	I
RBG	-	-	-	-	-	-	-	-	**X**	-	-	1	-	-	-	-	-	1	A	I
CW	-	-	-	-	-	-	-	-	-	**X**	-	-	-	-	-	-	1	1	C	n.a.
BRR	-	-	-	-	-	1	-	-	-	-	**X**	-	-	-	-	-	-	1	A	III
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
Total	1	0	48	27	0	1	0	43	1	0	0	3	1	2	1	2	1	131		

Annex 1.6 Rank orders observed in experimental group PR 21/1 a) before and b) after removal of the dominant egg layers GGB and PGG based on the number of agonistic interactions initiated. The reproductive individual is underlined. Age refers to the age at the time when the respective observation started. If callow workers eclosed during observations could not be re-identified after the end of the observations, their ovarian condition was assigned n.a.

a)

Dominant	Subordinate																				Age
	RYY	**RRO**	**GOR**	**YGR**	**YYR**	**CW1**	**BOB**	**RGO**	**GGY**	**GYR**	**RYR**	**GRY**	**YRG**	**YGO**	**BBO**	**YYY**	**CW2**	**ROG**	**PPP**	**BYY**	**Total**	
RYY	**X**	-	-	-	3	-	9	-	-	14	7	9	1	1	-	-	-	-	-	-	44	C
RRO	-	**X**	20	3	-	-	14	2	-	-	-	-	-	-	1	-	-	-	-	-	40	C
GOR	-	-	**X**	10	5	-	-	12	-	-	-	-	-	1	-	-	-	-	-	-	28	C
YGR	4	-	-	**X**	18	-	-	-	-	2	2	1	-	-	-	-	-	-	-	-	27	B
YYR	3	-	-	-	**X**	-	2	-	-	1	-	-	-	-	-	1	-	-	-	-	7	B
CW1	-	-	-	-	-	**X**	-	-	-	-	-	-	-	-	-	-	4	2	1	-	7	C
BOB	-	-	-	-	-	-	**X**	-	-	-	-	1	-	-	-	-	-	-	-	1	2	B
RGO	-	-	-	1	-	-	-	**X**	-	-	-	-	-	-	-	-	-	-	-	-	1	C
GGY	-	-	-	1	-	-	-	-	**X**	-	-	-	-	-	-	-	-	-	-	-	1	C
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
Total	7	0	20	15	26	0	25	14	0	17	9	11	1	2	1	1	4	2	1	1	157	

b)

Dominant	Subordinate																		Age	Ovar
	RRO	**GOR**	**BOB**	**YGR**	**BGR**	**GRB**	**RGO**	**GRY**	**YYR**	**RYR**	**YGO**	**GYR**	**RYY**	**YRR**	**YRG**	**YRY**	**BRB**	**Total**		
RRO	**X**	18	-	15	-	-	12	4	6	1	1	4	-	-	-	-	-	61	B	I
GOR	1	**X**	-	7	-	-	3	2	3	3	1	4	2	1	1	1	-	29	B	I
BOB	2	1	**X**	-	-	-	-	-	2	-	-	-	-	-	-	-	-	5	B	IV
YGR	-	-	-	**X**	-	-	-	-	1	1	-	-	-	1	-	-	-	3	B	I
BGR	1	-	-	-	**X**	-	-	-	1	-	-	-	-	-	-	-	-	2	C	II
GRB	-	-	-	-	-	**X**	-	-	-	-	-	-	-	-	-	-	1	1	A	I
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
Total	4	19	0	22	0	0	15	6	13	5	2	8	2	2	1	1	1	101		

Annex 1.7 Rank orders observed in experimental group PR 22/1 a) before and b) after removal of the dominant egg layer RBB based on the number of agonistic interactions initiated. The reproductive individual is underlined. Age refers to the age at the time when the respective observation started. If callow workers eclosed during observations could not be re-identified after the end of the observations, their ovarian condition was assigned n.a.

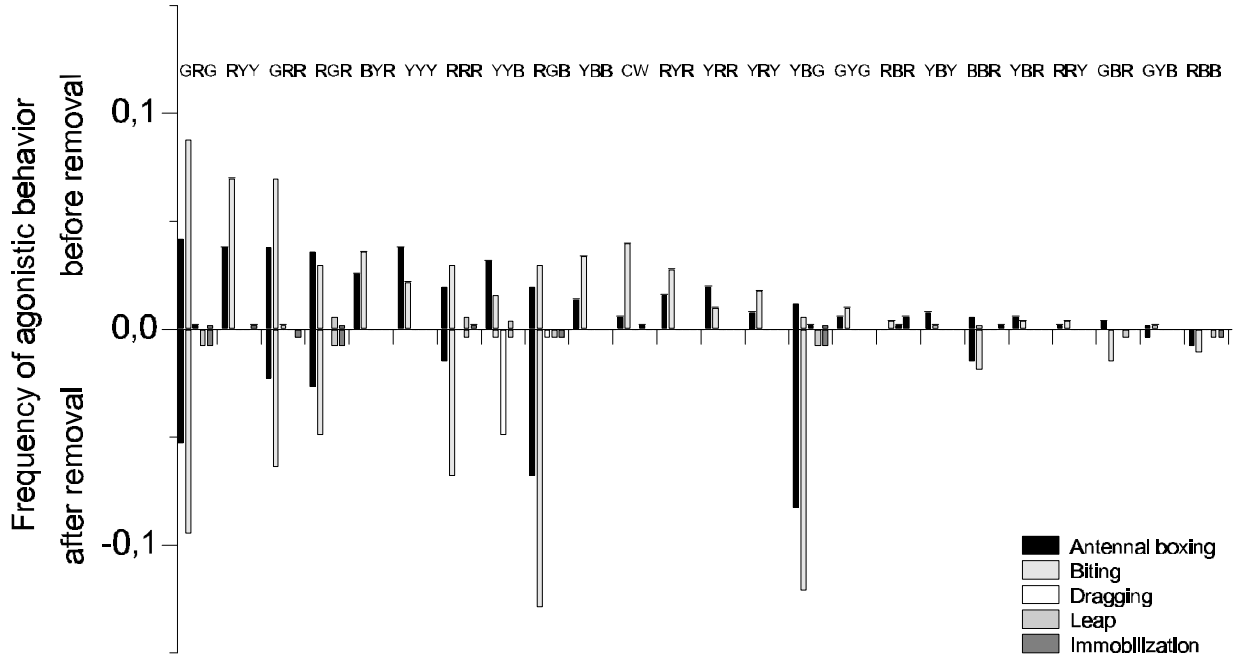
a)

Dominant \ Subordinate	RRB	YGG	BRR	RRO	YRY	YYG	BRB	RBR	RGR	GBG	GYG	CW1	RGG	RYR	CW2	GGB	RRR	CW3	GGR	GRG	RBB	BBR	YYR	YRG	YGR	BGB	GOG	YGY	GGG	RRG	RRY	GGY	BGG	GBB	YRR	Total	Age
RRB	X	-	51	-	-	-	88	20	93	2	-	52	1	-	-	-	-	-	-	-	14	2	-	-	-	2	-	-	-	-	-	-	-	-	-	325	C
YGG	-	X	-	41	21	1	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	36	23	24	-	3	2	-	-	-	-	-	-	-	156	C
BRR	-	-	X	-	-	-	-	4	12	-	-	-	-	-	9	16	-	-	-	-	-	1	-	-	-	-	-	-	1	2	-	-	-	-	-	45	C
RRO	-	-	-	X	6	1	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	4	4	13	-	1	1	-	-	-	-	-	-	-	33	C
YRY	-	-	-	-	X	1	-	-	-	-	11	-	-	1	-	1	-	-	-	-	-	-	-	1	2	-	3	3	-	-	1	-	-	-	-	24	C
YYG	-	-	-	-	-	X	-	-	-	-	-	-	-	2	-	1	-	-	-	-	-	-	-	-	-	-	6	5	-	-	6	1	-	-	-	21	C
BRB	-	-	2	-	-	-	X	5	-	-	-	-	4	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	18	B
RBR	2	-	-	-	-	-	-	X	-	3	-	-	3	-	-	5	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	15	C
RGR	-	-	1	-	-	-	-	-	X	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	1	2	-	-	1	-	-	7	A
GBG	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	2	-	-	-	1	-	-	-	1	-	5	B
GYG	-	-	-	-	-	2	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	5	C
CW1	-	-	1	-	-	-	-	-	3	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	C
RGG	-	-	-	-	-	-	-	-	-	1	-	-	X	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	3	A
RYR	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	1	3	C
CW2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	C
GGB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	B
RRR	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	B
CW3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	C
GGR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	A
GRG	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	B
RBB	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	B
BBR	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	1	B
YYR	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	1	C
YRG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	1	-	-	-	-	-	-	-	1	C
YGR	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	1	C
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	
Total	3	1	56	41	27	5	88	29	108	8	20	52	8	3	9	24	2	2	1	0	15	11	40	28	39	8	14	13	3	6	7	1	1	2	1	676	

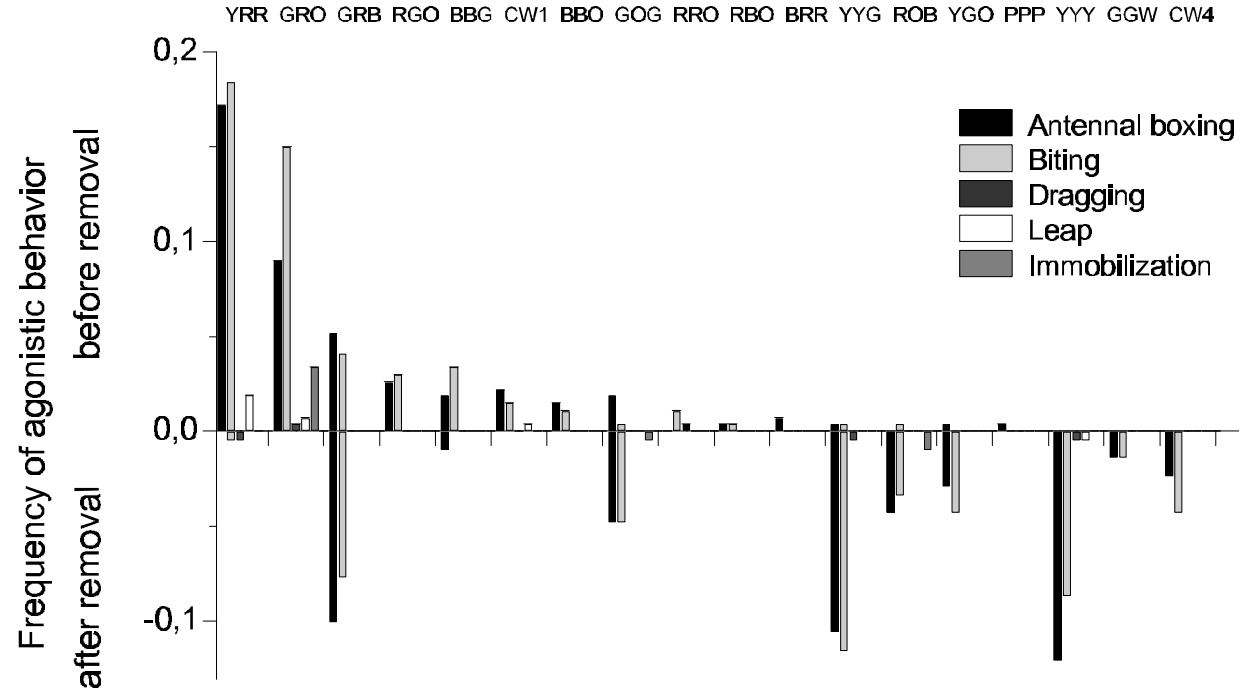
b)

Dominant \ Subordinate	RRO	YRY	YYG	GYO	RYR	YRG	YGG	GYY	RRB	YGY	YGR	RRY	YYR	GOG	YYY	BOB	YRR	Total	Age	Ovar
RRO	X	49	18	17	4	22	1	-	-	9	27	4	35	3	1	2	-	192	B	II
YRY	-	X	5	10	2	34	-	1	-	4	38	-	37	2	-	1	-	134	C	II
YYG	1	5	X	3	1	15	-	-	-	5	8	2	7	1	-	-	-	48	B	IV
GYO	-	-	4	X	1	5	1	-	-	2	2	2	1	2	-	1	1	22	C	I
RYR	1	-	1	-	X	-	-	-	-	-	-	-	3	1	1	1	-	8	B	I
YRG	-	-	1	-	-	X	-	-	-	1	-	-	-	1	-	1	-	4	C	II
YGG	1	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	1	B	n.a.
GYY	-	-	-	-	-	-	-	X	-	-	-	-	-	1	-	-	-	1	B	n.a.
RRB	-	1	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	1	A	n.a.
Others	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
Total	3	55	29	30	8	76	2	1	0	21	75	8	83	11	2	6	1	411		

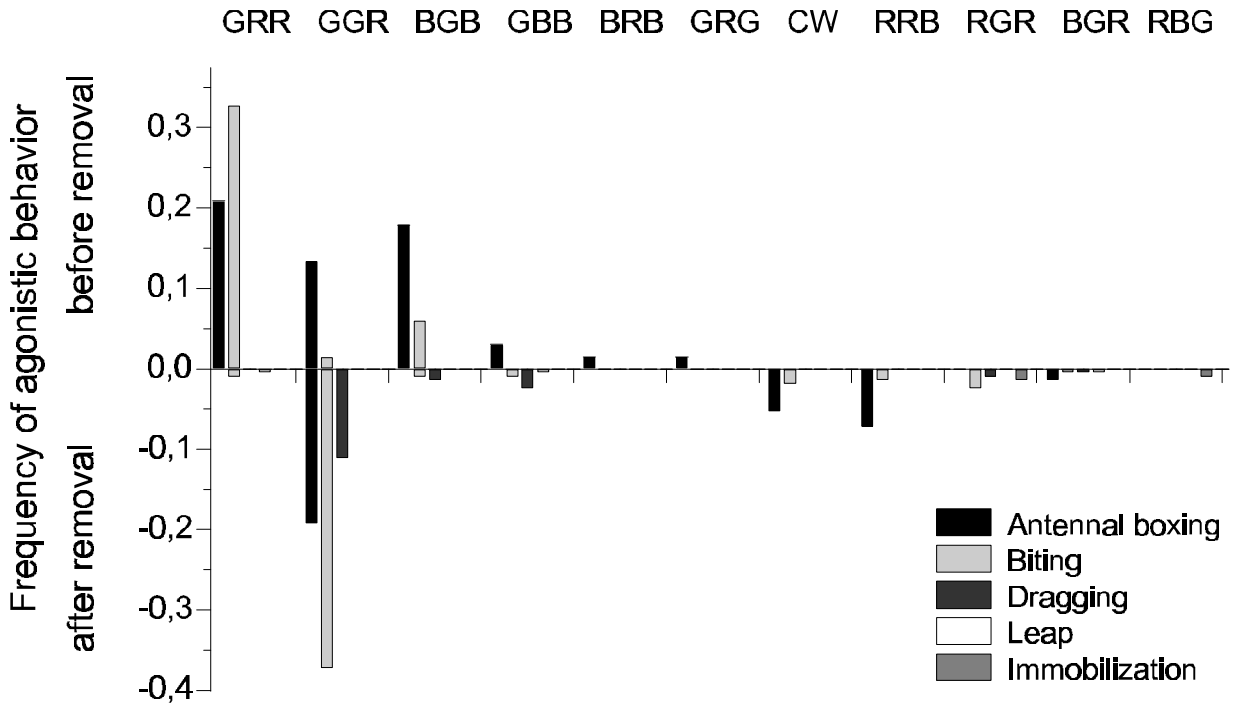
Annex 1.8 Rank orders observed in experimental group PR 4/2 a) before and b) after removal of the dominant egg layer GRR based on the number of agonistic interactions initiated. The reproductive individual is underlined. Age refers to the age at the time when the respective observation started. If callow workers eclosed during observations could not be re-identified after the end of the observations, their ovarian condition was assigned n.a.



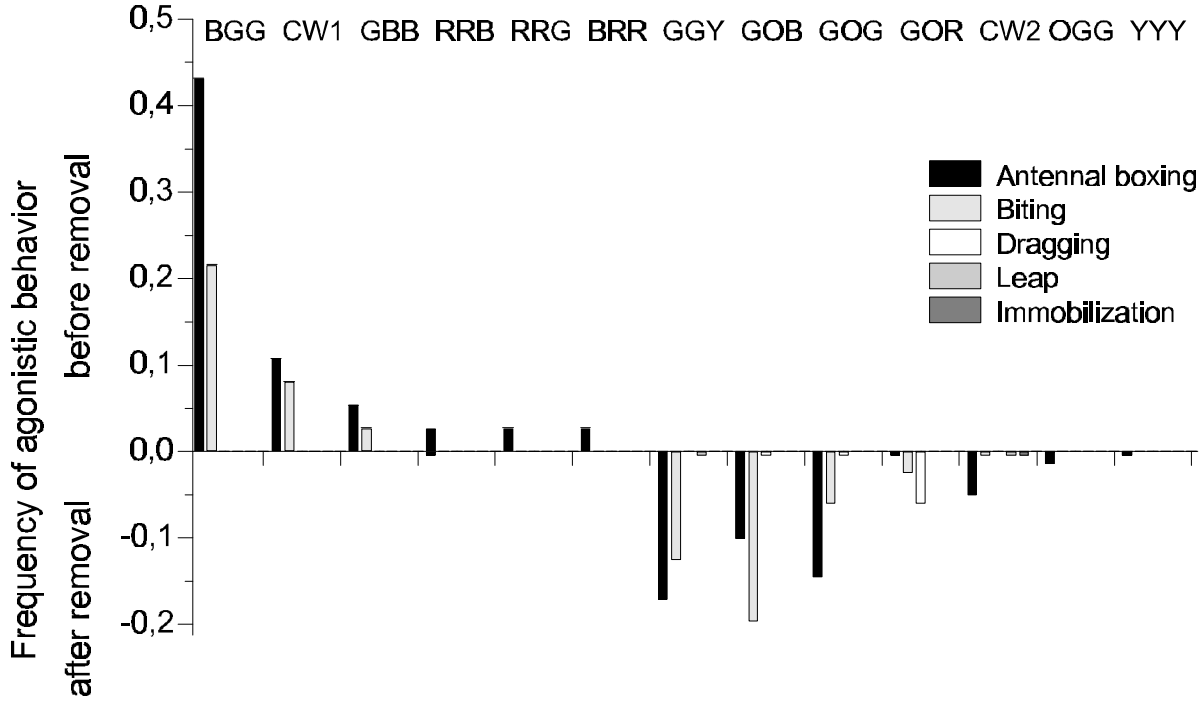
Annex 2.1 Individual aggressive profiles for 24 workers in experimental group ABS 27/1 before and after removal of the reproductive individual GGB. Only individuals that initiated agonistic dyads are included.



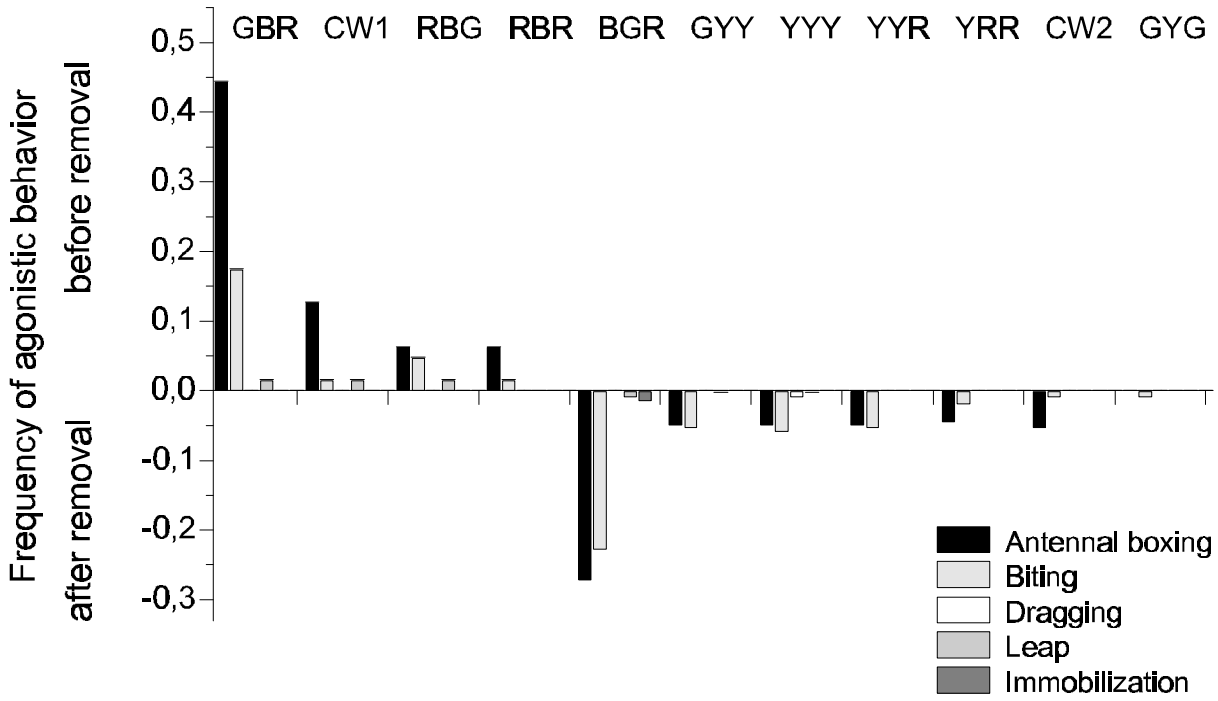
Annex 2.2 Individual aggressive profiles for 18 workers in experimental group ABS 8/4 before and after removal of the reproductive individual GGR. Only individuals that initiated agonistic dyads are included.



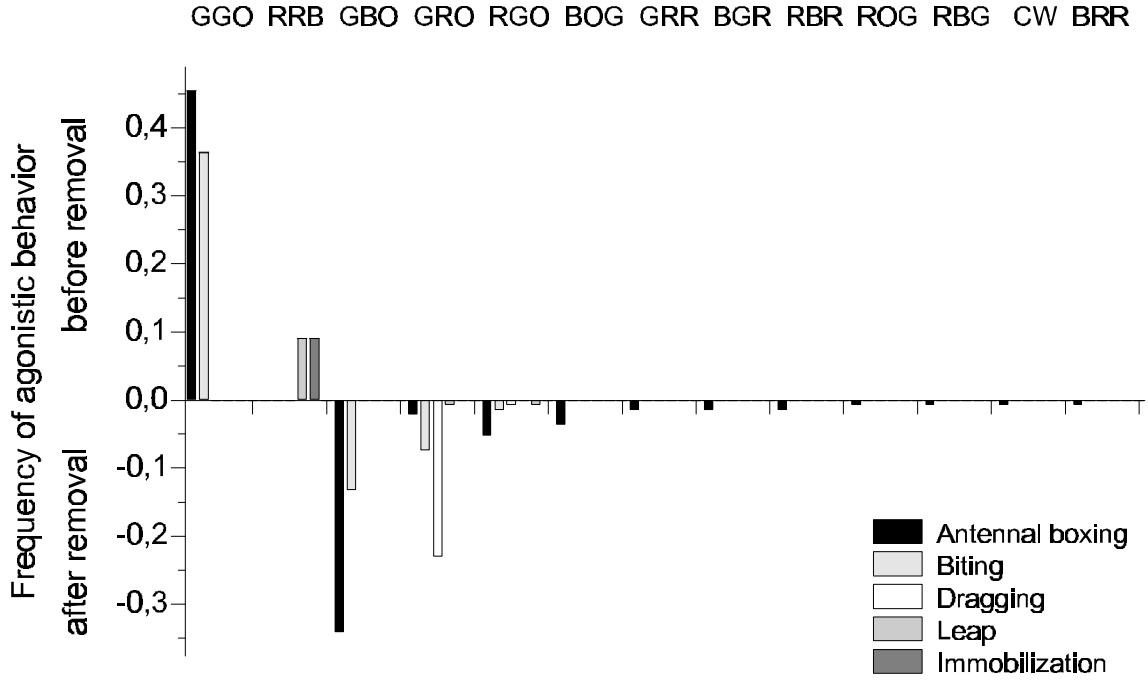
Annex 2.3 Individual aggressive profiles for 11 workers in experimental group PR 11/1 before and after removal of the reproductive individual RRR. Only individuals that initiated agonistic dyads are included.



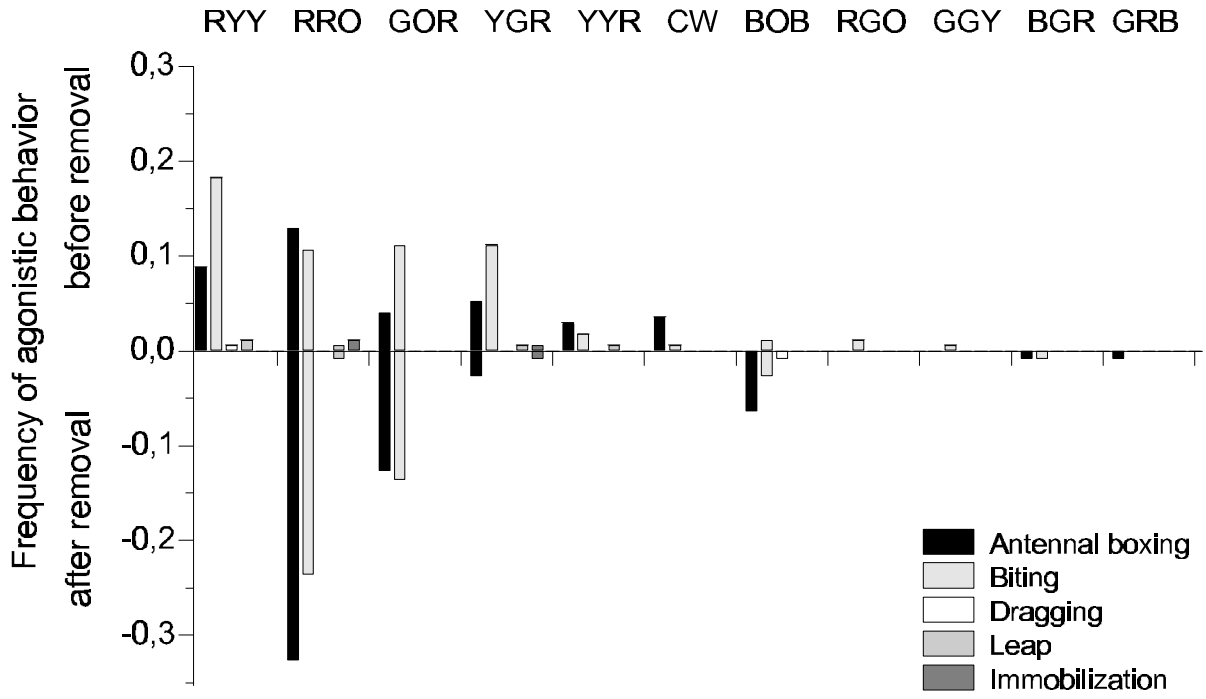
Annex 2.4 Individual aggressive profiles for 13 workers in experimental group PR 19/1 before and after removal of the reproductive individual GGR. Only individuals that initiated agonistic dyads are included.



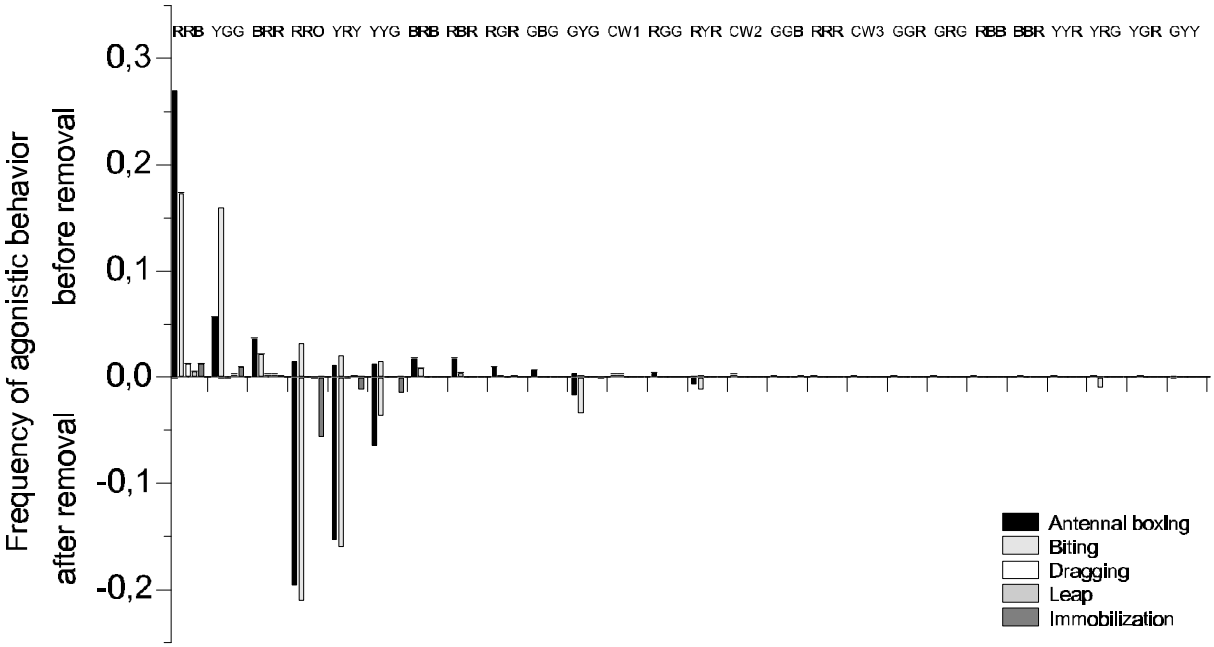
Annex 2.5 Individual aggressive profiles for 11 workers in experimental group PR 2/1 before and after removal of the reproductive individual GGR. Only individuals that initiated agonistic dyads are included.



Annex 2.6 Individual aggressive profiles for 13 workers in experimental group PR 21/1 before and after removal of the reproductive individuals GGB and PGG. Only individuals that initiated agonistic dyads are included.



Annex 2.7 Individual aggressive profiles for 11 workers in experimental group PR 22/1 before and after removal of the reproductive individual RBB. Only individuals that initiated agonistic dyads are included.



Annex 2.8 Individual aggressive profiles for 26 workers in experimental group PR 4/2 before and after removal of the reproductive individual GRR. Only individuals that initiated agonistic dyads are included.

12 Publications

- **Reviewed journal articles**

Schilder, K., J. Heinze and B. Hölldobler, 1999. Colony structure and reproduction in the thelytokous parthenogenetic ant *Platythyrea punctata* (F. Smith) (Hymenoptera; Formicidae). *Insectes Soc*. 46: 150-158.

Schilder, K., J. Heinze, R. Gross and B. Hölldobler, 1999. Microsatellites reveal clonal structure of populations of the thelytokous ant *Platythyrea punctata* (F. Smith) (Hymenoptera; Formicidae). *Mol. Ecol.* 8: 1497-1507.

Braude, S., K. Schilder and J. Muli, 1999. Interspecific interactions between ants and naked mole-rats. *Afr. J. Ecol.*, 37: 242-246.

- **Conference participation**

Schilder, K., J. Heinze and B. Hölldobler, 1995. Colony and population structure in the thelytokous parthenogenetic ant *Platythyrea punctata* (F. Smith) (Hymenoptera; Formicidae). *Verh. Dtsch. Zool. Ges.* 88 (1): p 47.

Schilder, K., J. Heinze and B. Hölldobler, 1995. Koloniestruktur der thelytok parthenogenetischen Ameise *Platythyrea punctata* (F. Smith) (Hymenoptera; Formicidae). 15. Tagung der Internationalen Union zum Studium Sozialer Insekten IUSSI (27. - 31.08.95), Utrecht: p 84.

Schilder, K., J. Heinze and B. Hölldobler, 1997. Reproductive tactics in the thelytokous parthenogenetic ant *Platythyrea punctata* (F. Smith) (Hymenoptera; Formicidae). K. Crailsheim and A. Stabentheiner (eds.), 16. Tagung der Internationalen Union zum Studium sozialer Insekten IUSSI (17. - 21.08.97), Schloß Seggau bei Graz: p 81.

Schilder, K., J. Heinze and B. Hölldobler, 1999. Reproductive organization and population-genetic structure in the thelytokous parthenogenetic ant *Platythyrea punctata* (F. Smith) (Hymenoptera; Formicidae). 13. Congress of the International Union for the Study of Social Insects (29.12.98 - 03.01.99), University of Adelaide, Australia.

13 Curriculum Vitae

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	Dissertation „Safer without Sex - Thelytokous Parthenogenesis and Regulation of Reproduction in the Ant *Platythyrea punctata* (F. Smith)“