Reproductive conflict among workers of the ant species *Pseudomyrmex gracilis* (Hymenoptera: Formicidae)

Universität Regensburg

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Für Simone

“Under carefully controlled experimental conditions, an animal will behave as it damned well pleases.”

Harvard Law of Animal Behaviour

Contents

1. Introduction

1.1 Inter- and intraspecific conflicts

From a gene-centred point of view, conflicts among organisms arise from the (unconscious) “striving” of each individual to maximise the propagation of its genes (Dawkins 1976). This “interest” collides with that of other, genetically different living beings.

Interspecific conflicts encompass cases with opposing requirements (e.g. predator-prey relationships and parasitism where need for nutrition stands against need for survival or health) as well as struggles for the same, non-social resources, e.g. food or shelter.

Food competition is a frequent phenomenon and occurs, for example, between cheetahs and lions which depend on the same prey as well as among different ant species competing for the same food resources, such as Homopteran honeydew (Blüthgen et al. 2000). Competition for shelter is, for example, known to occur between introduced and native crayfish species (Vorburger & Ribi 1999).

In contrast, conflicts within species are mostly fuelled by largely overlapping requirements of conspecific individuals with common physiology and sexual interests. Thus, intraspecific conflicts are dominated by contest concerning not only foraging and protection but also social resources such as mating partners.

Some animal species exhibit cooperative brood care, e.g. in birds (Arnold & Owens 1998), mammals (Jennions & Macdonald 1994) and social insects (see next section). In such cases, this social service, as a common good, is prone to overexploitation by selfishly acting individuals, leading to a so-called “tragedy of the commons” (Rankin et al. 2007). If every group member raises its reproduction at the cost of other necessary activities (e.g. brood care, defence), only few or even none of the offspring may survive in the end. Hence, natural selection should favour mechanisms that regulate the contingents of reproduction and other actions within the community (Wenseleers et al. 2003; Wenseleers & Ratnieks 2004; Gilbert et al. 2007). Under such

conditions, the individual share of the limited total reproductive output of the group is also subject to competition.

1.2 Eusociality – cooperation and conflict

Various advantages of group living (e.g. better defence against predators or more effective foraging and brood rearing) have led to numerous more or less complexly structured communities – from loose temporal hunting associations or swarms over more tightly bonded groups with varying interindividual relationships to highly developed social systems such as the human society and eusocial insect states. Eusociality is traditionally defined by three criteria: (i) reproductive partition of labour; (ii) cooperative brood care; (iii) overlapping generations (Wilson 1971).

In the animal kingdom, eusociality is known from naked mole rats (Alexander et al. 1991), sponge-dwelling shrimps (Duffy et al. 2000), ambrosia beetles (Kent & Simpson 1992), gall-making aphids (Stern & Foster 1996) and thrips (Crespi & Mound 1997), termites (Thorne 1997) and (aculeate) Hymenoptera. The latter two insect orders comprise the vast majority of known eusocial species, including ants. Within the Hymenoptera, eusociality developed at least seven times independently (Wilson & Hölldobler 2005).

Eusocial insects are a prime example for cooperation, having led to classifying colonies as new units of selection (Bourke & Franks 1995) and to scientists establishing the term “superorganism” (Hölldobler & Wilson 2009). Hamilton’s (1964) inclusive fitness theory provides a widely accepted ultimate explanation for this phenomenon but, at the same time, predicts conflicts among genetically different members of a social group (Ratnieks et al. 2006). This is the basic statement of kin conflict theory. Depending on individual reproductive capabilities, caste system and kin structure, such conflicts revolve, for example, around sex investment ratio, caste fate or male production (Ratnieks et al. 2006).

In the following, terms as “social insects“ or “social Hymenoptera” comprise mainly (although not exclusively) eusocial species that can be compared with the study organism of this thesis.

1.3 Conflicts over reproduction in social Hymenoptera

In most social Hymenopteran species, potential for conflict over male production exists in any colony with more than one possible egg layer, be it mated or not. This is due to the haplodiploid genetic system of the Hymenoptera (Bourke & Franks 1995) – leading to asymmetrical kin relationships – and the widespread capability of unmated females (including workers) to lay unfertilised eggs that develop into males (Bourke 1988; Choe 1989; Frumhoff & Ward 1992). Consequently, each female individual is more closely related to its own offspring (life-for-life relatedness coefficient $r = 0.5$, Bourke & Franks 1995) than to any other males in the population (except for clonal societies), be it a brother ($r = 0.25$) or a nephew ($r = 0.375$), the son of a full sister ($r = 0.75$). As long as there are no drawbacks, each female should thus aim to be the exclusive male producer.

Besides the sole potential for conflict among all females within a colony, kin conflict theory also makes predictions about coalitions among individuals with partly common "interests" and about methods of conflict resolution. Applied to male production, this means: if relatedness among female workers is below 0.5, e.g. due to synchronous coexistence of multiple patrilines (in monandrous colonies headed by a multiply mated queen), each female should favour the queen's male offspring (her brothers, $r = 0.25$) over the progeny of other workers (average $r < 0.25$). This can result in a conflict resolution mechanism called 'worker policing', i.e. mutual prevention of reproduction among workers, either through egg eating or aggression against workers with developed ovaries (Ratnieks 1988; Heinze 2004; Wenseleers et al. 2004). Worker policing, in turn, may favour self restraint, i.e. workers refraining from reproduction, because worker policing reduces the pay-off of ovarian development (Wenseleers et al. 2004; Wenseleers & Ratnieks 2006).

If, in contrary, a colony is monogynous (single queen, see Hölldobler & Wilson (1977) for terminology) and monandrous (single patriline), relatedness values alone would predict workers to tolerate each other's reproduction because their full nephews ($r = 0.375$) are genetically more valuable than brothers ($r = 0.25$) (Whitfield 2002). So, unless the queen is able to physically enforce worker altruism ('queen policing'; Oster & Wilson 1978; Ratnieks 1988), all or at least a high proportion of the male offspring

of a colony are expected to be produced by workers (‘relatedness hypothesis’; Hammond & Keller 2004). However, empirical data on worker reproduction in queenright colonies is ambiguous and colony-level efficiency might overrule relatedness, resulting in worker policing and self-restraint even under monogynous, monandrous conditions (‘efficiency hypothesis’; Hammond & Keller 2004).

In case that a colony is hopelessly orphaned, e.g. because of queen death and lack of replacement sexuals, theory predicts that workers, if capable, produce males as a last resort before the colony dies (Bourke 1988; Choe 1989). However, due to efficiency reasons, reproduction should be monopolised by only a fraction of the worker force (or else the produced male brood could not be reared), again revealing the omnipresent conflict among all females. For deciding which individuals take the role of the former queen, workers of various social insect species establish dominance hierarchies through aggressive behaviour (e.g. Heinze 1996; Heinze et al. 1996, 1997; Blatrix & Herbers 2004).

While the relationship between dominance success (or rank) and reproductive status has been examined in many studies (see references above), the dynamics of hierarchy formation are less well understood. This may partly be due to the lack of appropriate analytical tools. Usual methods for analysing dominance rely on interaction matrices (such as those presented in the Appendix, sections 7.2 and 7.3) which require a certain minimum of content to allow confident conclusions (Neumann et al. 2011). Moreover, they are influenced by (i.e., not independent of) the number of interacting individuals which may vary over time (Neumann et al. 2011).

Therefore, especially in cases with few observations, more or less large observation periods have to be pooled, thus greatly reducing temporal resolution of the data. Such pooling may be impeded by fluctuation of group composition, further complicating dynamics analysis. Music notation graphs as introduced by Chase (2006) may be helpful in some cases but allow mainly qualitative examination and can be rather confusing when applied to large datasets with numerous interactions and/or individuals.

Recently, the so-called ‘Elo rating’ – developed by Elo (1961, 1978) for rating chess players and suggested for application to animal social relationships by Albers & de

Vries (2001) – was promoted as a candidate that might be able to overcome these difficulties (Neumann et al. 2011). However, to the knowledge of the author, this type of dominance analysis has up to date not been employed in ants to elucidate the dynamics of social hierarchy formation.

Finally, as a further mechanism of conflict resolution, reproducing individuals are hypothesised to chemically advertise their fertility and/or dominance (‘fertility signalling’; Keller & Nonacs 1993 Cuvillier-Hot et al. 2004; Monnin 2006). The possibility to assess the productivity of an established egg layer with the help of an honest signal (Keller & Nonacs 1993) enables workers to decide for the most adaptive action (e.g. self-restrain and support a productive egg layer for maximising colony output, or struggle for dominance if the signal is gone). So far, some – mostly correlative – evidence for cuticular hydrocarbons (CHCs) to act as fertility signals has been accumulated in five subfamilies of ants, polistine and vespine wasps, and bumblebees (Monnin 2006).

1.4 Aims of the present study

Although the past decade has seen a wealth of studies on the conflicts described above, some hypotheses are still debated (e.g. relatedness against efficiency hypothesis). Moreover, the empirical basis of kin conflict theory is taxonomically patchy, with most studied species aggregating in only a small number of Hymenopteran clades (e.g. *Apis*, *Bombus*, *Vespula*, *Vespa*; among ants: mainly Formicoxenini and Ponerinae). If ignored, such taxonomic bias may lead to erroneous conclusions (Hammond & Keller 2004), and in any case it allows only cautious generalisations.

For these reasons, a comprehensive study on kin conflict was conducted in the ant species *Pseudomyrmex gracilis*. It belongs to the subfamily Pseudomyrmecinae which has, despite its species richness (200 species; Ward & Downie 2005), caught little attention by sociobiologists. Colonies of this widespread neotropical species (Wetterer 2010) usually inhabit dead twigs (Ward 1993), appear to be frequently polydomous, i.e. they occupy more than one nest site (Clement 2005; personal observation), and are mostly headed by a single queen (Clement 2005; Kautz et al. 2009; at most "occasionally weakly polygynous", P. Ward, personal communication), whereas the mating frequency of queens has been unknown so far.

Specifically, this study aimed to answer the following questions:

1) How are colonies of *P. gracilis* genetically and socially structured?
2) Do workers reproduce under queenright or queenless conditions?
3) Do workers establish dominance hierarchies when orphaned, with dominant individuals monopolising reproduction?
4) How – in particular how fast – do dominance hierarchies develop?
5) Do workers in queenright colonies police against reproducing nest mates?
6) Can fertility, measured as ovary size, be assessed by the CHC profile of an individual?

These questions were examined (i) by scoring microsatellite markers, (ii) by conducting manipulative behavioural experiments and observations followed by dominance analyses using both a matrix-based dominance index and the Elo rating method, (iii) and by extracting and quantifying CHCs from experimental individuals.

2. Material and Methods

2.1 Microsatellite primer establishment

For genetic analyses, microsatellites were used as markers. Since there were no previously established microsatellites for *Pseudomyrmex gracilis* and the only published primers for a congeneric species (*P. pallidus*, Peters 1997) did not perform sufficiently well in preliminary tests, new primers were developed.

For this purpose, specimens were collected from dead twigs in Southern Brazil and from swollen-thorn acacias in Southern Mexico in 2007, and stored in 96% ethanol for transportation. DNA was extracted from 36 workers (six colonies), following a

modified cetyltrimethyl ammonium bromide (CTAB) protocol (Sambrook & Russell 2001), and pooled. Genomic DNA was restricted with Tsp 509 I (New England Biolabs) and ligated using two adaptors (MWG Biotech; Tsp AD short and Tsp AD long, Tenzer et al. 1999). After purification (Ultrafree-4 spinning columns, Millipore), fragments were amplified (32 polymerase chain reactions (PCR), 25 µl each) containing 0.5 µl restricted and ligated product, 1.25 Units Taq DNA polymerase (MBI Fermentas), 1 µM Tsp AD short, 1× Taq buffer (containing 100 mM Tris-HCl pH8.8, 500 mM KCl, 0.8% Noidet P40; MBI Fermentas), 1.5 mM $MgCl_2$ (Fermentas), and 250 µM of each dNTP (Fermentas). Thermal cycling was performed in a TGradient Thermocycler (Whatman-Biometra): 20 cycles of 93 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, preceded by 72 °C for 5 min to synthesize the nick between the linker and the genomic DNA, and a final elongation at 72 °C for 10 min.

To enrich repeat motifs, $(GA)_{13}$ biotinylated probes were linked to streptavidin-coated magnetic beads (Dynabeads® M-280m Streptavidin; Dynal) and probes were subsequently hybridised to the DNA. Hybridisation and washing were carried out following Tenzer et al. (1999). Enriched DNA was recovered from the beads and amplified again using the same settings as before but without the initial extension step. The PCR was directly performed with 1 µl of bead solution as template.

Subsequently, PCR fragments were cloned using the TOPO-TA Cloning Kit (Invitrogen) following the manufacturer's protocol. White colonies (*n* = 159) were identified and dot-blotted on nylon membranes (Hybond™-N+Amersham). These clones then were probed with $(GA)_{13}$ oligonucleotide labelled with fluorescein (MWG Biotech) and detected by Gene Images CDP-Star detection module (Amersham Life Science). Out of 129 positive clones, 83 were sequenced using the BigDye™ Cycle Sequencing version 1.1 Ready Reaction Kit (PE Biosystems) and T7 or M13 reverse primers (MWG Biotech). Samples were run on an ABI PRISM® 310 Genetic Analyser used with a 310 Genetic Analyses Capillary 47 cm and POP4-Polymer (PE Biosystems). Sequences were assembled and edited in Sequencing Analysis 3.4.1 (PE Biosystems) and visually checked for microsatellites. Repeat motifs were identified in 64 clones; primers were designed manually and tested for 46 loci.

PCR amplification was performed in 20-µl reactions containing 1-50 ng DNA template, 0.5 Units Taq polymerase (Fermentas), 0.5 µM of each forward and reverse primer (MWG Biotech), 1× Taq buffer (Fermentas, content see above), 1× Enhancer (PEQLAB), 2 mM $MgCl_2$, 250 µM of each dNTP (Fermentas) using a T-Gradient Thermocycler (Whatman-Biometra). Conditions were 4 min at 94 °C, 35 cycles of 1 min 15 s at 95 °C, 1 min at 55 °C, 45 s at 72 °C and 10 min at 72 °C. In cases of successful PCR with four individuals, the PCR was repeated with the forward primer 5′-labelled with 6-FAM, TET or HEX (MWG Biotech).

The labelled products were diluted with water, mixed with Genescan-500 (Tamra) size standard and scored on an ABI PRISM® 310 Genetic Analyser used with a 310 Genetic Analyses Capillary 47 cm and POP4-Polymer. Loci were genotyped using GeneScan® 3.1 (PE Biosystems). To assess variability of microsatellites, DNA was extracted from individual ants from one population (17°06′ N, 094°55′ W, South Mexico). Each primer pair was tested on 9-32 workers.

Sixteen primer pairs were flanking polymorphic loci that comprised two to 20 alleles (Table 1). Observed and expected heterozygosities and exact Hardy-Weinberg probability tests (using the Markov chain method with default parameters) were calculated with the GenePop software (Raymond & Rousset 1995). No significant deviations between expected and observed heterozygosities were detected. Using the software Micro-Checker (Shipley 2003), no evidence for null alleles was found. No linkage disequilibrium between the loci was detected based on Fisher’s exact test as implemented in the online version of the GenePop software (Raymond & Rousset 1995).

Table 1 Primer sequences and characteristics of 16 microsatellite loci for *P. gracilis*. Forward primers (the first primers given) were 5′-fluorescent labelled in PCRs. The repeat motif is given for the cloned allele. Size range for loci Pg10-16 resulted from additional data of another test population that was not used for calculating heterozygosity. GB: GenBank; T_a: annealing temperature; *n*: number of individuals screened; *A*: observed number of alleles; H_E: expected heterozygosity; H_O: observed heterozygosity.

Locus	GB accession	Primer sequence	Repeat motif	T_a (°C)	Size range (bp)	*n*	*A*	H_E	H_O
Pg01	FJ463656	5' TET-CGGTCCGCCATTCACATAGTA GCATGGAAACGTTTTCCTATG	$(GA)_{12}G(GA)_{22}A_2(GA)_2G_2AG$	55	141-160	32	8	0.77	0.78
Pg02	FJ463657	5' 6-FAM-ATTCCCCACTTCTCTTATTAAA ACAGAAATAATACTGGAGTGG	$(GA)_3GTA_4(GA)_{28}$	52.5	91-109	32	10	0.80	0.69
Pg03	FJ463658	5' HEX-AACTGCGCGCACTTGGATATC ATCCCAATGGAAGTTAATCGG	$(GA)_2A(GA)_9A_2(GA)_{28}A(GA)_6$ $A(GA)_7A_2(GA)_3A_2(GA)_2A_2(GA)_2$	52.5	115-137	32	7	0.62	0.53
Pg04	FJ463659	5' HEX-GCCTCCGTTTATCCAGAAGTT CGACAGTCGTCGGTACAAAGC	$GACA(GA)_2CA(GA)_7G_4(GA)_{19}$ $AGAG_2A_3$	47.5	129-164	32	4	0.69	0.72
Pg05	FJ463660	5' TET-ATCTACACGCGATCGCGATGC ATGGTTTACACTGTAGGTTTC	$AGA_3(GA)_{10}G_3AG_3A_4G(GA)_3A_2$	50	111-113	32	2	0.25	0.28
Pg06	FJ463661	5' HEX-GATACACGGGTTGGTCTG AAGATTGGCCTTCAACATTGC	$(GA)_{20}A_2(GA)_7G$	55	77-142	32	20	0.92	0.88
Pg07	FJ463662	5' 6-FAM-TCTACAAATGACATTCCTGAT AATACCCTTACACGGATGGTC	$GA_3(GA)_{33}A_2(GA)_2A_2(GA)_3$	52.5	104-174	32	13	0.87	0.88
Pg08	FJ463663	5' HEX-ATATCGAAATGTCCATGCACG AATTACGTCAACATTCTAAAC	$(GA)_{25}A_2G$	55	77-102	32	5	0.77	0.90
Pg09	FJ463664	5' 6-FAM-CTTGCTTCGAGAATATACAGC TTAAATATCAAGGGGTTTCGC	$(GA)_{20}G_2A_2(GA)_7A_2(GA)_2$ CAG_2 A_2GA	55	86-147	32	13	0.74	0.72
Pg10	FJ463665	TGGCGCTCTGCTCTGGATGTT GGCGGAATAGAAAGATGGGTG	$(GA)_{27}$	55	73-109	11	2	0.42	0.55

Table 1 continued.

Locus	GB accession	Primer sequence	Repeat motif	T_a (°C)	Size range (bp)	*n*	*A*	H_E	H_O
Pg11	FJ463666	TGTGCTAAGTGTACATACATT ACAGTACAAGACAAACGTAAG	$GAG_2A_4(GA)_{18}A_2(GA)_2A_2GA_5(GA)_4G_3$ $A_5GA_3(GA)_8A_2(GA)_5A_2(GA)_6A_2(GA)_4$	52.5	96-116	12	4	0.31	0.33
Pg12	FJ463667	TCGAAAATCTGTGATTATATC ATTTGCCCGCGCAAAGTATCA	$(GA)_6G_2(GA)_{15}A_2(GA)_{15}G_3AG$	55	95-173	13	9	0.90	0.92
Pg13	FJ463668	AACTCGTTAGTTGCACGTTAG GAAAGTTTTCACTTTAGTAAA	$A(GA)_{38}A_4(GA)_4$	50	116-192	12	1	-	-
Pg14	FJ463669	GTCAATCGGTATACTCACGTC TGGTATAGCAAATGTTTGTTA	$AGA_2(GA)_{14}A_4GA_3(GA)_8A_2(GA)_6$ $A_2(GA)_9$	50	186-208	12	2	0.43	0.42
Pg15	FJ463670	TACAAGTTAACGCGATGTCGC GAAAACGATCTTTTTATCGAA	$(GA)_6A_2(GA)_{15}A_2(GA)_6A_2(GA)_2A_2$ $(GA)_8A_2(GA)_9A_2GA_3(GA)_2$	52.5	183-281	11	6	0.82	0.90
Pg16	FJ463671	GAACTTGCTCAAACTGATACT TAGGTCGGTGATCTTTGAGAA	$A(GA)_4A_2(GA)_{19}A_2(GA)_3G_2A_2(GA)_5$ $A_2(GA)_2G$	47.5	97-161	9	5	0.75	0.89

2.2 Colony sampling and maintenance

Colonies for Experiments 1 and 3 were collected from October 2008 to January 2009 at Joaquina beach (27°37’37” S, 48°26’59” W), Florianópolis, SC, southern Brazil, by breaking up twigs and, to a large extent, the conspicuous withered infructescence stems of the bromeliad species *Vriesea friburgensis* (Figure 1) where colonies could be reliably found in high frequency. Additional colonies (used in Experiment 2) were collected during April 2009 in Florida, USA (colony PS-Fl1: 24°32’ N, 81°48’ W; PS-Fl2: 24°44’ N, 80°59’ W), and transported to the University of Regensburg for behavioural observations. For avoiding the occurrence of satellite nests of the same colony in the dataset, only nests with a least distance of ca. 3 m to each other were used for analysis.

Seven percent of 75 collected Brazilian field nests contained more than one queen (identified by physogastry, i.e. swollen abdomen, and distinct thorax morphology), 47% were queenless. Most nests (70%; $n = 67$) contained less than 30 workers upon collection. For experiments, 26 Brazilian and two Florida colonies were transferred to wood nests with carved nest chambers (3-4 mm deep, total area about 45 cm^2), covered with glass plates (Figure 2). Nests were put in plastic boxes (1.5-2.5 l).

Colonies used in Experiments 1 and 3 were stored in the laboratory at the Federal University of Santa Catarina (UFSC) where also all observations were conducted. Nest boxes were placed near the north windows where they were exposed to natural light and climate conditions (about 12-14 hours light per day, 14-30 °C, 50-70% humidity).

Colonies used in Experiment 2 were stored in a climatic chamber (24 °C, 60% humidity) at the University of Regensburg until three days before onset of observations.

All colonies were maintained by exchanging water and providing fresh food (honey, dried mosquito larvae and a mixture of tuna, sardines and mincemeat) *ad libitum* twice a week. They were kept under these conditions at least two weeks before they were used for an experiment.

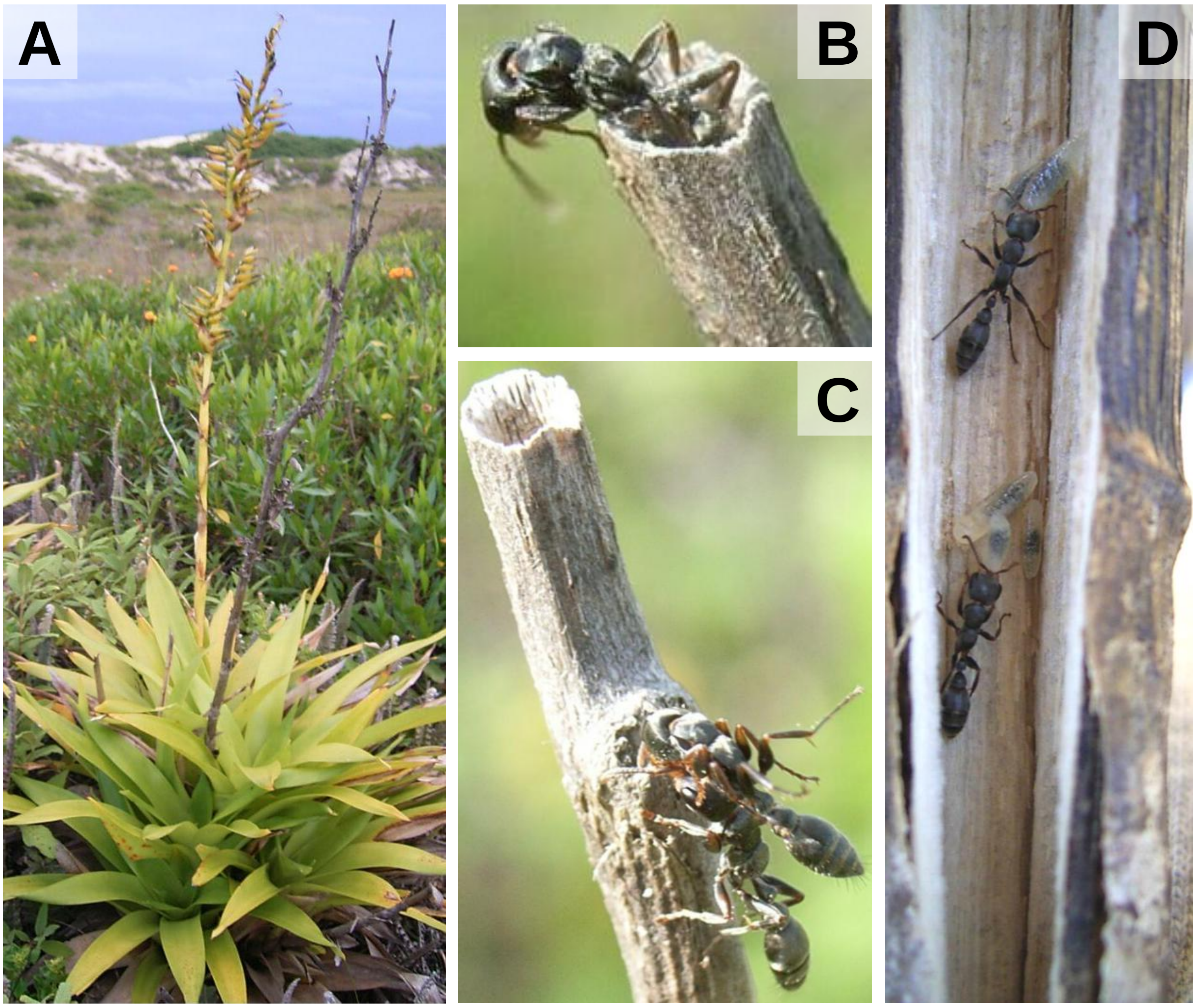


Figure 1 Field nests of *P. gracilis*. **A:** Group of bromeliad rosettes (*Vriesea friburgensis*) with one inflorescence (stalk to the left, most flowers withered) and one old infructescence (stalk to the right, probably from the previous year) containing a colony of *P. gracilis*. **B:** Worker of *P. gracilis* leaving the nest at the infructescence tip. **C:** Worker of *P. gracilis* carrying a putative nest mate towards nest entrance. **D:** Nest part of *P. gracilis* in broken-up bromeliad infructescence stem.

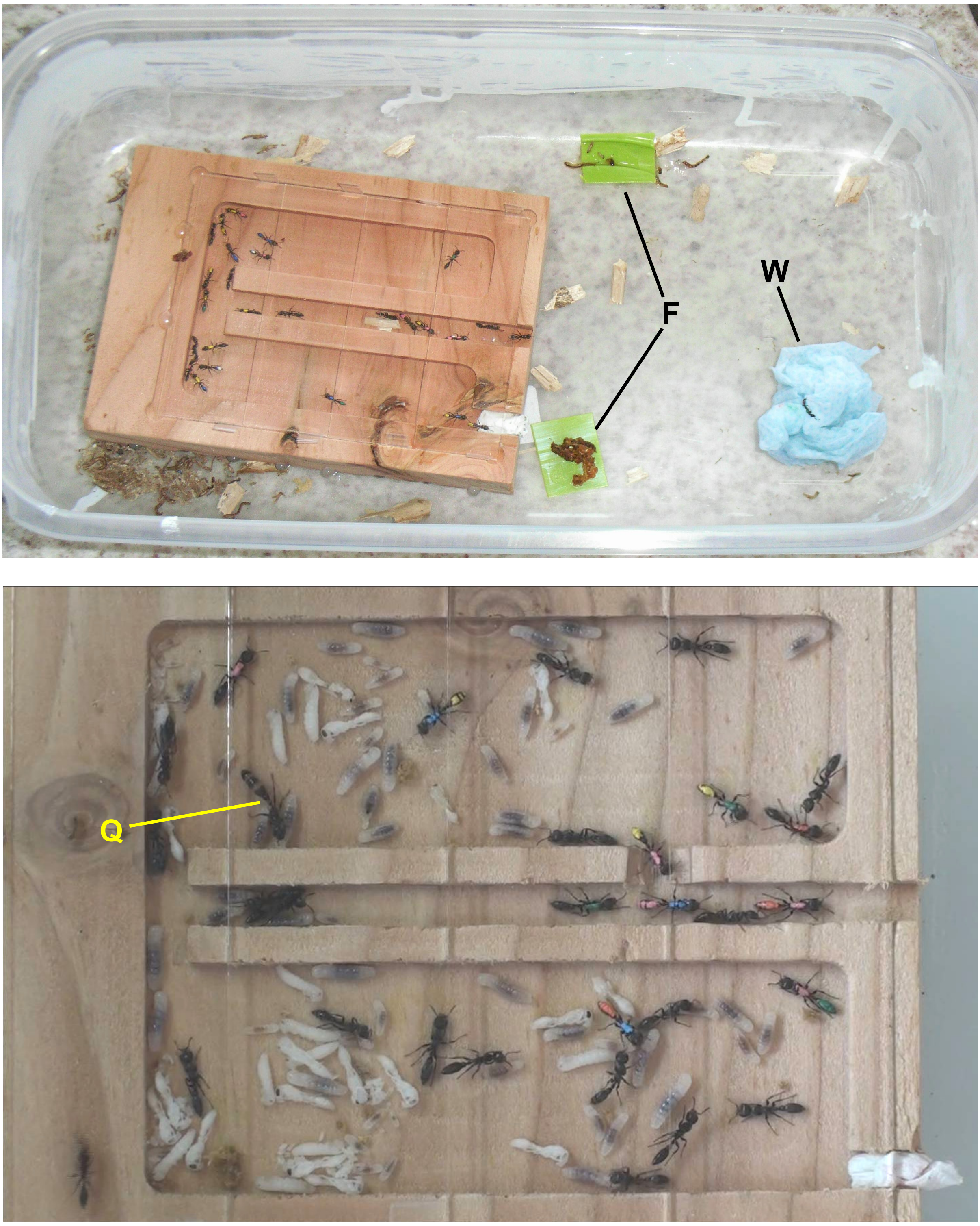


Figure 2 Laboratory ant nests. **Above:** Wooden nest box in opened plastic container. F: food on plastic plates. W: Wet paper towel for water supply. **Below:** Video screenshot of colony PS104 in phase 3. Q: queen.

2.3 Genetic colony structure

To derive parental and sister relationships, 145 workers and 25 queens (defined by physogastry and thorax morphology) of 15 laboratory colonies (11 monogynous, four polygynous with two, three, three and nine queens, respectively) were chosen for genetic analyses. From 13 of those colonies, 11 to 12 workers were examined. The two remaining colony samples consisted solely of the queens (three and nine, respectively). Additionally, 16 males from three monogynous colonies (PS008, PS013, PS086) were analysed for identifying the sources of male progeny. Samples were preserved in 95% ethanol for transportation before DNA was extracted.

DNA extraction, PCR amplification and microsatellite scoring followed the same protocols as described above (Section 2.1). Six to eight microsatellite markers per individual were used: Pg02-03, Pg07-09, Pg12-13 and Pg15 (Table 1).

Kin relationships, along with missing parental genotypes, were inferred with the software COLONY 2.0 (Jones & Wang 2010) which uses maximum-likelihood estimation and allows for genotyping errors. Polygamy was assumed for both sexes as well as inbreeding (thus allowing the program to estimate the degree of inbreeding). Allele frequencies were derived by the software and updated during the search for the maximum likelihood configuration. Runs were set to “very long” with the full-likelihood method and maximum likelihood precision. Genotyping error rate was arbitrarily estimated as 0.02. (For further details of COLONY analysis see Appendix, Section 7.1.) Thirteen genotypes of queens were entered as candidate mothers of 157 female offspring genotypes (including the two queen-only samples). When COLONY suggested genotyping errors, the corrected genotypes provided by the software were used for calculating pairwise relatedness among parents.

To estimate average and pairwise relatedness among nest mates, queens and their mates, the software RELATEDNESS 5.0.8 (Goodnight & Queller 2001) was employed. For estimation of population-wide average within-colony relatedness, 145 workers from 13 colonies were analysed. For colony-specific values, two samples consisting only of putative queens (PS068 and PS084) were added, mounting up to

157 individuals from 15 colonies. For colony sample sizes, see Table 1 in the results section.

Allele frequencies were calculated by RELATEDNESS (based on the input data), weighted by colony and bias-corrected with respect to colony membership. Standard errors of r means and 95% confidence intervals were estimated by jackknifing by locus ($n = 8$). The regression relatedness coefficients (r') calculated by the software were converted into life-for-life relatedness (r) according to Bourke & Franks (1995), i.e. values between females and males were divided by 2 ($r = r'/2$) whereas all other values (among females and among males) remained unchanged ($r = r'$).

2.4 Dominance hierarchies (Experiments 1 and 2)

Preliminary observations had revealed that, after orphaning, workers began to exhibit a distinct behaviour: the actor quickly moved towards the recipient and back (once or twice), touching it with its antennae, mouthparts and forelegs. This behaviour was tentatively interpreted as ritualised aggression used to resolve the reproductive conflict among workers. Because it resembled “antennal boxing” as observed in other ant species (e.g. Sommer and Hölldobler 1992; Monnin and Peeters 1999), this term (or in short just “boxing”) was adopted for the ritualised aggression in *P. gracilis*.

Experiment 1

For examining boxing behaviour under queenless relative to queenright conditions, 36 workers in each of ten queenright, monogynous colonies were colour-marked (see Figure 2). Single workers were transferred into a plastic basin with foam-covered ground and fixed by pressing them into the foam with a mesh. Through the gaps in the mesh, varnish colour (Edding® paint marker 751) was applied to thorax and/or gaster with a fine needle. Individuals were named by this colour code (e.g. gror = green orange). To provide enough time for the colour to dry without being removed through allo-grooming by nest mates, marked individuals were isolated for 1-3 h before putting them back into their nest. Right afterwards, marked workers were allo-groomed by nest mates with above-average rate but after one day the allo-grooming rate dropped to the same level as before marking (Daniela Wanke, unpublished data, *n* = 1 colony).

At least one week after colour marking, observation of each colony started by registering aggressive acts in the course of four to eight days (phase 1). Then, colonies were split in two parts and each was transferred into a new nest. The queenless part contained all coloured workers (13-29) that had survived until colony separation and several brood items (excluding eggs and small larvae to ease discovery of worker-laid eggs). Colony separation marked the beginning of phase 2, which lasted until at least one worker-laid egg was present (after 11-23 days). Finally, to find out whether reproductive workers are policed against under queenright conditions, the separated colony parts were reunited (phase 3) by placing all workers, brood items and the queen in the original nest. Again, all aggressive events were recorded (now including biting, dragging, and stinging, which had not occurred before). Two days after reunion, the whole colony was frozen for subsequent ovary dissections.

Observations were made partly directly and partly by video recording (Sony HDR-SR10E). Eight to nine hours of observation per phase and colony added up to a total of 292 h, 156.5 h of that by video capture. Within each phase, observations were distributed over several days, each session lasting 0.5-2 h. Boxing acts were recorded by opportunistic sampling: the whole nest area was observed while concentrating on spots with many individuals and conspicuous movements.

In order to assess ovary development, individual ants were dissected and ovaries were assigned each to one of five ordinal size scores (sometimes treated as nominal variable, depending on analysis):

1 – very small ovaries that did not show any signs of development;
2 – small ovaries that were elongated and thickened but did not contain maturing eggs;
3 – middle-sized ovaries with slightly swollen ovarioles, the oocytes being hardly large enough for measurement;
4 – large ovaries with at least one maturing egg;
5 – most elongated ovaries with numerous enlarged egg chambers.

To derive dominance orders from the ritualised aggressions for each colony, the frequency-dependent dominance index (*FDI*) published by Bang et al. (2010) was employed. This index takes into account the number of aggressive interactions between a focal individual and its nest mates as well as second-level interactions between those nest mates and other individuals of the colony.

The *FDI* for every (focal) individual was calculated with the following formula (Bang et al. 2010):

$$FDI = \frac{\sum_{i=1}^{n} B_i + \sum_{n=1}^{n} \sum_{j=1}^{m} b_{ij} + 1}{\sum_{i=1}^{p} L_i + \sum_{i=1}^{p} \sum_{j=1}^{q} l_{ij} + 1},$$

composed of the following terms:

$\sum B_i$: the total number (frequency) of boxing acts recorded for the focal individual;

n: number of nest mates that were boxed by the focal individual;

$\sum b_{ij}$: sum of frequencies with which all individuals i boxed by the focal individual in turn showed boxing behaviour towards nest mates (j);

m: for each b_{ij} summation, m represents the number of nest mates j towards which each individual i showed boxing behaviour (i in turn being boxed by the focal individual);

$\sum L_i$: total frequency with which the focal individual was boxed by nest mates;

p: number of nest mates (i) that boxed the focal individual;

$\sum l_{ij}$: sum of frequencies with which all individuals i that boxed the focal individual in turn were boxed by nest mates (j);

q: for each l_{ij} summation, q represents the number of nest mates j that boxed each individual i (which in turn boxed the focal individual).

For inter-colony comparisons, *FDI* values were normalised in relation to the within-colony maxima which were set equal to 1.

Experiment 2

To examine dominance hierarchy formation, two additional queenright colonies (PS-Fl1 and PS-Fl2) collected in Florida, USA (see above), were subjected to uninterrupted around-the-clock behavioural observations. Individuals were colour-marked and colonies split as described above (see Experiment 1), with 17 marked individuals from colony PS-Fl1 and 20 from PS-Fl2 placed each in a new wood nest. To ensure undisturbed observations, the nest boxes were moved to another room upon colony splitting (three days after colour marking), which marked the onset of observations. As that room lacked climate control (i.e., temperature and humidity were lower than in the climatic chamber) and because the nest box had to remain open the whole time, water was supplied on a daily basis as wet paper towel in the plastic box and additionally by wetting a paper plug in the wood nest block, adjacent to one of the carved nest chambers. Room lights necessarily remained switched on during the whole observation period. Ants were prevented from escaping by placing the plastic box in a basin filled with water.

Observations were conducted either directly as described above (see Experiment 1, maximal 2 h per day) or with digital video recording (DigiMicro 1.3, Drahtlose Nachrichtentechnik GmbH). Video images were captured in realtime with VirtualDub 1.9.8 (Lee 2009). This way, boxing behaviour was recorded for 32 days (PS-Fl1) and 28 days (PS-Fl2), and video captures were examined without gaps until 12 (PS-Fl1) and 15 (PS-Fl2) days after colony separation, respectively. Later recordings were examined only to an extent of mostly 3-12 h per day (gaps between examinations are visualised in Figure 15 and 16, see Appendix, Section 7.3). Altogether, 4800 boxing events were recorded in colony PS-Fl1 and 3639 in colony PS-Fl2.

In colony PS-Fl1, the individual that was identified as egg layer on day 18 was removed on day 25 to find out whether another worker would take over its role. (However, no new eggs were discovered afterwards until the end of the experiment.) Four workers of colony PS-Fl1 and nine of PS-Fl2 died during the observation period.

To derive dominance scores from observed boxing behaviour, the Elo rating method was employed as proposed by Albers & de Vries (2001) and Neumann et al. (2011).

This method assigns an arbitrarily chosen initial rating (1000) to each individual at the beginning of the analysis. Afterwards, the rating of each individual is updated with each dyadic interaction it participates in, using the following formulas:

I) Higher-rated individual wins:

Winner: $Elo_{new} = Elo_{old} + k \cdot (1 - p_w)$

Loser: $Elo_{new} = Elo_{old} - k \cdot (1 - p_w)$

II) Lower-rated individual wins:

Winner: $Elo_{new} = Elo_{old} + k \cdot p_w$

Loser: $Elo_{new} = Elo_{old} - k \cdot p_w$

k: arbitrarily chosen constant;

p_w: estimated winning probability for the higher-rated individual in the observed interaction.

p_w is estimated as a function of the most recent ratings that the interacting individuals had before the current interaction. The larger the absolute difference between the ratings of two individuals, the higher p_w will be. p_w is calculated from a z score based on a fixed SD = 200 (see Elo 1978 or Supplementary 1 of Neumann et al. 2011).

Inserting p_w in the formulas as described above leads to giving an expected outcome (e.g. a win of a highly rated individual against a lowly rated one) less weight (resulting in a slighter change of the ratings) than an unexpected outcome. If, for example, individual A is rated with $Elo_{old} = 1200$ and wins against B with $Elo_{old} = 800$, the new ratings (choosing $k = 100$) will be 1208 for A and 792 for B. If, however, the lower-rated B wins against A, the ratings change more drastically: 1108 for A and 892 for B. In both cases, the winning probability for A was estimated as $p_w = 0.92$.

The arbitrarily chosen value for the constant k has only little influence on general trends (Albers & de Vries 2001). However, it may lead to a faster and stronger diversification of Elo ratings in the initial phase of observations (compare, for example, the y-axis scalings of Figures 15A and 15B or 16A and 16B, respectively, in the Appendix, Section 7.3). Additionally, with low k values, established Elo ratings are

less sensitive to unexpected outcomes of single interactions (as illustrated by Figures 15 and 16 where the only difference between A and B is the choice of *k*).

In the observations conducted with *P. gracilis*, dyadic encounters each consisted of one boxing event with the actor (boxing individual) defined as winner and the recipient (boxed individual) as loser of the interaction. Boxing 'interactions' in *P. gracilis* do not constitute an extensive contest with both interactants actively trying to defeat each other but instead boxing is a short and more or less unidirectional event. Consequently, it appears reasonable not to attribute much weight to a single boxing action, and *k* was set to 10 for analysis. However, for comparison, graphs with ratings based on *k* = 50 are presented, too (see Appendix, Section 7.3).

As threshold for an individual's Elo rating to be regarded as established (in contrast to 'provisional', see Neumann et al. 2011) a minimal number of 10 interactions was set. In both colonies, one individual (PS-Fl1: or/gr; PS-Fl2: gror) did not meet this criterion and was excluded from subsequent correlation analysis. At the end of the experiment, colonies were frozen and the workers' ovaries dissected as described for Experiment 1.

2.5 CHC profiles (Experiment 3)

To determine whether the ants' CHCs are correlated with reproductive status, 16 colonies were studied (four of which were polygynous with two, three, three and nine queens, respectively). They were treated the same way as in Experiment 1 except that workers were not marked and colonies were frozen immediately at the end of phase 2 (which lasted 18-23 days), i.e. when worker-laid eggs were present. CHCs were extracted by individually soaking each worker from an orphaned colony part, the queen(s) and three workers from each queenright part in 200 µl pentane (CHROMASOLV®, GC-grade, Sigma-Aldrich, Germany) for 10 min. Afterwards, ovaries of these individuals were dissected and scored as described above. After evaporation of the pentane, the CHC extracts were transported to the University of Regensburg, Germany, for further examination.

Extracts of one worker and queen per ovary size score (as available) from each queenless and queenright colony part were selected for GC-MS analysis. Score 2 was omitted because categories 1 and 2 were the most similar ones and it was intended to maximise contrasts in the data, while at the same time keeping sampling and analysing effort at a reasonable level. In total, extracts of 57 workers and 17 queens (two queens from the same colony) were used. Workers in queenright parts had all ovaries of size 1, with one exception of size 3.

To each sample, 100 ng heneicosane (Sigma, St Louis, MO, USA) was added as internal standard to assess absolute CHC amounts (heneicosane was not detected in individual samples). Subsequently, the samples were resuspended in n-hexane (Fluka Chemie GmbH, Buchs, Switzerland) and evaporated to approximately 10 µl (under a constant stream of nitrogen) 2 µl of which were injected into an Agilent 6890N Series gas chromatograph coupled to an Agilent 5973 inert mass selective detector (Agilent Technologies, Böblingen, Germany).

The GC was equipped with a RH-5ms+ fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Capital Analytical Ltd., Leeds, England). Temperature was programmed from 70 °C to 220 °C with a 40 °C/min heating rate, then to 310 °C with 5 °C/min, and finally held for 12 min at 310 °C. Helium was used as carrier gas with a constant flow of 1 ml/min. Injection was carried out at 250 °C in the splitless mode for 1 min. Electron impact mass spectra (EI-MS) were recorded with an ionization voltage of 70 eV, a source temperature of 230 °C and an interface temperature of 315 °C. The software MSD ChemStation for Windows (Agilent Technologies, Palo Alto, CA, USA) was used for data acquisition and analysis.

For each GC spectrum, all peaks were manually integrated. Saturated, unsaturated and methyl-branched hydrocarbons were identified based on retention indices and characteristic fragment ions in the mass spectra (reference). Some peaks had to be combined for further analysis since they could not be clearly separated in all chromatograms. This procedure is conservative with regard to the hypotheses tested. Only peaks that were present in more than 30% of all samples and accounted for more than 0.5% of the total peak area in at least three samples were included in subsequent analyses. The smallest peak area in the whole dataset was defined as detection

threshold and added to all peak areas. Subsequently, relative amounts of the components were calculated (peak area / total peak area per individual). Because the relative amounts constitute compositional data, they were transformed to logcontrasts prior to analysis (Aitchison 1986): $Z_{ij} = \log_{10}(Y_{ij}/g(Y_j)+1)$. Here, Z_{ij} is the standardised peak area i for individual j, Y_{ij} is the relative peak area i for individual j, and $g(Y_j)$ is the geometric mean of all relative peak areas for individual j. For multivariate analyses of the chemical profiles (cluster and discriminant analyses as well as MANOVA, see next section), only such transformed relative peak areas were used.

A first inspection of the chromatograms (Figure 3, Table 2) and a subsequent cluster analysis with the chemical profiles (Figure 4) indicated the existence of two distinct classes, called “chemomorphs” henceforward. As colonies were grouped into these chemomorphs by the order of collection, this was assumed to be a methodological artefact related to either collection or laboratory conditions. However, it was not possible to associate this phenomenon with a certain methodological element, such as climate, the lot of wood for artificial nests or the charge of food. To consider variation in CHC profiles caused by this divergence, “chemomorph” was treated as an additional independent variable within which the colony variable was nested.

For estimation of absolute substance amounts, first the amount corresponding to the added standard (heneicosane, with a molar mass of 296.58 g/mol = 296.58 ng/nmol) was calculated: $M_{\text{standard}} = \frac{100}{296.58}\,\text{nmol} \approx 0.34\,\text{nmol}$. (Here, the symbol M is used for substance amount instead of the international symbol n to avoid ambiguity.) This amount was multiplied for each substance in each sample by that substance’s untransformed peak area $A_{\text{substance}}$ and divided by the peak area of heneicosane A_{standard} in the same sample:

$$M_{\text{substance}} = M_{\text{standard}} \cdot \frac{A_{\text{substance}}}{A_{\text{standard}}}\,.$$

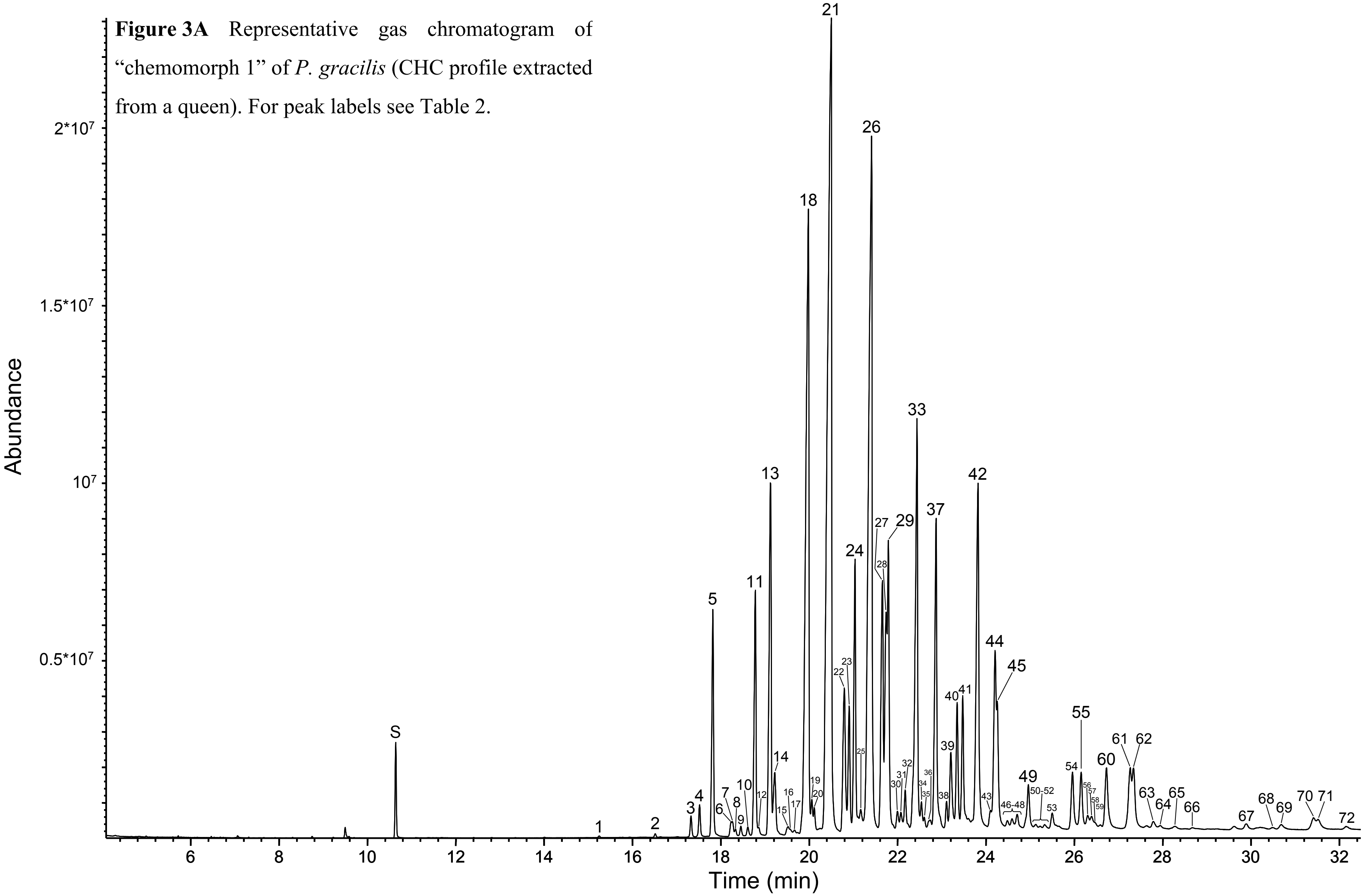


Figure 3A Representative gas chromatogram of "chemomorph 1" of *P. gracilis* (CHC profile extracted from a queen). For peak labels see Table 2.

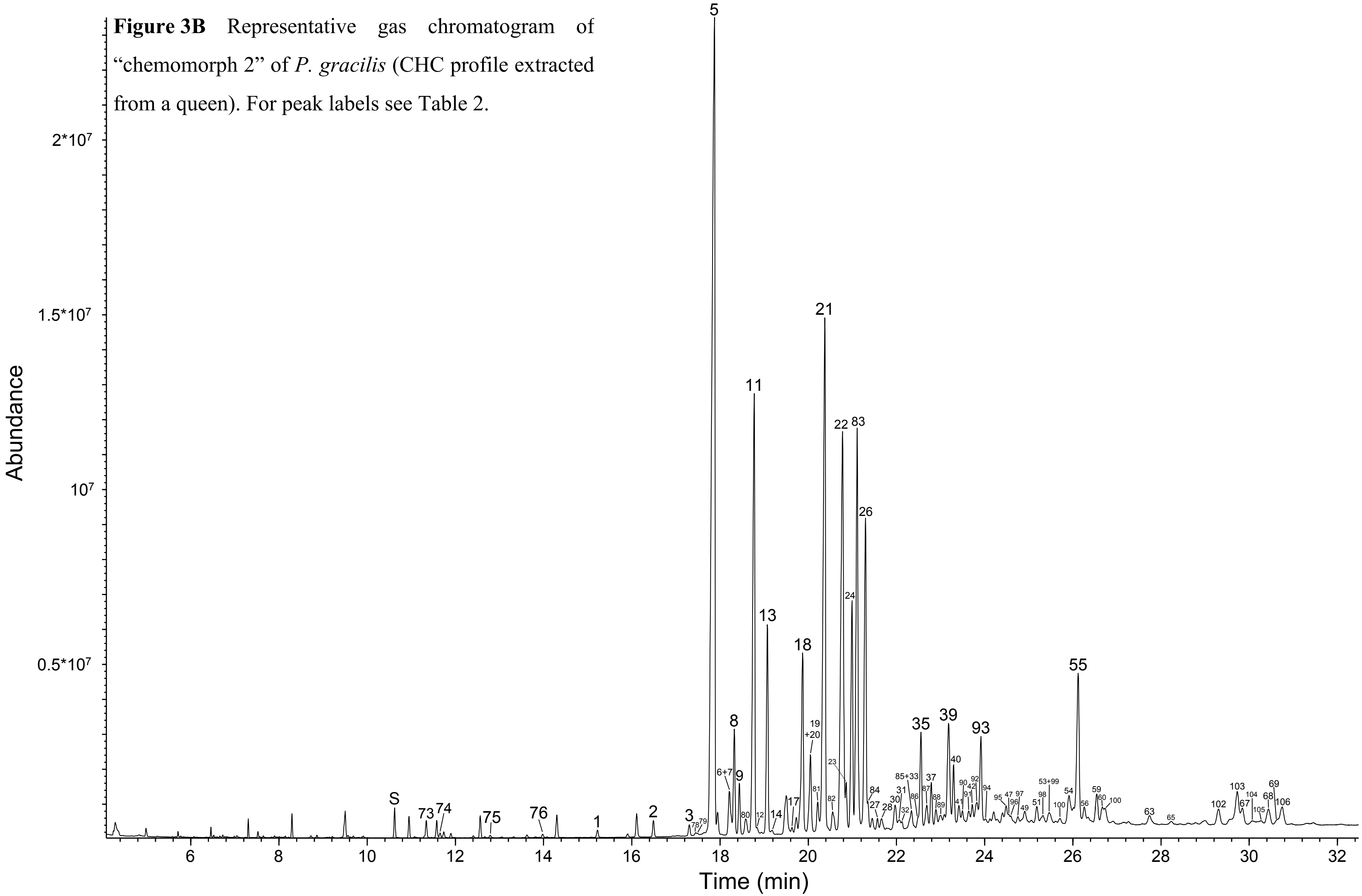


Figure 3B Representative gas chromatogram of "chemomorph 2" of *P. gracilis* (CHC profile extracted from a queen). For peak labels see Table 2.

Table 2 Retention times and relative (untransformed) peak areas of compounds that were used in the statistical analyses of CHC profiles of *P. gracilis*. Slashes denote mixtures of related substances (e.g. 13/11me-C_{27} which is a mixture of 13me-C_{27} and 11me-C_{27}), ordered by ascending retention time. Peak labels used in Figure 3 are given in parentheses. *RT*: Retention time (in minutes); *Q1*: 25% quartile; *Q2*: median; *Q3*: 75% quartile; Median diff.: difference between medians of chemomorph 2 and chemomorph 1; Diff. rank: order by absolute median differences (1 = largest difference).

Peak labels of identified compounds omitted from analysis (ordered by *RT*): C_{21} = standard (S); C_{23} (75); C_{25} (1); C_{26} (2); 4me-C_{26} (3); 4me-C_{27} (10); 6me-C_{28} (17); 6me-C_{32} (48); 4me-C_{32} (49).

		***RT* range**		**% Peak area of chemomorph 1**			**% Peak area of chemomorph 2**				
Substance or group	**Pooled compounds (Peak label)**	**Min**	**Max**	***Q1***	***Q2***	***Q3***	***Q1***	***Q2***	***Q3***	**Median diff.**	**Diff. rank**
Carbon acid	-	12.11	12.14	0.00	0.01	0.06	0.00	0.00	0.13	0.00	51
Carbon acid	-	14.50	14.54	0.01	0.04	0.44	0.00	0.01	0.51	0.03	46
Group 1	C_{27}:1 (4); unknown (78)	17.41	17.53	0.04	0.16	0.55	0.03	0.06	0.21	0.10	38
C_{27}	(5)	17.77	18.03	0.51	1.83	2.34	15.20	18.87	22.32	-17.04	1
Group 2	13/11me-C_{27} (6); 9me-C_{27} (7)	18.19	18.27	0.09	0.22	0.46	0.61	0.79	1.10	-0.57	23
7me-C_{27}	(8)	18.30	18.40	0.02	0.07	0.17	0.06	0.13	0.30	-0.06	41
5me-C_{27}	(9)	18.42	18.51	0.04	0.08	0.19	0.07	0.10	0.27	-0.02	47
3me-C_{27}	(11)	18.72	18.91	0.12	0.37	0.78	8.18	9.86	11.74	-9.49	2
C_{28}	(13)	19.05	19.16	0.72	1.59	2.51	3.04	4.61	5.76	-3.02	10
3,7dime-C_{27}	(14)	19.16	19.24	0.16	0.66	1.12	0.11	0.15	0.18	0.51	25
13me-C_{28}	-	19.46	19.50	0.01	0.10	0.37	0.00	0.00	0.00	0.10	37
Group 3	11me-C_{28}; 10me-C_{28} (15); 8me-C_{28} (16)	19.47	19.58	0.01	0.09	0.19	0.66	0.72	0.93	-0.64	22
4me-C_{28}	(18)	19.84	20.01	4.38	6.33	8.25	1.73	2.00	2.38	4.33	7
Group 4	3me-C_{28} (19); C_{29}:1 (20); C_{29}:2; two unknown	19.99	20.30	0.20	0.65	0.96	0.66	0.77	0.91	-0.12	36
C_{29}	(21)	20.32	20.63	5.57	13.20	18.21	5.97	7.73	9.43	5.47	4
Group 5	unknown (82); C_{30}:1; carbon acid	20.54	20.71	0.13	0.59	3.31	0.46	0.64	1.41	-0.05	44
Group 6	15/13/11/9me-C_{29} (22); 7me-C_{29} (23)	20.72	20.97	7.27	16.50	20.68	10.74	12.59	15.20	3.91	8
5me-C_{29}	(24)	20.96	21.15	0.92	1.86	2.81	0.32	0.43	0.92	1.44	14
Group 7	4me-C_{29} (25); 11,15dime-C_{29} (83); three unknown	21.03	21.19	0.62	0.78	2.13	6.40	8.91	11.24	-8.13	3
Group 8	Unknown (84); 3me-C_{29} (26); two unknown	21.21	21.51	4.69	7.92	10.63	7.29	9.39	11.99	-1.48	13
C_{30}	(27)	21.56	21.67	0.36	0.80	1.69	0.12	0.15	0.18	0.64	21

Table 2 continued.

		RT range		% Peak area of chemomorph 1			% Peak area of chemomorph 2				
Substance or group	**Pooled compounds (Peak label)**	**Min**	**Max**	***Q1***	***Q2***	***Q3***	***Q1***	***Q2***	***Q3***	**Median diff.**	**Diff. rank**
Group 9	3,11dime-C_{29} (28); 3,7dime-C_{29} (29); unknown	21.64	21.80	2.59	5.43	10.90	0.34	0.49	0.75	4.94	5
Group 10	16/14/12me-C_{30} (30); 8me-C_{30} (31); unknown	21.94	22.11	1.08	1.47	2.33	0.82	1.03	1.39	0.44	27
6me-C_{30}	(32)	22.12	22.18	0.06	0.16	0.27	0.00	0.00	0.01	0.16	35
Group 11	dime-C_{30} (85); 4me-C_{30} (33); three unknown	22.23	22.47	0.61	1.25	2.90	0.23	0.30	0.45	0.95	17
Group 12	5me-C_{30} (86); 3me-C_{30} (34); unknown	22.47	22.55	0.04	0.17	0.31	0.15	0.23	0.30	-0.05	43
C_{31}:2	(35)	22.54	22.69	0.00	0.01	0.01	0.00	0.01	0.23	0.00	52
C_{31}:1	-	22.63	22.76	0.01	0.01	0.12	0.00	0.00	0.00	0.01	50
Group 13	Unknown (87); 4,12dime-C_{30} (36)	22.67	22.79	0.25	0.57	0.79	0.37	0.48	0.62	0.09	40
C_{31}	(37)	22.78	22.97	0.50	1.53	3.27	0.21	0.29	0.54	1.24	15
Unknown	(88)	22.88	22.97	0.00	0.01	0.01	0.22	0.36	0.52	-0.35	31
Group 14	Carbon acid; unknown (89); unknown; 16/14me-C_{31} (38)	22.93	23.17	1.21	4.02	10.04	0.35	0.79	1.30	3.23	9
Group 15	15/13/11me-C_{31} (39); 7me-C_{31} (40)	22.31	23.40	5.39	7.71	14.95	2.50	3.06	4.14	4.64	6
5me-C_{31}	(41)	23.41	23.50	0.47	0.80	1.03	0.00	0.00	0.08	0.79	18
Group 16	Unknown (90); 7,17dime-C_{31}; 11,12dime-C_{31}; four unknown	23.43	23.72	1.66	2.55	3.59	0.29	0.53	0.83	2.02	12
Group 17	3me-C_{31} (42); unknown (91)	23.64	23.82	0.01	0.23	0.95	0.55	0.73	0.87	-0.50	26
5,17dime-C_{31}	-	23.79	23.88	0.01	0.39	0.96	0.00	0.00	0.00	0.38	30
Group 18	Unknown (92); unknown	23.80	23.88	0.00	0.01	0.01	0.40	0.56	0.82	-0.56	24
Group 19	Unknown (93); unknown (94)	23.90	24.07	0.00	0.01	0.01	1.54	2.21	3.21	-2.20	11
C_{32}	(43)	24.03	24.13	0.01	0.01	0.18	0.00	0.00	0.00	0.01	49
Group 20	3,11dime-C_{31} (44); 3,7dime-C_{31} (45); carbon acid; unknown	24.12	24.33	0.35	0.64	1.07	0.33	0.48	0.80	0.17	34
Group 21	16/14/12me-C_{32} (46); two unknown	24.40	24.51	0.01	0.20	0.43	0.13	0.26	0.34	-0.06	42
Group 22	Unknown (47); unknown (95)	24.48	24.63	0.00	0.01	0.09	0.46	0.75	1.06	-0.74	20
Group 23	Unknown (52); unknown (98)	25.29	25.38	0.01	0.01	0.02	0.28	0.41	0.54	-0.40	29
Group 24	C_{33} (53); unknown (99)	25.45	25.56	0.01	0.01	0.31	0.00	0.00	0.17	0.01	48
15me-C_{33}	(54)	25.89	26.07	0.36	1.26	2.50	0.31	0.50	0.75	0.77	19
7me-C_{33}	(55)	26.09	26.35	0.01	0.12	0.68	0.01	0.17	0.93	-0.05	45
Group 25	11,12dime-C_{33} (58); two unknown (56, 57); two unknown	26.25	26.50	0.35	1.05	1.79	0.00	0.01	0.29	1.04	16
Unknown	(59)	26.53	26.67	0.01	0.01	0.42	0.14	0.21	0.59	-0.20	33
Group 25	3me-C_{33} (60); unkown (101)	26.65	26.85	0.01	0.01	0.39	0.00	0.10	0.49	-0.10	39
Unknown	(63)	27.72	27.83	0.00	0.01	0.01	0.14	0.22	0.38	-0.21	32
Unknown	(102)	29.29	29.38	0.00	0.01	0.01	0.29	0.45	0.67	-0.44	28

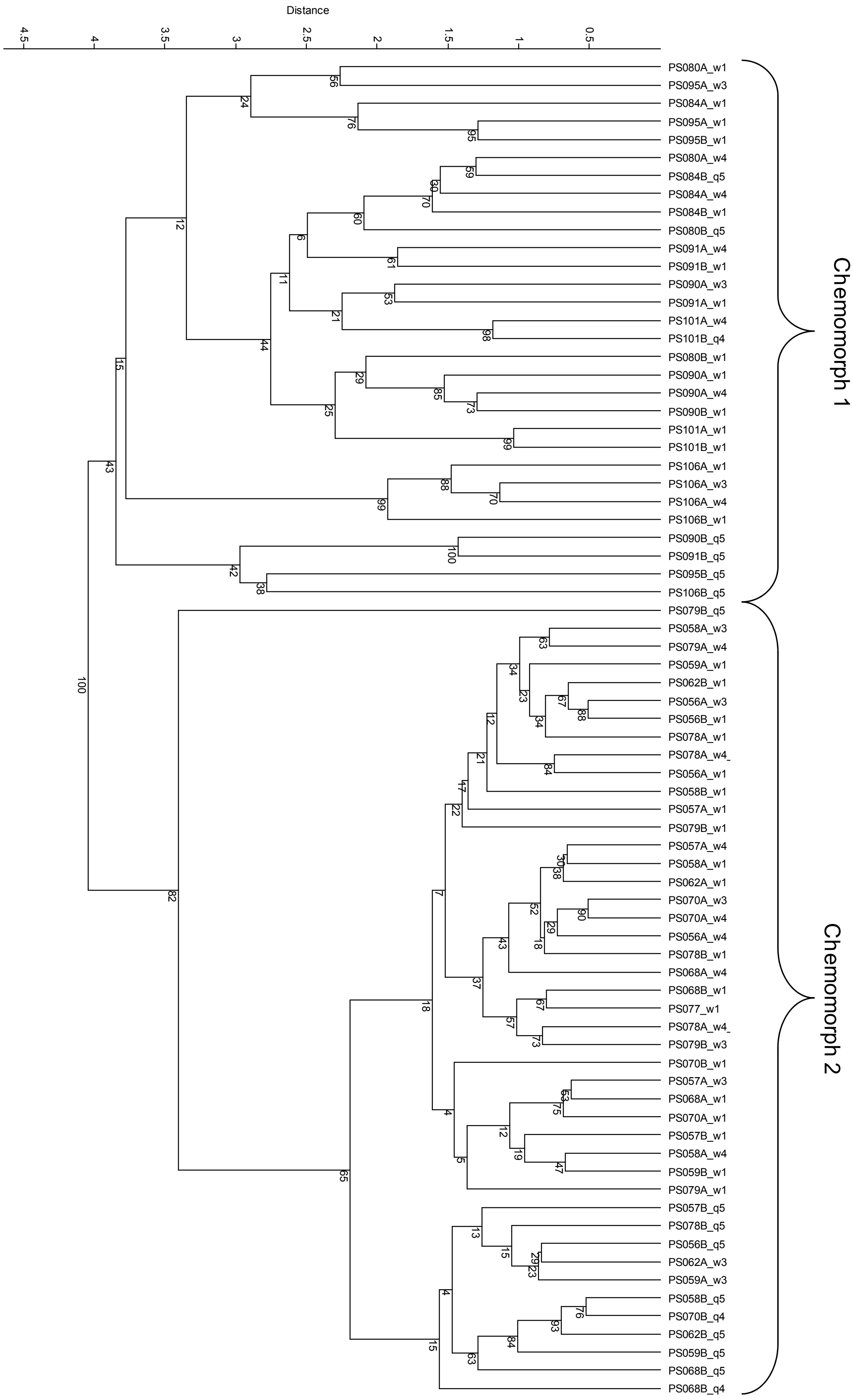


Figure 4 Dendrogram of cluster analysis, computed with PAST 1.97 (Hammer et al. 2001). Algorithm: Paired group; similarity measure: Euclidean; Boot n: 1000. Sample labelling pattern: PS{colony number}_{caste: queen/worker}{ovary size}. Colony number concurs with order of collection. Fifty-two variables (compounds) of 56 workers and 17 queens were analysed.

2.6 Statistical analyses

Boxing rates of ants were compared among experimental phases with pairwise Mann-Whitney U tests (performed with JMP 9.0, SAS Institute Inc.) whose *p* values were subjected to a stepwise Bonferroni correction (Holm 1979).

For correlating ovary size with other variables, gamma correlations (Statistica 9, StatSoft, Inc., USA) were used because the data contained many tied scores. For correlations of ovary size with dominance order, *FDI* values were averaged over each ovary size score within each colony. Numbers of workers attacked in phase 3 (after colony reunion) were expressed as percentages of all individuals with the same ovary size within the same colony.

For multivariate analyses of the chemical profiles, the transformed relative peak areas were used (see previous section). To reduce the number of variables in the CHC data for subsequent multivariate analyses, principal components were extracted in principal component analyses with varimax rotation (selection criterion: Eigenvalues > 1). To test for differences among CHC profiles in relation to caste (queen/worker), colony identity, colony part membership (queenright/queenless part) and ovary size, discriminant analyses (DAs) were performed. Furthermore, to combine possible influences of various factors in one model, MANOVAs (multivariate analyses of variance) were conducted with caste, chemomorph, colony (nested within chemomorph) and ovary size as independent variables and principal components as dependent variables. Additionally, to further explore the effect of chemomorph and of ovary size independently from caste membership, MANOVAs with reduced datasets (workers only and separated by chemomorph) were performed (for number of principal components and sample sizes see Table 6).

Finally, to investigate the influence of ovary size on single substances, the MANOVAs were repeated with the whole set of selected compounds instead of principal components. One substance that showed significant effects in most MANOVAs was examined in more detail. All PCAs, DAs, and MANOVAs were performed with SPSS 17.0 (SPSS, Inc.).

Probabilities of statistical tests presented in this monograph are always two-tailed.

3. Results

3.1 Colony structure and relatedness

Inferred parental genotypes were mostly considered reliable: single-locus genotypes of 22 fathers had average probabilities of 0.8590 +/- 0.1428 (mean +/- SD; $n = 8$ loci), inferred genotypes of five mothers had probabilities of 0.6813 +/- 0.2211 (Figure 5).

Twelve out of the 15 examined colonies comprised one full-sister group (i.e. one matriline and one patriline) containing all examined individuals of the respective colony (Table 3). Within the remaining three colonies, two to six full-sister groups were detected due to coexistence of different matrilines and/or patrilines. Two paternal half-sister dyads were inferred with probabilities (P) of 0.76 and 0.92 (all other dyads: $P < 0.5$), both within the same colony (PS095) where one male most probably had mated with both residing queens. Life-for-life relatedness between this male and the two queens was $r = 0.2784$ ($r' = 0.5567$) and $r = 0.3194$ ($r' = 0.6387$), respectively. COLONY inferred 53 highly probable ($P > 0.75$) maternal half-sister relationships within two colonies (PS008, PS095) and between PS080 and PS084. All female offspring within each colony were related as either full or half siblings, except for one individual in colony PS008. The inferred genotype of this alien individual's mother had distinctly low probabilities for all loci (< 0.3, Figure 5B), so it was not used to analyse r among mothers and fathers.

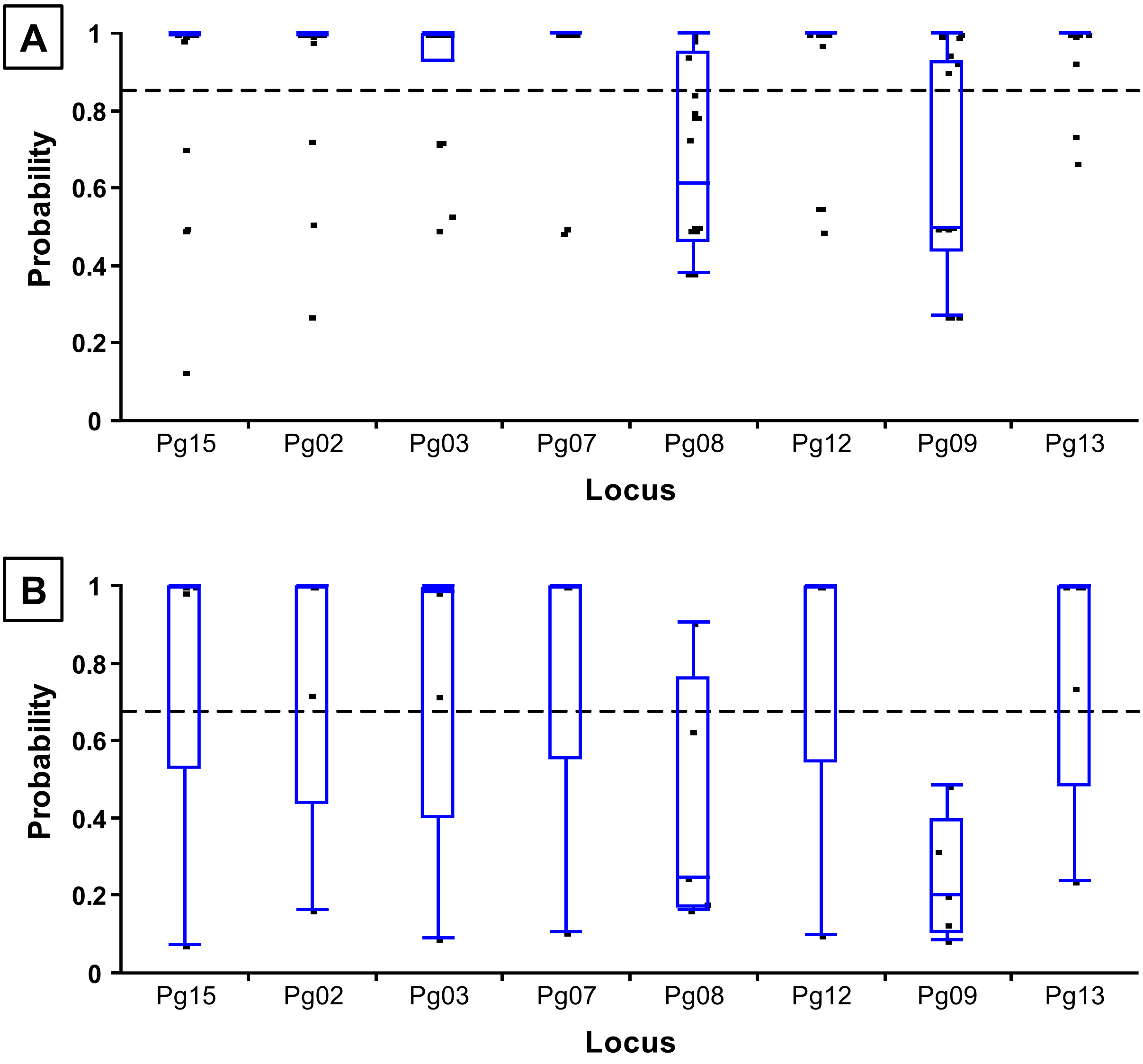


Figure 5 Probabilities for correct inference of father (**A**; *n* = 21) and mother (**B**; *n* = 5) genotypes by locus. Data points were jittered for better visibility. Box plots give medians (middle line) with quartiles (box); whiskers represent the range of all points that fall within the following limits: upper quartile + 1.5 · (interquartile range), lower quartile – 1.5 · (inter-quartile range). The dashed line represents the overall mean. The lowest probability value of each mother genotype locus (B) belongs to the mother of a single foreign individual of colony PS008.

Overall (i.e. estimated population-wide) relatedness among analysed female offspring within colonies was *r* = 0.7353 +/- 0.0431 (mean +/- jackknife-derived SEM; *n* = 8 loci). In 13 out of 15 colonies, *r* was not significantly different from 0.75 (Table 3).

Relatedness among queens of the same colony (excluding colony PS008) was *r* = 0.7632 +/- 0.1065 (mean +/- SD; *n* = 4 colonies). Between queens and their male mates, $r = 0.1320$ +/- 0.0975 ($r' = 0.2640$ +/- 0.1950; $n = 15$ colonies), ranging from 0.0070 to 0.3072 (Table 3; multiple queen/mate relationships within the same colony were averaged, see Figure 6). Relatedness among the males that mated with one or more queens of the same colony was 0.1258 +/- 0.2872 ($n = 3$ pairwise male combinations) for colony PS008, 0.5243 +/- 0.1375 ($n = 6$) for colony PS095 and 0.0215 ($n = 1$) for colony PS080 (Figure 6).

COLONY estimated the inbreeding coefficient as 0.065.

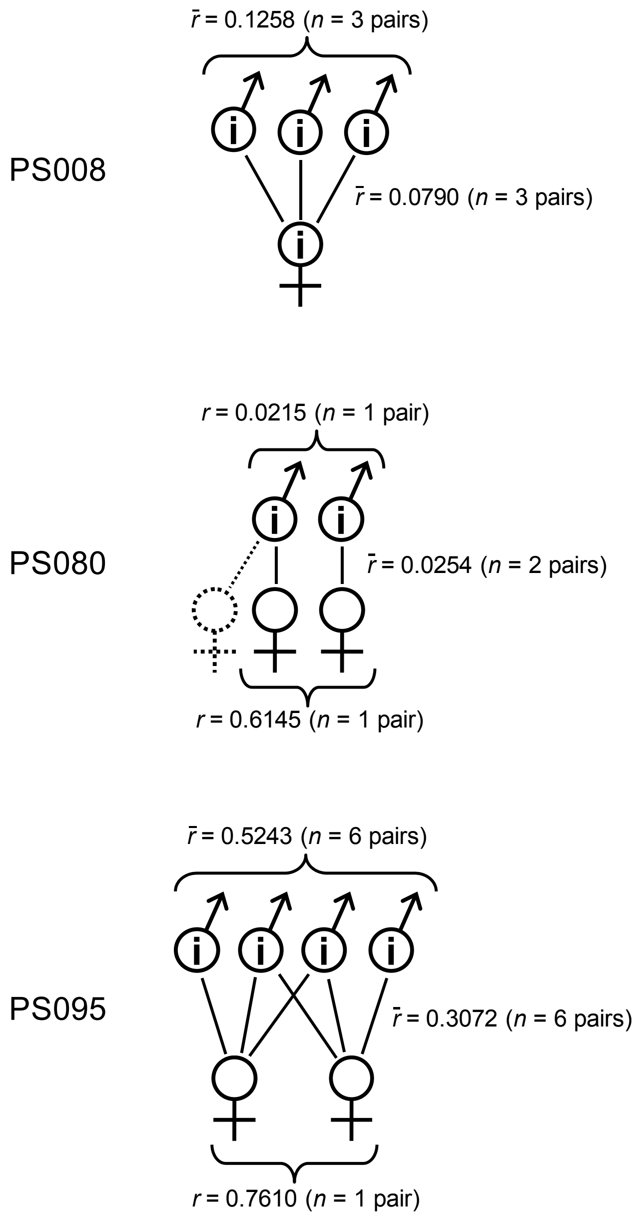


Figure 6 Relationships (pairwise r) among mothers and fathers of three *P. gracilis* colonies with multiple matrilines and/or patrilines. Dotted lining indicates the third queen of colony PS080 whose genotype did not differ from one of the other queens (next to it in the figure) at the analysed loci, so it is possible but not verifiable that this third queen produced some of the sampled workers. "i" indicates genotypes completely inferred by the software COLONY. Among individuals of the same sex, all possible pairs were used for average pairwise r calculation. Between mothers and fathers, only those pairs whose mating was inferred by COLONY (solid lines) were taken into consideration, resulting in an estimation of r between actual (and not potential) pairs of mating partners. (For standard deviations see Table 3.)

Table 3 Genetic analyses of 15 colonies of *P. gracilis*. "# queens / # candidates": number of queens upon collection / number of queens analysed as candidate mothers; *r*: life-for-life relatedness coefficient; "*r* between parents" = *r* between mother(s) and father(s) (= *r*′/2); "inferred" = mother genotype completely inferred; "nest queen(s)" = queen(s) of same nest assigned as mother(s); multiple queen/mate relationships within the same colony were averaged, see Figure 6. *r* among female offspring is given as means +/- jackknife-derived 95% confidence intervals, *n* = 8 loci; other *r* values are given as mean +/- SD if *n* > 2; if neither SD nor *n* is given, *n* = 1.

Colony	# queens / # candidates	# offspring analysed	# matri-lines	# patri-lines	*r* among female offspring	*r* among mothers	*r* among fathers	*r* between parents	mother assignment
PS008	0/0	11	2	4	0.4717 +/- 0.1379	-	0.1258 +/- 0.2872 (*n* = 3)[1]	0.0790 +/- 0.1081 (*n* = 3)[1]	inferred
PS013	0/0	11	1	1	0.6689 +/- 0.1560	-	-	0.0744	inferred
PS056	1/1	12	1	1	0.8422 +/- 0.2025	-	-	0.1968	nest queen(s)
PS057	1/1	10	1	1	0.8192 +/- 0.1413	-	-	0.2207	nest queen(s)
PS062	1/1	12	1	1	0.7777 +/- 0.1332	-	-	0.1158	nest queen(s)
PS068[2]	11/0	9	1	1	0.8330 +/- 0.1711	0.8226	-	0.1668	inferred
PS070	1/1	12	1	1	0.8125 +/- 0.2128	-	-	0.1889	nest queen(s)
PS078	1/1	10	1	1	0.7458 +/- 0.0454	-	-	0.0878	nest queen(s)
PS079	1/1	10	1	1	0.7560 +/- 0.1193	-	-	0.0546	nest queen(s)
PS080[3]	4/3	12	2	2	0.4145 +/- 0.1818	0.6145	0.0215 (*n* = 2)	0.0254 (*n* = 2)	nest queen(s)
PS084[4]	3/0	3	1	1	0.8639 +/- 0.1566	0.8548	-	0.1541	queen of PS080 assigned as mother
PS086	1/1	11	1	1	0.8640 +/- 0.1539	-	-	0.3038	nest queen(s)
PS090	1/1	10	1	1	0.8352 +/- 0.1348	-	-	0.0379	nest queen(s)
PS091	1/0	12	1	1	0.7647 +/- 0.1207	-	-	0.0070	inferred
PS095[5]	2/2	12	2	4	0.7371 +/- 0.2803	0.7610	0.5243 +/- 0.1375 (*n* = 6)	0.3072 +/- 0.0531 (*n* = 6)	nest queen(s)

[1] mother and father of a single foreign worker excluded from *r* calculations

[2] nine queens and no workers analysed as offspring

[3] all three queens analysed as candidate mothers (two genetically identical)

[4] three queens and no workers analysed as offspring

[5] Two of the four fathers mated twice, leading to six father-mother pairings (see Figure 6)

3.2 Origin of males

Throughout the whole study, no clear evidence for worker reproduction in the presence of the queen was found. Two out of 176 workers of eight experimental colonies had ovaries of size 3. Those two workers were from the same colony where egg laying by workers started distinctly earlier than in other colonies (within one or two days after queen removal). Hence, in this colony, the queen might have been less productive than normally or even infertile, leading to worker ovarian development even in her presence. The analysed males were, by visual comparison of genotypes, identified as progeny of the queens. After colony separation, in the queenless parts, workers began to lay eggs after 7-15 days (with exception of the colony mentioned above) (Figure 7). These developed into males within a few weeks.

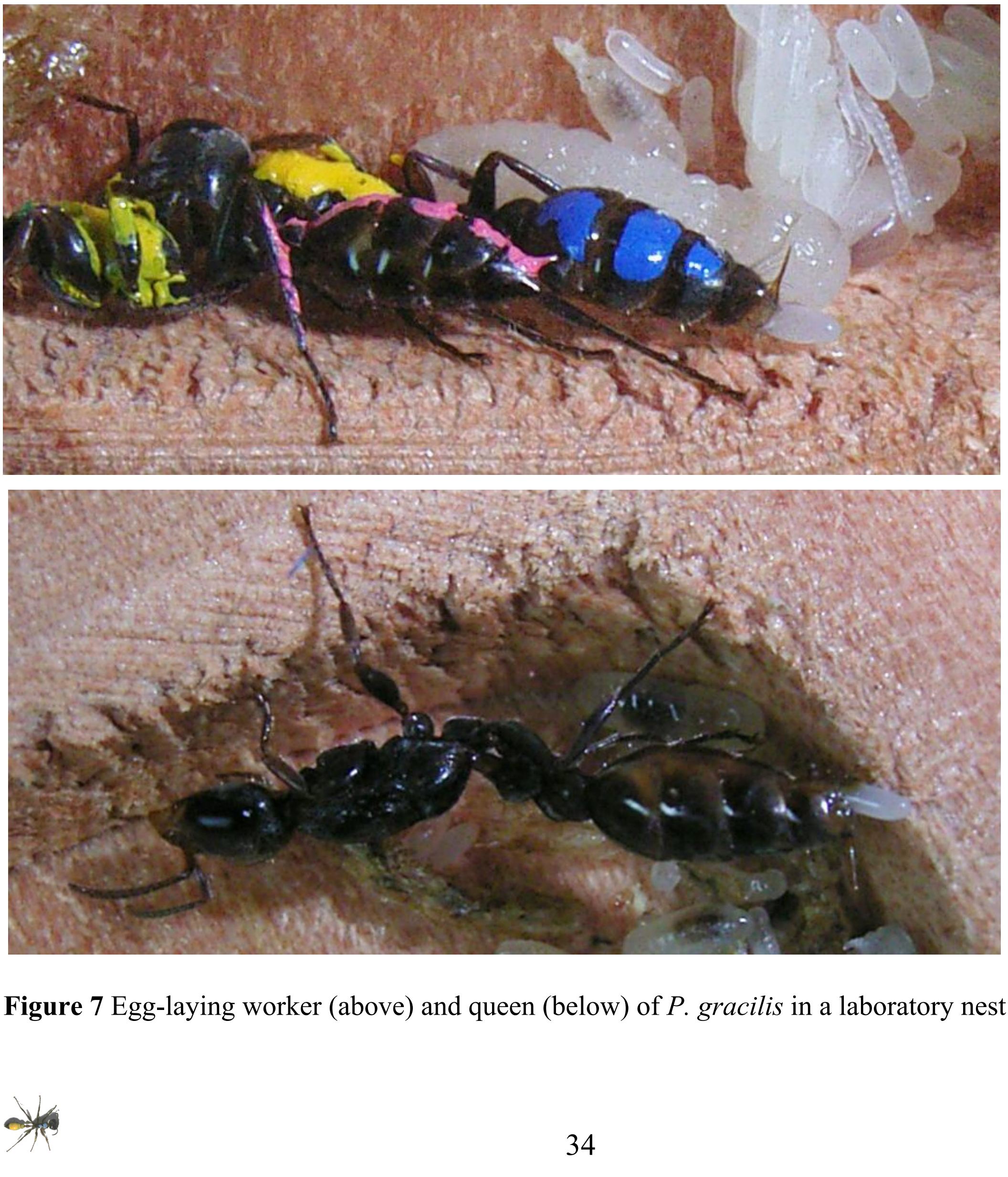


Figure 7 Egg-laying worker (above) and queen (below) of *P. gracilis* in a laboratory nest.

3.3 Dominance hierarchies and worker policing (Experiments 1 and 2)

Experiment 1

During all experimental phases, queens exhibited neither boxing nor any behaviour that appeared aggressive. They remained mostly inactive, rarely moved around in the nest, and contact with workers consisted exclusively of passive allo-grooming and trophallaxis. Aggressions other than boxing were only observed in phase 3.

Boxing rates among workers rose from a median of 2 interactions per hour before colony splitting to 17.3 actions/h during phase 2 and decreased again, dropping back to low levels with 2.5 actions/h after colony reunion (Figure 8, Table 4). In queenless colony halves, first eggs were discovered after six to 15 days (*n* = 15 colonies from Experiments 1 and 3), with 4.2 +/- 2.5 eggs (mean +/- SD) added per day. By that time, aggression levels had begun to decrease.

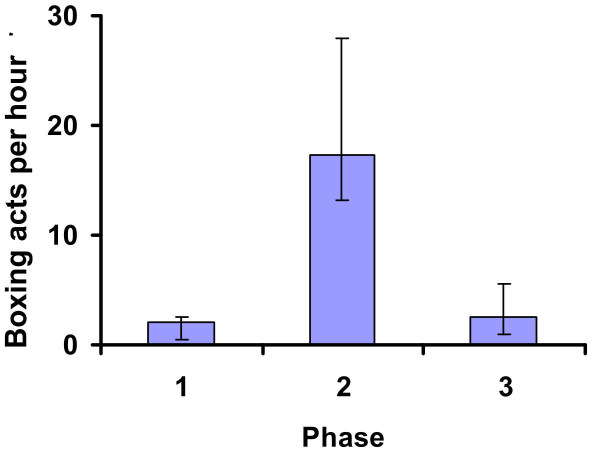
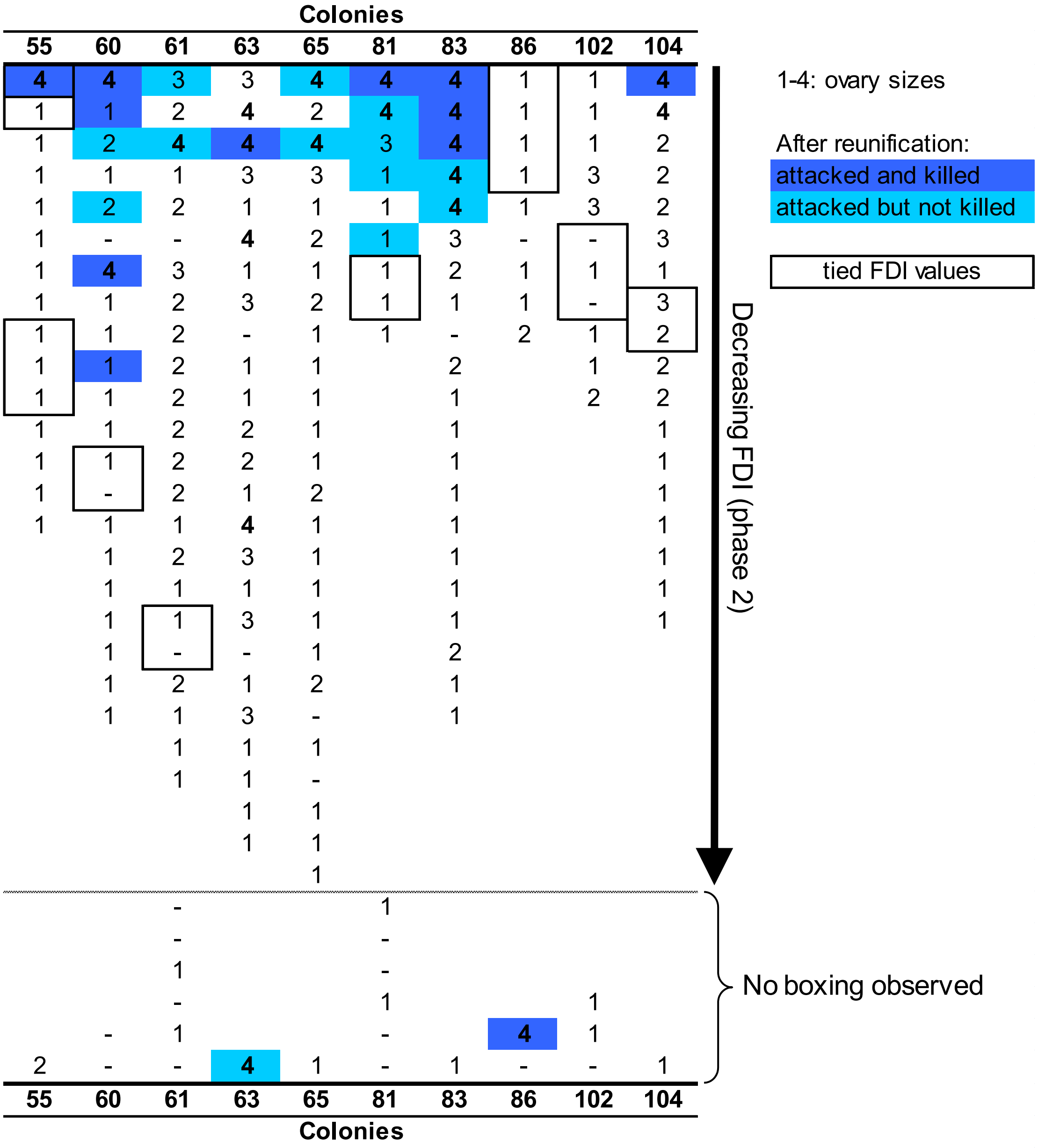


Figure 8 Rates of boxing behaviour of *P. gracilis* workers during experimental phases (1: before colony splitting; 2: in queenless colony part; 3: after reunification). Columns show medians, whiskers represent inter-quartile range. *n* = 10 colonies.

Table 4 Bonferroni-corrected results of Mann-Whitney U tests comparing boxing rates between experimental phases (*n* = 10 colonies; see Figure 8). The pairwise tests were ordered by *p* value to ease Bonferroni correction. *df*: degrees of freedom; corrected *α*: significance level 0.05 divided by the number of hypothesis tests remaining before the actual test has been Bonferroni-corrected (i.e. tests counted from the actual test to the bottom of the table); *: significant after Bonferroni correction.

Comparison	χ^2	*df*	corrected *α*	*p*
Phase 1 / Phase 2	13.19	1	0.01667	0.0003*
Phase 2 / Phase 3	11.06	1	0.025	0.0009*
Phase 1 / Phase 3	0.57	1	0.05	0.4488

In phase 2, the distribution of boxing frequencies of workers varied among colonies. Two workers of colony PS055 accounted for almost all recorded boxing acts while in colony PS083 most individuals exhibited this behaviour (see Appendix, Section 7.4). Absolute *FDI* values also varied distinctly within as well as among colonies; tied values occurred rarely (Figure 9).



55	60	61	63	65	81	83	86	102	104
4	4	3	3	4	4	4	1	1	4
1	1	2	4	2	4	4	1	1	4
1	2	4	4	4	3	4	1	1	2
1	1	1	3	3	1	4	1	3	2
1	2	2	1	1	1	4	1	3	2
1	-	-	4	2	1	3	-	-	3
1	4	3	1	1	1	2	1	1	1
1	1	2	3	2	1	1	1	-	3
1	1	2	-	1	1	-	2	1	2
1	1	2	1	1		2		1	2
1	1	2	1	1		1		2	2
1	1	2	2	1		1			1
1	1	2	2	1		1			1
1	-	2	1	2		1			1
1	1	1	4	1		1			1
	1	2	3	1		1			1
	1	1	1	1		1			1
	1	1	3	1		1			1
	1	-	-	1		2			
	1	2	1	2		1			
	1	1	3	-		1			
		1	1	1					
		1	1	-					
			1	1					
			1	1					
				1					

No boxing observed

55	60	61	63	65	81	83	86	102	104
		-			1				
		-			-				
		1			-				
		-			1			1	
	-	1			-		4	1	
2	-	-	4	1	-	1	-	-	1

Figure 9 Rankings of worker ants by *FDI*, based on boxing behaviour during the queenless phase, and levels of aggression against workers after colony reunification in 10 colonies of *P. gracilis*.


In phase 3, during the first hours and partly until the next day, additional forms of aggression occurred: certain workers were bitten into legs, antennae or petioles, stung, or dragged around in the nest or out of the nest. Up to four individuals per colony were killed in the process (Figure 9).

Such aggression was directed almost exclusively towards the workers from the formerly queenless parts (exception: six short episodes of aggression against a worker from a queenright part were observed). In most cases, workers from the formerly queenright part acted against workers from the formerly queenless part, but in two colonies, workers from the formerly queenless part fought among themselves (29% and 35% of aggressions, respectively).

Subsequent dissections showed that attacks were directed preferentially towards workers with developed ovaries and not randomly towards all workers from the formerly queenless part (Figure 9). Hence, aggression can be considered as policing rather than nest mate discrimination.

Worker ovaries were scored to sizes 1 to 4 (throughout all three experiments); score 5 did only occur in queens. Worker ovary size was positively correlated with dominance status ($n = 33$; $\mathit{gamma} = 0.4877$; $p = 0.0005$; Figure 10A) and as well with the percentage of attacked individuals after colony reunion ($n = 38$; $\mathit{gamma} = 0.7305$; $p < 0.0001$; Figure 10B).

Egg eating was never observed in any of the three experimental phases.

Experiment 2

Within the first days after colony separation, Elo ratings strongly diversified (Figure 11), owing to unbalanced aggression levels among the workers (see Tables 7 and 8 in the Appendix, Section 7.3). While the course of rating development of some workers was rather monotonic (especially those individuals that were rated highest in the end, Figure 11), other individuals' dominance rating rose within the first days but then settled far below their former maxima.

After less than 15 days, the Elo ratings of the three finally highest-rated workers in both observed colonies had reached a level that remained almost constant until the end of the experiment (Figure 11, see also Figures 15 and 16 in the Appendix, Section 7.3).

In colony PS-Fl1, 73% of the boxing acts observed between day 14 and the end of the experiment were exchanged between the two top-rated individuals (or/or and bl/gr, see Table 7 in the Appendix, Section 7.3) with 56% and 44% of boxings exhibited by those two workers, respectively. In colony PS-Fl2, the last days (20-30) of the experiment were largely dominated by the highest rated worker (nobl) which accounted for 80% of all observed boxings (see Table 8 in the Appendix, Section 7.3).

In both colonies, the highest-rated workers had above-average ovary size scores (Figure 11). In colony PS-Fl1, this resulted in a significant association between Elo rating and ovary size ($n = 16$; $gamma = 0.7179$; $p = 0.0018$). In colony PS-Fl2, the existence of workers with middle-sized ovaries among the lowly rated individuals led to the corresponding correlation being weak and not significant ($n = 17$; $gamma = 0.0910$; $p = 0.6809$).

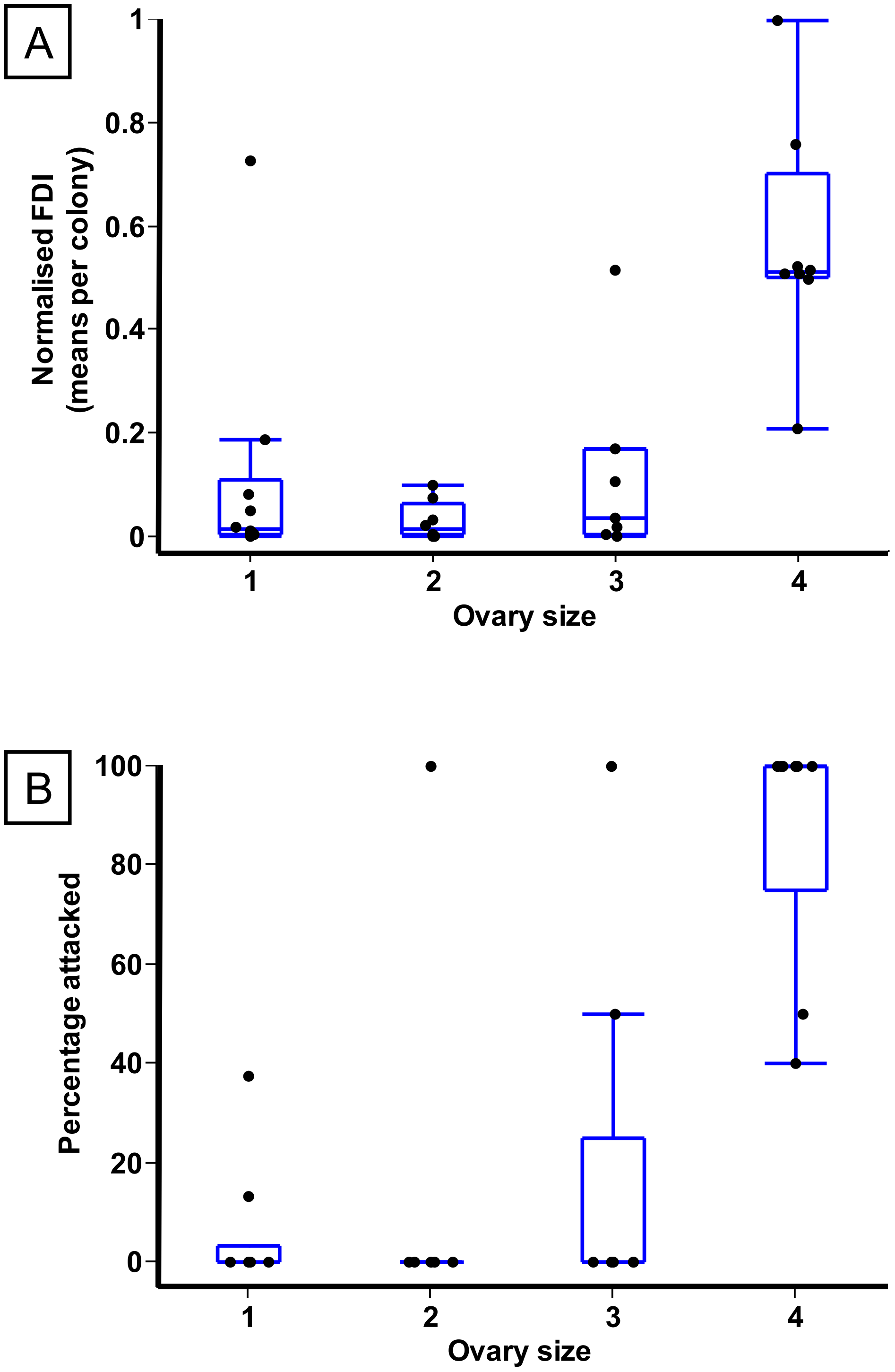


Figure 10 Dominance and policing of workers of *P. gracilis*, depending on ovary size. Data points were jittered for better visibility. **A:** Dominance (measured as normalised *FDI*) of workers in orphaned colonies. **B:** percentage of attacked workers from orphaned colonies after colony reunion (phase 3). Box plots show median (middle line) and inter-quartile range (box); whiskers represent the range of all points that fall within the following limits: upper quartile + 1.5 · (interquartile range), lower quartile – 1.5 · (inter-quartile range).

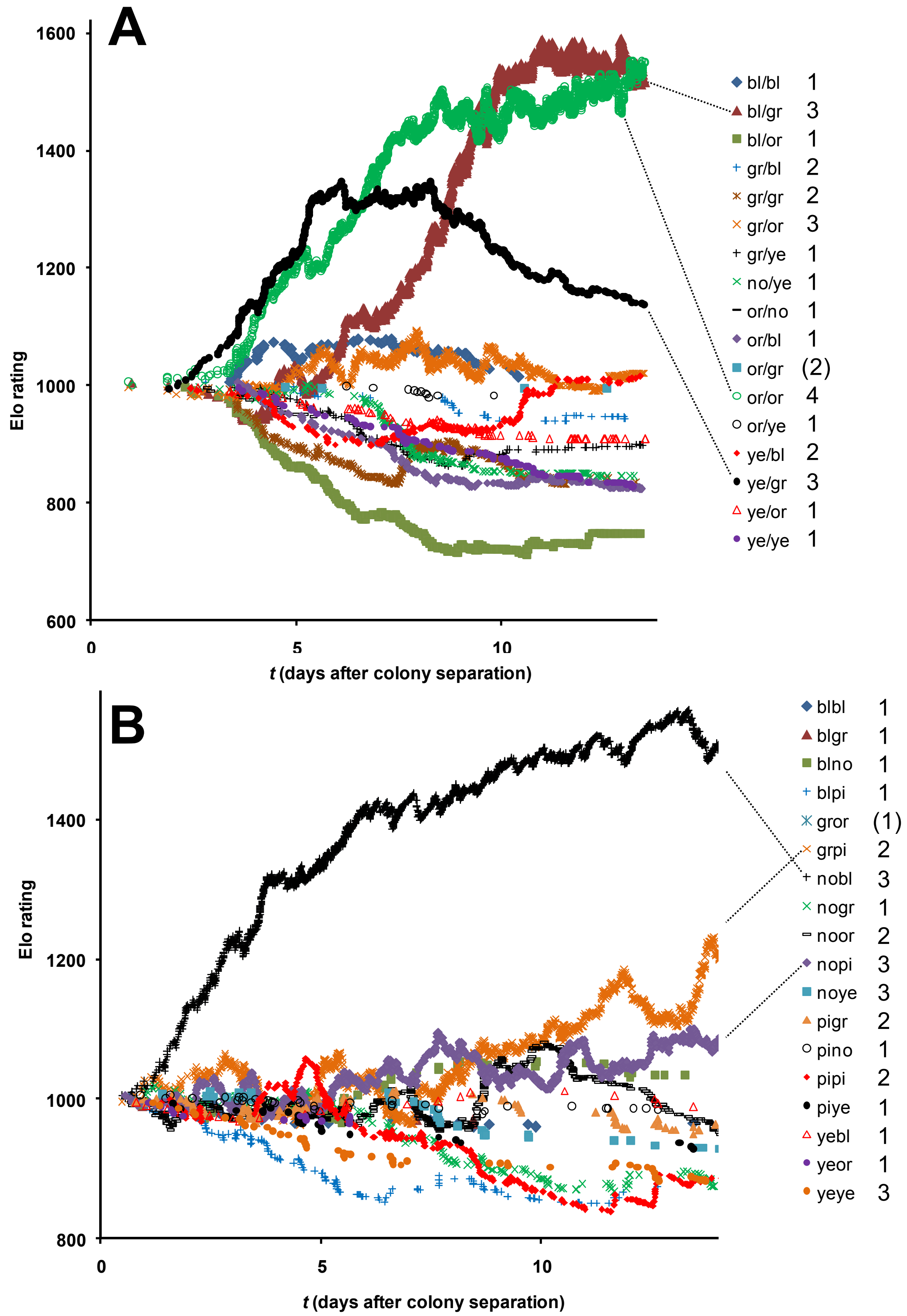


Figure 11 Development of Elo ratings for colonies PS-Fl1 (**A**) and PS-Fl2 (**B**) during the first 14 days of Experiment 2. Ovary size scores are presented behind the individual names (parentheses indicating that not more than 10 interactions were observed for the corresponding individual). Final ratings obtained after the complete observation periods are presented in Figures 15 and 16 as well as Tables 7 and 8 (see Appendix, Section 7.3).

3.4 CHC profiles (Experiment 3)

In total, 151 substances were differentiated based on their mass spectra; of these, 52 compounds or groups of compounds were included in subsequent analyses (Table 2). The total substance amounts of the CHC profiles in queens (median of 51.2 nmol) were significantly higher than in workers (12.2 nmol) (Mann-Whithey U test: $\chi^2 = 23.26$; *df* = 1; $p < 0.0001$; Appendix, Section 7.4, Figure 13).

From the whole dataset (including queens), eight principal components (PCs) with Eigenvalues > 1 were extracted (containing 86.3% of the variance). Based on the reduced dataset containing only workers, nine PCs were extracted (88.1% of variance), as well as 10 PCs within chemomorph 1 (91.3% of variance) and six PCs within chemomorph 2 (81.7% of variance).

Discriminant analyses based on the whole dataset yielded significant differences between castes, colonies, queenless/queenright colony parts and ovary sizes (Table 5, Figure 12). In the reduced dataset (only workers), colonies were significantly separated while ovary size groups and colony parts were not (Table 5).

Table 5 Results of discriminant analyses based on principal components (PCs) extracted from relative amounts of 52 CHCs (see also text). *n*: number of samples (individuals).

Dataset	Discriminator variable	*Wilks' Lambda*	χ^2	*df*	*p*	Correctly classified
Whole (*n* = 74; 8 PCs)	Caste	0.254	93.2	8	< 0.001	94.6%
	Colony	< 0.001	525.8	128	< 0.001	64.9%
	Colony part	0.730	21.7	8	0.006	70.7%
	Ovary size	0.212	104.1	24	< 0.001	55.4%
Workers only (*n* = 57; 9 PCs)	Colony	< 0.001	592.8	144	< 0.001	77.6%
	Colony part	0.919	4.34	9	0.888	-
	Ovary size	0.563	29.0	27	0.360	-

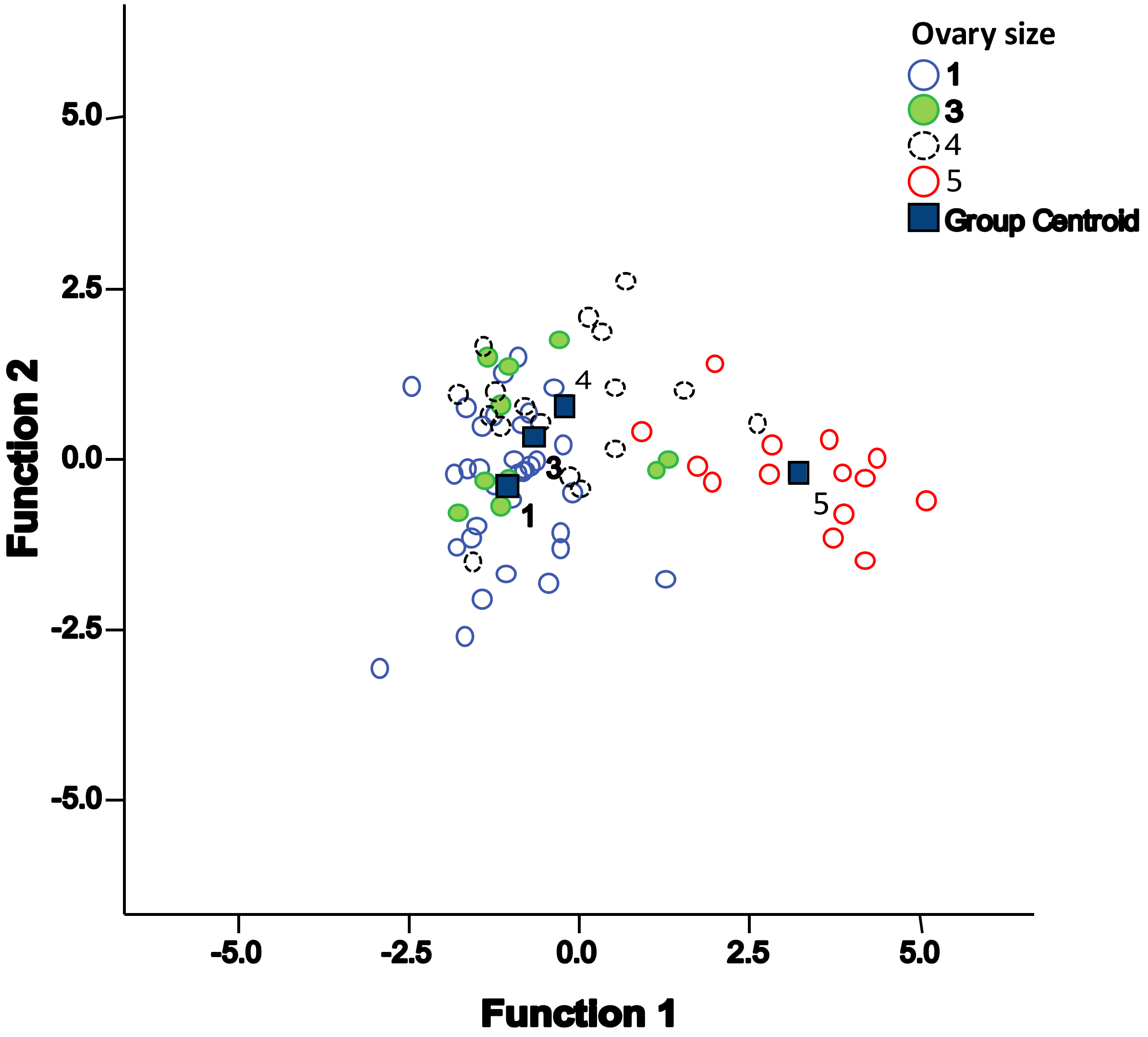


Figure 12 Discriminant analysis of ovary size by CHC profiles (whole dataset, see main text and Table 5 for details).

In the MANOVAs with principal components as dependent variables, caste, chemomorph and colony always had a significant effect on the chemical profiles while ovary size was not significant in any case (Table 6). The MANOVAs with all single compounds as dependent variables yielded similar results, with the exception that caste had no significant effect (Appendix, Table 9). Caste and ovary size had distinctly higher *p* values than in the analysis with principal components and were thus far from significant.

Among the substances that were significantly associated with ovary size, 7me-C_{33} stood out because in the single-substance MANOVA with the whole dataset it had the lowest *p* value of all compounds for the factor caste and the fifth lowest for ovary size (Appendix, Section 7.4, Table 9). Within workers only, this substance's relative amount was also most significantly influenced by ovary size in chemomorph 1 but not in chemomorph 2 (here, only one substance, C_{33}, had a significant *p* value: $p = 0.044$).

Table 6 Results of MANOVAs with principal components (PCs) of the CHC data as dependent variables. Factor: independent variable; *Wilk*: Wilk's Lambda; *df (H)*: degrees of freedom (hypothesis); *df (E)*: degrees of freedom (error); *n*: number of samples (individuals).

Dataset	**Factor**	***Wilk***	***F***	***df (H)***	***df (E)***	***p***
Whole (*n* = 74; 8 PCs)	Caste	0.718	2.253	8	46	0.040
	Chemomorph	0.021	267.2	8	46	<0.001
	Colony[chemomorph]	0.002	3.777	120	340	<0.001
	Ovary size	0.534	1.349	24	134	0.145
Workers only (*n* = 57; 9 PCs)	Chemomorph	0.011	296.3	9	30	<0.001
	Colony[chemomorph]	<0.001	4.877	135	249	<0.001
	Ovary size	0.541	1.200	18	60	0.290
Worker only, chemomorph 1 (*n* = 23; 10 PCs)	Colony	<0.001	7.686	60	31	<0.001
	Ovary size	0.058	1.572	20	10	0.233
Worker only, chemomorph 2 (*n* = 34; 6 PCs)	Colony	0.036	1.558	54	91	0.031
	Ovary size	0.507	1.146	12	34	0.358

In the whole dataset, the relative amount of 7me-C_{33} monotonically increased with ovary size (Figure 14). However, in size score 5 (entirely consisting of queens) it was about five times higher than in score 4 (which contained three queens and 14 workers) while the differences among categories 1, 3 and 4 were distinctly smaller. Queens had an absolute amount of 0.94 nmol of 7me-C_{33} on their cuticles, workers 0.0026 nmol (Mann-Whitney U test: $\chi^2 = 38.36$; $df = 1$; $p < 0.0001$; Appendix, Section 7.4, Figure 13). This inter-caste difference was significant even under consideration of variation in total profile amount (see analysis of relative amounts in Appendix, Table 9).

4. Discussion

4.1 Colony structure

According to the results on kin structure and relatedness, *Pseudomyrmex gracilis* appears to be a mostly monogynous and monandrous species with occasional functional polygyny (by full-sister queens) and polyandry. (“Functional”, in this context, refers to the presence of matrilines/patrilines in the worker genotypes in contrast to the mere observation of more than one queen or more than one mating event per queen. A colony may, for example, be considered polygynous because it contains several dealate queens; however, if only one of them reproduces, the colony is functionally monogynous.)

Published data on colony structure of Pseudomyrmecinae is, on the one hand, not scarce but, on the other, probably not substantial enough to derive confident generalisations for the whole subfamily (not least due to the mostly low or not presented sample sizes, see Appendix, Section 7.5, Table 10). From the literature available up to date, one may get the impression that most pseudomyrmecine species are monogynous (Janzen 1966, 1973) but (at least facultative) polygyny is not rare either (Appendix, Section 7.5, Table 10). Similarly, in other ant subfamilies, neither monogyny nor polygyny are uncommon and can frequently both be found within a species (e.g. Buschinger 1968, 1974; Hölldobler & Wilson 1977; Ward 1989a; Mackay et al. 1990; Frumhoff & Ward 1992; Keller 1993; Heinze 2008). The present results on *P. gracilis* confirm this provisional view, adding data about the southern edge of this species’ wide distribution range.

Polygyny can be adaptive if the habitat is saturated with ant colonies (and probably other insects) competing for the same kind of nest site (Heinze & Tsuji 1995), especially if the nest sites constitute an ephemeral resource (Hölldobler & Wilson 1977). At the site of collection in Brazil, colonies of *P. gracilis* and other ant species were frequently found in withered bromeliad infructescences and dead parts of other plants (e.g. *Actinocephalus polyanthus*, Cereto et al. 2011, or *Epidendrum sp.*, personal observation). Such plant material decays within one or two years (personal observation) and thus provides small, ephemeral cavities. Additionally, at the collection site, the low vegetation cover on sandy soil might well facilitate saturation with competing ant colonies. Hence, queens of *P. gracilis* might sometimes fare better by seeking readoption in the mother nest than by trying to found a new colony on their own. (Colony foundation by budding – i.e. one or more queens leaving the colony in company of a group of workers – has not been reported in this species, so the winged queens of *P. gracilis* should be assumed to found solitarily.)

As far as queens (of both monogynous and polygynous colonies) were genetically analysed as putative mothers, they could be assigned to offspring which means that they were inseminated. Only in the two (polygynous) cases where queens were treated as offspring, it remains unclear whether they were inseminated and produced own progeny because neither their spermathecae were dissected nor any putative offspring was analysed.

Regarding the number of patrilines per colony, functional monandry (one patriline among the whole colony offspring) seems to be prevalent in social Hymenoptera: The "mean effective paternity frequency" was reported to lie between 1 and about 1.5 in 57 other ant species (as well as bees and wasps) (Boomsma & Ratnieks 1996) and corroborated by later studies (e.g. Murakami et al. 2000; Villesen & Boomsma 2003; see also Heinze 2008 and references therein). The average number of patrilines in *P. gracilis* (slightly higher than 1) falls well in this range. According to Boomsma & Ratnieks (1996), copulation frequency likely overestimates actual paternity frequency, so females of *P. gracilis* might in average mate with two or more males more often than the numbers of patrilines suggest. On the other hand, this appears unlikely regarding the low colony sizes of *P. gracilis* together with the positive correlation between colony size and mating frequency found in other species (Boomsma & Ratnieks 1996; Murakami et al. 2000).

The data of this study indicate that virgin queens of *P. gracilis* may, though rarely, mate with closely related males (probably their brothers) although this does apparently not lead to substantial population-wide inbreeding. This small amount of inbreeding, however, might account for most colonies yielding relatedness levels slightly higher than 0.75.

When close relatives are involved, neither polygyny nor polyandry substantially decrease within-colony relatedness. This explains why relatedness in one colony with multiple matrilines and patrilines was nevertheless high. Only two colonies had among-worker *r* values significantly lower than 0.75. In one case (colony PS008), this was caused by a single worker within the dataset completely unrelated to its “nest mates”. This individual might have been accidentally sampled together with the other workers or might have been accidentally accepted by the colony in the field. In another colony (PS080), the existence of two matrilines and patrilines, together with relatedness between mating partners and among males being closed to zero, led to its low *r* value. Such cases, however, seem to constitute a minority within the population.

The high percentage of queenless nests is best explained by polydomy, i.e. colonies being spread over several nest sites, a common trait among pseudomyrmecine (e.g. Janzen 1966; Buschinger et al. 1994; Debout et al. 2007; Kautz et al. 2009; see also Appendix, Section 7.5, Table 10) and other ants (Debout et al. 2007). This would render the natural occurrence of isolated, queenless colony parts more likely. For example, the destruction of the queenright part of a polydomous colony will leave all other nests in the same condition that was examined in this study. To elucidate the putative polydomy of *P. gracilis*, genetic and/or nest mate discrimination analyses of all nests found within a small range would be appropriate but this was not within the scope of the present study.

4.2 Worker reproduction and dominance hierarchies

Under orphaned conditions, workers of many social Hymenopteran species begin to lay eggs which develop into viable males (Bourke 1988, Choe 1989), Pseudomyrmecinae being no exception (Mintzer 1982; Choe 1989). This, in turn, is an important assumption for the examination of reproductive conflicts among workers and could be confirmed for *P. gracilis* in this study. Furthermore, the lack of any aggression by queens rules out physical queen control as mechanism to control worker reproduction.

In orphaned colonies of other ant species, behaviours similar to the boxing behaviour described here have been interpreted as aggressive in the context of hierarchy establishment (e.g. Cole 1981; Sommer & Hölldobler 1992; Peeters & Tsuji 1993; Monnin and Peeters 1999; Gobin et al. 2003). Hence, the same function can be attributed to the boxing of *P. gracilis*. Under this assumption, the results presented above suggest that, by means of this behaviour, dominance hierarchies are established among workers in orphaned colonies of *P. gracilis*. The high rankings of individuals with developed ovaries indicate that dominance behaviour serves to regulate reproduction among potential egg layers, as similarly (concerning either dominance hierarchies or at least observation of aggressive behaviour) reported for other social Hymenoptera (e.g. *Pachycondyla inversa*, Kolmer & Heinze 2000; *Parischnogaster nigricans serrei* (Vespidae), Turillazzi & Pardi 1982; various ant and non-ant taxa, Bourke 1988, Röseler 1991).

In some of the queenless colony parts, more than one worker with well developed ovaries was found, suggesting that several high-ranking workers may share reproduction, as similarly reported for other species (e.g. *Harpagoxenus sublaevis*, Bourke et al. 1988; *Pa. apicalis*, Oliveira & Hölldobler 1990; *Pa. villosa*, Heinze et al. 1996). However, whether this indeed occurs or whether males are always the progeny of one single individual can only be unequivocally determined by genetic comparison of male offspring with putative egg layers (as done in *Pa. villosa*; Trunzer et al. 1999).

The timing of aggressive interactions in relation to queen removal and emergence of first eggs can vary greatly among species. While in *Pa. villosa* aggression among workers begins to increase ca. seven weeks after colony orphaning together with the first eggs being laid (Heinze et al. 1996), the frequency of dominance interactions among workers of *P. gracilis* rose only few days after colony separation and decreased with the start of egg laying (one to two weeks after orphaning).

It must be added that the period until egg laying by workers reported for *Pa. villosa* only holds for groups of workers that were isolated not only from the queen but also from any brood, in contrast to this study. When larvae were present in the orphaned colony parts of *Pa. villosa*, no eggs were found even after “more than 105 days” (Heinze et al. 1996), rendering the difference to *P. gracilis* even more pronounced.

Such “larval inhibition” which can partly be explained by eggs being fed to larvae (Heinze et al. 1996) does obviously not occur in *P. gracilis* (since egg numbers increased despite the presence of larvae and because no egg-feeding behaviour was observed).

Hierarchy formation may be viewed as self-organising process that cannot be accurately predicted by independently analysing dyadic relationships because social context may have considerable influence on how individuals fare in dominance contests (Chase 1980; Chase et al. 2002; Chase & Seitz 2011). Furthermore, intrinsic dominance strength of individuals might change over time without identifiable causes (e.g. due to aging processes or diseases). This calls for analysing sequences of interactions rather than pooling them in matrices – a method that assumes stability of properties like dominance strength (or, accordingly, dyadic winning probabilities) over the pooled period of time.

Dynamics of hierarchy formation have been studied in ants but because analyses were based on interaction matrices large periods of time had to be summarised (e.g. Oliveira & Hölldobler 1990; Monnin & Peeters 1999). This technique allows only studying of hierarchy changes after conspicuous events such as (experimentally or naturally) altered group composition (Oliveira & Hölldobler 1990; Monnin & Peeters 1999). Examination of changes within shorter time spans is not possible with such temporally “coarse” matrices.

To analyse hierarchy formation in more detail, Elo rating appears to be a formidable tool because ratings are quickly established and steadily updated with every new interaction, thus providing high temporal resolution (Albers & de Vries 2001). Moreover, a single rating value integrates the complete interaction history of an individual (accounting for the dominance strength of an interaction partner at the time of each encounter), thus allowing – with a certain degree of confidence – comparisons of individuals that were never directly observed interacting (Albers & de Vries 2001).

However, since only two colonies were sampled, any of the following conclusions based on Elo ratings obtained from Experiment 2 must be treated with caution.

Nevertheless, the results can be regarded as helpful for interpretation of other data and conduction of future studies.

The Elo ratings corroborate the conclusion of Experiment 1 that the most dominant (or aggressive) individuals usually have the largest ovaries and represent the egg layers of orphaned colonies of *P. gracilis*. To reveal such overall patterns, a reduced dataset as obtained in Experiment 1 appears to be sufficient (as long as enough colonies and individuals per colony are sampled) because in both examined colonies of Experiment 2 the future top rankers consistently exhibited the largest proportions of boxings and early reached a stable rank.

On the other hand, whenever relationships between certain individuals are the subject of interest, matrix-based dominance measures such as dominance indices may lead to erroneous conclusions because of the dynamics in hierarchy formation as especially observed during the first days after colony separation. Furthermore, rank differences in colonies of *P. gracilis* appear to be more pronounced among top-ranking individuals and between top- and low-ranking workers than among low-rankers, as also reported for the ponerine ant species *Pa. apicalis* (Oliveira & Hölldobler 1990; Blacher et al. 2010).

Another reason for cautious interpretation of the results of Experiment 2 are inconsistencies with Experiment 1 both in experimental design and in the data obtained. In Experiment 2, colonies were exposed to 24 h light per day and probably also to unfavourable humidity levels. While the latter might not be problematic (as *P. gracilis* naturally occurs in wet as well as dry habitats), the unnatural light conditions might have influenced the behaviour of the ants and might therefore be responsible for such phenomena as the prolonged period of high boxing frequency and late (or even lacking in colony PS-Fl2) appearance of worker-laid eggs compared with Experiment 1. With this in mind, one might speculate that under natural conditions dominance relationships are settled earlier than observed in Experiment 2. To confirm this, further experiments should be conducted with natural light rhythms, accepting gaps in observational data which might be partly countered by increasing the constant *k*.

4.3 Worker policing

The high levels of aggression in reunited queenright colonies (phase 3) might partly be explained by chemical divergence of the split colony parts during separation (phase 2), leading to discrimination against former nest mates that – at that moment – were recognised as aliens. However, workers with developed ovaries suffered significantly more from attacks than their nest mates with small ovaries from the same, orphaned colony part. This shows that, in *P. gracilis*, worker reproduction is not tolerated when a fertile queen is present. Moreover, policing acts on the level of adult individuals instead of worker eggs, which may be laid but eaten as an alternative policing mechanism. Both forms of policing are well documented from other social Hymenopteran species (e.g. *Aphaenogaster cockerelli*, Smith et al. 2009; queenless ponerine ants, Monnin & Ratnieks 2001; various ant, bee and wasp species, Ratnieks & Wenseleers 2005 and Ratnieks et al. 2006; *Vespula atropilosa*, Landolt et al. 1977; *Vespula spp.*, Wenseleers et al. 2005).

The absence of workers with developed ovaries in the experimental queenright colonies (and also in further experimental and natural colonies, unpublished data) might be interpreted as “self restraint” (Wenseleers et al. 2004; Wenseleers & Ratnieks 2006) which means that the workers refrain from reproduction even though they would be principally capable to lay eggs. This behavioural strategy can evolve when the costs of reproduction (e.g. the risk of being policed) outweigh the benefits (Wenseleers et al. 2004) and may be a possible evolutionary route (Ratnieks 1988; Ratnieks & Reeve 1992) to complete worker sterility occurring in several ant species (e.g. *Linepithema humile*, Passera et al. 1988; a number of ecitonine and myrmicine species, Bourke 1988; several ponerine species, Villet et al. 1991).

The lack of ovary development in queenright colonies, together with the existence of worker policing in *P. gracilis*, and the exclusive assignment of queens as mothers of all genetically examined males, strongly indicate that no or only a small proportion of the males in natural colonies are produced by workers. Thus, *P. gracilis* is an example for a monogynous, monandrous species with self-restraining and mutually policing workers, as also reported for other social Hymenoptera (e.g. *Aphaenogaster smythiesi*, Iwanishi et al. 2003; *Diacamma* sp., Kikuta & Tsuji 1999; *Polistes chinensis*, Saigo & Tsuchida 2004; *Vespa crabro*, Foster et al. 2002).

Altogether, the results of the present study are clear evidence against the relatedness hypothesis of male origin which has received much support (Ratnieks et al. 2006) but apparently cannot account for all social Hymenopteran societies (Hammond & Keller 2004). Specifically, it assumes that worker reproduction is not associated with costs that lower the total reproductive output of the colony. However, the results on *P. gracilis* indicate that reproduction costs override the benefits from relatedness alone, thus favouring the efficiency hypothesis (Hammond & Keller 2004).

4.4 Fertility signalling

As the previous section emphasised, workers of *P. gracilis* are obviously capable to discriminate nest mates according to their reproductive status. Most probably, they use chemical cues present on the cuticulae of their nest mates. Evidence available up to date – mostly correlative in nature – suggests CHCs, mainly alkenes and branched alkanes, as possible fertility signals (reviewed by Monnin 2006 and complemented by later studies, e.g. Smith et al. 2008 and 2009). Recently, Holman et al. (2010) reported the existence of a queen primer and releaser pheromone – 3-methylhentriacontane – that affects worker ovarian development and behaviour in the formicine ant species *Lasius niger*. Moreover, the concentration of this substance on queen cuticulae was reduced after an immune challenge, revealing its production as costly. Thus, CHCs can indeed be considered as serious candidates for honest fertility signalling in ants.

In this study, CHC profiles of *P. gracilis* were investigated, revealing that queens are well distinguishable from workers, which have less developed ovaries, on the basis of their CHC profiles. Consequently, clear differences among castes (and thus among fertility levels due to segregation of castes by ovary size) could be detected. So, the CHC profiles provide sufficient information to serve as caste and probably also fertility signals.

However, among-worker differences associated with ovary development were weak, regarding mainly substances whose amount rose with ovary size (e.g. 7me-C_{33}) but also compounds that are apparently produced in high amounts by non-reproducing individuals while lacking in reproductive individuals (tentatively identified as carbon acids, unpublished data). The latter phenomenon was found in at least one other species (the ponerine *Streblognathus peetersi*, Cuvillier-Hot et al. 2004; however, the corresponding substances were not selected by PCA for statistical analysis), raising "the possibility that the disappearance, rather than the elevation, of a subset of compounds marks the fertile individuals" (Le Conte & Hefetz 2008).

The weak statistical evidence for a fertility signal might be due to: (1) the high inter-individual variability of CHC profiles, including the chemomorph phenomenon; (2) the limited duration of the orphaned-colony phase in the experiment; or (3) the conservative approach to pool substances that were in many cases not clearly separated during gas chromatography.

Indeed, published CHC profiles of other species appear to be mostly less complex (regarding number of compounds) than those of *P. gracilis* (e.g. Sledge et al. 2001; Dietemann et al. 2003; Hartmann et al. 2005), so the statistical noise may be stronger in the CHC analysis of the present study. The two chemomorphs found in the dataset of this study pose a substantial problem to the analysis and interpretation of the data because chemical differences among ovary sizes were (within workers) significant in only one of them (with the smaller sample size). As this phenomenon appears to be a laboratory artefact, natural colonies should be chemically analysed to elucidate occurrence and significance of this kind of variation. In a first, small sample of *P. gracilis* workers taken directly from field nests, CHC profiles appeared to differ at least as much as laboratory samples, with chromatograms looking similar to the two described chemomorphs and one or two additional morphs (unpublished data). This suggests that there may be an even greater variability in CHC profiles within this ant population than recognised in the present study.

For this reason, it would be of interest to examine the extent and the causes of this complex chemomorph phenomenon. Natural co-occurrence of multiple chemomorphs might be linked to cryptic speciation. Chemical signalling is known to play a major role in such processes, e.g. in the myrmecophilous and morphologically very similar hover fly species *Microdon mutabilis* and *M. myrmicae* (Schönrogge et al. 2002,

2008). Moreover, CHC profiles proved to be a reliable taxonomic tool for discriminating among species of ants and other insects with strongly resembling morphology (Akino et al. 2002; Lucas et al. 2002; Martin et al. 2008; Kather & Martin 2012). Hence, similar ecologically or behaviourally driven divergences might occur in *P. gracilis*, for example upon choice of mating partners or in the context of nest mate or parasite recognition. However, testing the study population for cryptic speciation would require large-scale genetic and chemical analyses going beyond the scope of this study.

The time after which individuals developing their ovaries become chemically distinguishable from non-reproductive nest mates varies among social Hymenopteran species from two days to a few weeks (Monnin 2006). In the present study, the duration of the separation experiments (until egg production in the queenless parts) was obviously sufficient to enable the ants to recognise the changed fertility state of some of their nest mates. Hence, if the assumption that CHCs are used as fertility recognition cues is true the data analysis should have revealed such differences.

However, besides the statistical noise mentioned above, there is another confounding factor: The technical equipment used (gas chromatograph and mass spectrometer) might have been less sensitive to differences in CHC amounts than the ants were in the experiments. This is not unlikely owing to the exceptional chemical sensitivity of insect antennae (Schroth et al. 2001). If the ants had been more sensitive than the equipment, the end of the experiments might have fallen in a period when the putative fertility signal was strong enough to be detected by the ants but not by the machines. Further experiments with prolonged experiment duration should reveal whether the mere candidates for fertility cues suggested in this study can be significantly attributed to changes in ovarian development. The probability of increased chemical among-worker differentiation after a longer trial period is high, regarding that the experiments of this study were terminated after the first eggs had appeared (indicating that workers had not yet reached their full reproductive capability) and that in other ant species chemical signals are not a mere on/off signal but gradually correlate with fertility (Cuvillier-Hot et al. 2004). Additionally, a prolonged temperature program (with slower heating rate) combined with a better performing column in gas

chromatographic separation of CHC compounds might produce higher resolution and improve the statistical analysis.

4.5 Conclusions

In summary, the predictions of kin conflict theory are, as far as tested in this study, met in the ant species *P. gracilis*, representing the subfamily Pseudomyrmecinae, a taxon of social insects not thoroughly investigated for kin conflicts up to now.

Thus, this study inserts an important empirical part in the taxonomic gap between those clades that contain most of the studied species: the subfamilies of the poneroid group on the one hand and, on the other, the Formicinae and Myrmicinae which are, within the formicoids, only distantly related to the Pseudomyrmecinae (Brady et al. 2006; Moreau et al. 2006). However, whether *P. gracilis* represents the rule rather than an exception among pseudomyrmecines can only be determined by examining a number of additional species that might together be regarded as a sufficient surrogate for this species-rich ant subfamily.

Because of the difficulties of CHC analysis in the obtained samples, further separation experiments with extended duration as well as bioassays and GC-coupled electroantennograms with candidate compounds will be needed to support or reject the existence of a fertility signal (e.g. 7me-C_{33}) in *P. gracilis*.

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6. Abstract / Zusammenfassung

Abstract

Insect societies in the insect order Hymenoptera (bees, wasps, ants) are characterised by a high degree of cooperation but also of conflict potential. However, this was – within the framework of kin conflict theory – studied only in relatively few taxa. To broaden the empirical basis for this theory, colonies of the ant species *Pseudomyrmex gracilis* (Formicidae: Pseudomyrmecinae) were examined.

In 12 of 15 partly polygynous colonies, all analysed workers were inferred as full sisters. This suggests monogyny and monandry to be predominant in the population. In three cases, though, offspring was assigned to more than one queen and/or more than one male.

In presence of the queen, workers did not produce male offspring. Workers that were separated from the queen began to produce males. Within several days, they established dominance hierarchies by mutual (antennal) boxing. Especially the top ranks were clearly separated from the lower ranks and remained stable after being established. The highest ranking workers had in average the largest ovaries and were severely attacked when reunited with the queenright colony part.

An analysis of cuticular hydrocarbons revealed significant differences between queens and workers. However, within the worker caste no clear evidence for a fertility signal could be found. This may partly be due to the high variability of the chemical profiles.

The present study confirms predictions derived from kin conflict theory for a species of the subfamily Pseudomyrmecinae that has, up to now, not been examined in this context. Additionally, the results contradict the hypothesis that in monogynous, monandrous colonies a significant part of the male progeny should be produced by workers, thus supporting the alternative view that colony-level costs may outweigh individual benefits and even egoistic workers may fare better by reproductive self-restraint to the good of the group.

Zusammenfassung

Insektenstaaten aus der Insektenordnung der Hautflügler (Hymenoptera: Bienen, Wespen, Ameisen) sind geprägt von einem hohen Grad an Kooperation, aber auch von großem Konfliktpotenzial. Dieses wurde im Rahmen der Verwandtenkonflikttheorie allerdings nur in relativ wenigen, eng umgrenzten Verwandtschaftsgruppen erforscht. Um die empirische Basis für diese Theorie zu erweitern, wurden Kolonien der Ameisenart *Pseudomyrmex gracilis* (Formicidae: Pseudomyrmecinae) untersucht.

In 12 von 15 zum Teil polygynen Kolonien wurden alle analysierten Arbeiterinnen oder Königinnen als Vollschwestern eingestuft, was für den Großteil der Population funktionelle Monogynie und Monandrie nahelegt. In drei Fällen wurden allerdings Nachkommen von mehr als einer Königin und/oder mehr als einem Männchen in derselben Kolonie nachgewiesen.

Arbeiterinnen, die von der Königin getrennt wurden, begannen, Männchen zu produzieren. Dabei etablierten sie binnen weniger Tage Dominanzhierarchien mittels eines ritualisierten „Box“-Verhaltens. Vor allem die obersten Ränge waren von den restlichen klar getrennt und blieben nach Etablierung stabil. Die ranghöchsten Arbeiterinnen hatten im Schnitt die größten Ovarien und wurden heftig attackiert und zum Teil getötet, wenn sie mit dem Kolonieteil mit Königin zusammengeführt wurden.

Eine Analyse der kutikulären Kohlenwasserstoffe ergab signifikante Unterschiede zwischen Königinnen und Arbeiterinnen. Innerhalb der Arbeiterinnen gab es keine eindeutigen Nachweise eines Fruchtbarkeitssignals, was zum Teil an der großen Variabilität der chemischen Profile liegen könnte.

Die vorliegende Studie bestätigt die Vorhersagen der Verwandtenkonflikttheorie für eine Art der bisher in diesem Kontext nicht untersuchten Unterfamilie Pseudomyrmecinae. Zusätzlich widersprechen die Ergebnisse der Hypothese, dass bei Monogynie und Monandrie ein großer Teil der Männchen von Arbeiterinnen produziert wird, und stützen die alternative Ansicht, dass Kosten, die für die Kolonie durch Arbeiterinnenproduktion entstehen, den individuellen Vorteil mehr als ausgleichen können.

7. Appendix

7.1 Further details of COLONY analysis

For assessing reliability of analysis, five independent runs with different random number generator seeds were conducted, all based on the same data. In all cases, the log-likelihood function (over progress of likelihood maximisation) stabilised well before the end of the run, with virtually equal log-likelihood values (mean: –1667.19; range: –1667.70 to –1666.32), indicating satisfactory performance. A closer inspection of the output files revealed no relevant differences among runs.

At two loci (Pg08 and Pg09), genotype data was largely fragmentary, except for colony PS086 in which data for these loci was complete but missing for loci Pg02 and Pg15. To examine whether these gaps in the data caused a strong bias, the analysis was repeated (same settings, again five runs) without colony PS086 and without loci Pg08 and Pg09. The results were qualitatively and quantitatively highly consistent with the analysis of the whole dataset and consequently not reported.

7.2 Interaction matrices of Experiment 1

The following pages contain interaction matrices for *P. gracilis* workers in orphaned colony parts, based on observations as described in the methods section and used for behavioural analysis (see, for example, Figures 9 and 10). In each cell of the matrix (besides the diagonal), representing a dyad of two workers, the number of boxing acts of the row individual (“actor”) towards the column individual (“recipient”) is given. Consequently, row and column sums represent the total per-individual number of boxing acts given and received, respectively. Below the row sums (to the right of the column sums), the total number of observed interactions is presented. The columns to the right of the interaction matrix provide information on ovary size and dominance rating (*FDI*) for each individual. Colours denote whether individuals were treated aggressively by nest mates upon reunification of formerly separated colony parts: dark blue – attacked and killed; bright blue – attacked but not killed.

attacked and killed

attacked but not killed

PS055

Actor	Recipient															
	nopi	orno	grbl	yebl	grno	oror	piye	yepi	yegr	blbl	grye	pibl	grgr	yeor	pior	gror	Sum
nopi	x	35	1	7	8	14		1	4	3	4	4	4	5	22		112
orno	3	x				3	1	3	1	2	1	2	4	5	8		33
grbl			x														0
yebl				x													0
grno					x												0
oror						x									3		3
piye							x										0
yepi								x									0
yegr									x								0
blbl										x							0
grye											x						0
pibl												x					0
grgr													x				0
yeor														x			0
pior															x		0
gror																x	0
Sum	3	35	1	7	8	17	1	4	5	5	5	6	8	10	33	0	148

Individual	**Ovary size**	**FDI**
nopi	4	3.8205
orno	1	3.8205
grbl	1	0.2
yebl	1	0.0909
grno	1	0.0833
oror	1	0.0714
piye	1	0.0270
yepi	1	0.0233
yegr	1	0.0227
blbl	1	0.0227
grye	1	0.0227
pibl	1	0.0222
grgr	1	0.0213
yeor	1	0.0204
pior	1	0.0112
gror	2	-

PS060

	Recipient																							
Actor	orgr	nopi	yeye	grye	blye	noor	noye	pibl	yegr	orno	grpi	grbl	blbl	orpi	gror	nobl	oror	yeno	piye	blgr	pior	blno	blor	Sum
orgr	x	2	6	1	6	4	5	4	1		1													30
nopi	1	x	7	4	4	24	11	1	5		8				1	2								68
yeye		4	x	5	13	11	8	4	10	4	3	2				4				3	1			72
grye			1	x	5	3	3	3	1	4	7					3	1	1		1	2			35
blye			3	4	x	21	2		6		5					1					1			43
noor			1	2	9	x	6	3	4	2	2							1	2	1				33
noye			2		1	2	x	4			1				1	3	2		1		2			19
pibl		1			2		3	x					1	1		5				2	1			16
yegr				1		1	1	2	x		4													9
orno				2			1			x	2								1		1			7
grpi				1		2				1	x													4
grbl												x				1								1
blbl													x											0
orpi														x										0
gror															x									0
nobl																x	1			1				2
oror																	x							0
yeno																		x						0
piye																			x					0
blgr																				x				0
pior																					x			0
blno																						x		0
blor																							x	0
Sum	1	7	20	20	40	68	40	21	27	11	33	2	1	1	2	19	4	2	4	8	8	0	0	339

Individual	**Ovary size**	**FDI**
orgr	4	36.6667
nopi	1	6.6400
yeye	2	1.5736
grye	1	1.0570
blye	2	1
noor	-	0.9300
noye	4	0.7422
pibl	1	0.7268
yegr	1	0.6359
orno	1	0.4314
grpi	1	0.2985
grbl	1	0.1739
blbl	1	0.0435
orpi	-	0.0435
gror	1	0.0200
nobl	1	0.0176
oror	1	0.0119
yeno	1	0.0110
piye	1	0.0081
blgr	1	0.0064
pior	1	0.0062
blno	-	-
blor	-	-

PS061

	Recipient																												
Actor	yeno	blor	orbl	grbl	nobl	nogr	orgr	oror	noye	orye	pibl	grgr	piye	blpi	blgr	pigr	grpi	blbl	yebl	gror	nopi	pino	grno	grye	noor	orno	yegr	yepi	yeye	Sum
yeno	x				2	2		4						1	2	1					1									13
blor		x			2			1	2			1	2	1	1								1							11
orbl			x		2			2	1			1		1		1														8
grbl				x														1												1
nobl					x				1			1				3														5
nogr						x	1	2			1		1	2					1											8
orgr					1		x			1	1			1		1		1		2										8
oror						1		x				1											1							3
noye							1		x												1									2
orye										x		1									1									2
pibl										1	x	1		2			1													5
grgr						1					1	x	2			1														5
piye												1	x	1																2
blpi														x	1	1														2
blgr															x					3										3
pigr							1									x						1	1							3
grpi																	x													0
blbl																		x												0
yebl																			x											0
gror																				x		1								1
nopi																					x									0
pino																						x								0
grno																							x							0
grye																								x						0
noor																									x					0
orno																										x				0
yegr																											x			0
yepi																												x		0
yeye																													x	0
Sum	0	0	0	0	7	4	3	9	4	2	3	7	5	9	4	8	1	2	1	5	3	2	3	0	0	0	0	0	0	82

Individual	**Ovary size**	**FDI**
yeno	3	38
blor	2	34
orbl	4	29
grbl	1	2
nobl	2	1.4545
nogr	-	1.3810
orgr	3	1.35
oror	2	1.2143
noye	2	0.9167
orye	2	0.8889
pibl	2	0.8333
grgr	2	0.7059
piye	2	0.5882
blpi	2	0.36
blgr	1	0.3571
pigr	2	0.3429
grpi	1	0.2
blbl	1	0.1667
yebl	-	0.1667
gror	2	0.1538
nopi	1	0.1
pino	1	0.0625
grno	1	0.0476
grye	-	-
noor	-	-
orno	1	-
yegr	-	-
yepi	1	-
yeye	-	-

PS063

	Recipient																									
Actor	pior	grgr	blor	yegr	grno	yebl	blbl	nogr	blye	pino	gror	yepi	orbl	noye	blpi	noor	yeye	nobl	blgr	orye	orgr	grbl	blno	piye	yeor	grpi	Sum
pior	x			6		2		1	1			2			1	2	2										17
grgr		x							1		1	1						2									5
blor			x			1																					1
yegr				x		3		2			7	1	3		3		4	1		2	2						28
grno					x	1			1						1	1											4
yebl				2		x		3	2		3	2	4		6		3	1		5	2						33
blbl					1		x						1						1								3
nogr					3	3		x	4	1	1	2	3	1	4	7	1	4		5	5	1			1		46
blye						1		3	x		4	1	2		3	3		1			2						20
pino					1			2		x					1			1				1	2	1	1		10
gror				11		4		4	6		x	7	5		8	8	4			5	4		1		1		68
yepi				2				1			1	x			3	3	1				2						13
orbl								3	1		2		x			3		2		1	1						13
noye													1	x		1											2
blpi									1	2	1	1			x	1				2	1				1		10
noor											1				1	x				2	1						5
yeye				1		1											x				2						4
nobl							1		1								1	x		2							5
blgr																			x								0
orye								2							2					x				1			5
orgr													1								x						1
grbl																						x					0
blno																							x				0
piye																								x			0
yeor																									x		0
grpi																										x	0
Sum	0	0	0	22	5	16	1	21	18	3	21	17	20	1	33	29	16	12	1	24	22	2	3	2	4	0	293

Individual	**Ovary size**	**FDI**
pior	3	177
grgr	4	112
blor	4	35
yegr	3	2.4409
grno	1	2.3548
yebl	4	2.0583
blbl	1	1.5000
nogr	3	1.4724
blye	-	1.4626
pino	1	1.3103
gror	1	1.2475
yepi	2	1.1812
orbl	2	1.1469
noye	1	0.9130
blpi	4	0.6333
noor	3	0.5422
yeye	1	0.5317
nobl	3	0.3363
blgr	-	0.3333
orye	1	0.3116
orgr	3	0.0636
grbl	1	0.0370
blno	1	0.0357
piye	1	0.0333
yeor	1	0.0120
grpi	3	-

PS065

		Recipient																											
		nogr	yepi	blor	noye	orgr	grye	yeye	nopi	grpi	pibl	pior	yebl	pipi	grbl	pigr	yeno	gror	grgr	yeor	piye	yegr	blye	nobl	noor	orye	orpi	blbl	Sum
Actor	nogr	x	1													1		1											3
	yepi		x		1		1		1		1	2				1	3	1			1								12
	blor			x					1			1				2													4
	noye				x				3		1	1	2	4	3	7	1	2			1								25
	orgr				1	x	1	1	1		1				2	2		1	1	1	1								13
	grye		2		1	1	x	2	4		1	2	3	13	5	4	2	3		1	4					1	2		51
	yeye				2	2	2	x						1	1	2		1	3		2			1					17
	nopi		1		3		2		x		1	1	4	1	4	3	1	2			2								25
	grpi					1	1	1		x		1				2				1									7
	pibl				1		1		2		x	2	1	2		4	1			1	3					1			19
	pior			1			1		4	1	3	x	1	2	4	7	3	3		2	3			1	1				37
	yebl						1	1	1			3	x	2	1	2		1		1	2			1			1		17
	pipi					4	1	1	4	1	2	4	8	x	5	5		4		2	7				3		1		52
	grbl					2			1		1	2	2	3	x	5	2	5	2	2			1						28
	pigr						2	2	6	2	2	7	4	5	4	x	1	2			1					1			39
	yeno								1		2		1	3	1		x	1									1		10
	gror					1		1				1	5	8		2	1	x			1		1						21
	grgr													1					x										1
	yeor				1					1						1				x	1	1							5
	piye													2		1		2	1		x								6
	yegr																					x							0
	blye																						x						0
	nobl																							x					0
	noor																								x				0
	orye																									x			0
	orpi																										x		0
	blbl																											x	0
	Sum	0	4	1	10	11	13	9	29	5	15	27	31	47	30	51	15	29	7	11	29	1	2	3	4	3	5	0	392

Individual	**Ovary size**	**FDI**
nogr	4	76
yepi	2	5.2340
blor	4	3.6552
noye	3	2.7184
orgr	1	1.7310
grye	2	1.5556
yeye	1	1.2893
nopi	2	1.2035
grpi	1	1.1972
pibl	1	1.1344
pior	1	1.0990
yebl	1	1.0034
pipi	1	0.9854
grbl	2	0.9781
pigr	1	0.9348
yeno	1	0.7723
gror	1	0.6339
grgr	1	0.6207
yeor	1	0.4346
piye	2	0.3785
yegr	-	0.0769
blye	1	0.0161
nobl	-	0.0141
noor	1	0.0127
orye	1	0.0120
orpi	1	0.0089
blbl	1	-

PS081

	Recipient															
Actor	orpi	grbl	pigr	piye	blbl	yebl	grno	gror	grye	blno	nopi	orno	pibl	pino	yeye	Sum
orpi	x	2	4	3					2							11
grbl		x	3	2	1	1			7							14
pigr		5	x	5	1	2	1	1	12							27
piye		1	7	x	1				5							14
blbl			2		x	1										3
yebl		2	7			x			1							10
grno							x									0
gror								x								0
grye				2		1			x							3
blno										x						0
nopi											x					0
orno												x				0
pibl													x			0
pino														x		0
yeye															x	0
Sum	0	10	23	12	3	5	1	1	27	0	0	0	0	0	0	82

Individual	**Ovary size**	**FDI**
orpi	4	70
grbl	4	1.4118
pigr	3	1.3333
piye	1	0.8493
blbl	1	0.8367
yebl	1	0.7971
grno	1	0.0400
gror	1	0.0400
grye	1	0.3590
blno	1	-
nopi	-	-
orno	-	-
pibl	1	-
pino	-	-
yeye	-	-

PS083

Actor	Recipient																					
	orpi	grno	yeor	pigr	noor	grbl	orgr	nobl	piye	nopi	yepi	nogr	pior	blor	orye	orbl	pibl	pino	grye	grpi	noye	yebl	Sum
orpi	x			1	2	2	5	2		1	1	1		2	1	1							19
grno		x		1	1		2	1	1	2	2	1											11
yeor	1	1	x	3	4		2					1		1	3		1						17
pigr		1		x		2	4	3	2	3	9	5		4	4	3	2	1	3				46
noor				1	x	4	1	7		7	4	1		3	2	1			1				32
grbl				3		x	1	2	3	5	1	5	1	7	3	5	3		5	1	1		46
orgr			2		2	3	x	1	1		3	4		2	1	1	2		4				26
nobl	1			7	2	8	7	x		4	9	2	1	6	3	3	5		3				61
piye						2		1	x	1	3	1	1	2		2		2	1				16
nopi			1	2	5	5	7	2		x	4	6		3	1	1	1		3				41
yepi					2	1	2	1	1	1	x	3	2	3	2		1						19
nogr				2	3	3	5		1	10	5	x	1	4	3	2	2		5				46
pior								3	1		2		x	1	2		1		1	1			12
blor					1			1	1	4	2	3		x	1	3				1			17
orye						1				2	1	2	3	1	x	1	1		1				13
orbl				1				2	2	3				3	2	x	1		3				17
pibl								2		1			1			1	x						5
pino													1					x					1
grye										1		1				1	3		x				6
grpi														1						x			1
noye																					x		0
yebl																						x	0
Sum	2	2	3	21	22	31	36	28	13	45	46	36	11	43	28	25	23	3	30	3	1	0	452

Individual	**Ovary size**	**FDI**
orpi	4	11.2941
grno	4	11.0741
yeor	4	2.7412
pigr	4	1.7222
noor	4	1.4053
grbl	3	1.2071
orgr	2	1.1795
nobl	1	1.1662
piye	-	1.0679
nopi	2	0.9924
yepi	1	0.9178
nogr	1	0.8863
pior	1	0.6864
blor	1	0.6701
orye	1	0.5899
orbl	1	0.5807
pibl	1	0.3764
pino	1	0.3684
grye	2	0.3547
grpi	1	0.2135
noye	1	0.0303
yebl	1	-

PS086

		Recipient										
		yepi	nogr	yegr	yeno	orno	blor	yeye	pibl	grno	yeor	pino	Sum
Actor	yepi	x	12	21	8	4	15	11		11			82
	nogr	6	x	2	4	3	12	9		6			42
	yegr	7	2	x	8	1	6	3	1	9			37
	yeno	2	5	9	x	1	6	3		18			44
	orno	2	1	1	2	x	1			1			8
	blor	4	2	2	7	1	x	4		4			24
	yeye						1	x					1
	pibl								x				0
	grno									x			0
	yeor										x		0
	pino											x	0
	Sum	21	22	35	29	10	41	30	1	49	0	0	238

Individual	**Ovary size**	**FDI**
yepi	1	1.5031
nogr	1	1.5031
yegr	1	1.5031
yeno	1	1.5031
orno	1	1.4969
blor	-	1.2646
yeye	1	0.1453
pibl	1	0.0270
grno	2	0.0048
yeor	4	-
pino	-	-

PS102

		Recipient													
		yegr	grpi	yeye	pior	blor	grgr	nobl	orye	gror	yebl	pigr	blpi	noor	orpi	Sum
Actor	yegr	x	2	2	14	6				1	5	1				31
	grpi		x	1	2	2										5
	yeye			x	1	1						1				3
	pior			1	x	1	1	1	1	1	1	1				8
	blor				1	x										1
	grgr						x									0
	nobl							x								0
	orye								x							0
	gror									x						0
	yebl										x					0
	pigr											x				0
	blpi												x			0
	noor													x		0
	orpi														x	0
	Sum	0	2	4	18	10	1	1	1	2	6	3	0	0	0	48

Individual	**Ovary size**	**FDI**
yegr	1	49
grpi	1	6
yeye	1	0.52
pior	3	0.3714
blor	3	0.2857
grgr	-	0.05
nobl	1	0.05
orye	-	0.05
gror	1	0.0476
yebl	1	0.04
pigr	2	0.0385
blpi	1	-
noor	1	-
orpi	-	-

PS104

	Recipient																			
Actor	gror	pino	blye	grno	piye	orpi	grgr	blpi	blor	blno	pibl	blbl	pigr	pipi	orno	pior	grye	orbl	blgr	Sum
gror	x	5	6	5	1	4	1	1	1	1	1	1	2	1		1		1		32
pino		x	4	3		3	1	2	2	6	1	6	2	5	7	3	4	3		52
blye		3	x	1		2						1	1		1	1	1	1		12
grno			1	x		2		1			1	3		1			1			10
piye					x															0
orpi				2		x					1									3
grgr							x													0
blpi								x										1		1
blor									x											0
blno										x										0
pibl											x					1				1
blbl												x						1		1
pigr													x							0
pipi														x						0
orno															x					0
pior																x				0
grye																	x			0
orbl																		x		0
blgr																			x	0
Sum	0	8	11	11	1	11	2	4	3	7	4	11	5	7	8	6	6	7	0	112

Individual	**Ovary size**	**FDI**
gror	4	113
pino	4	4.05
blye	2	2.5484
grno	2	0.6905
piye	2	0.5
orpi	3	0.3571
grgr	1	0.0909
blpi	3	0.0833
blor	2	0.0833
blno	2	0.0625
pibl	2	0.0571
blbl	1	0.0476
pigr	1	0.04
pipi	1	0.0370
orno	1	0.0357
pior	1	0.0333
grye	1	0.0270
orbl	1	0.0238
blgr	1	-

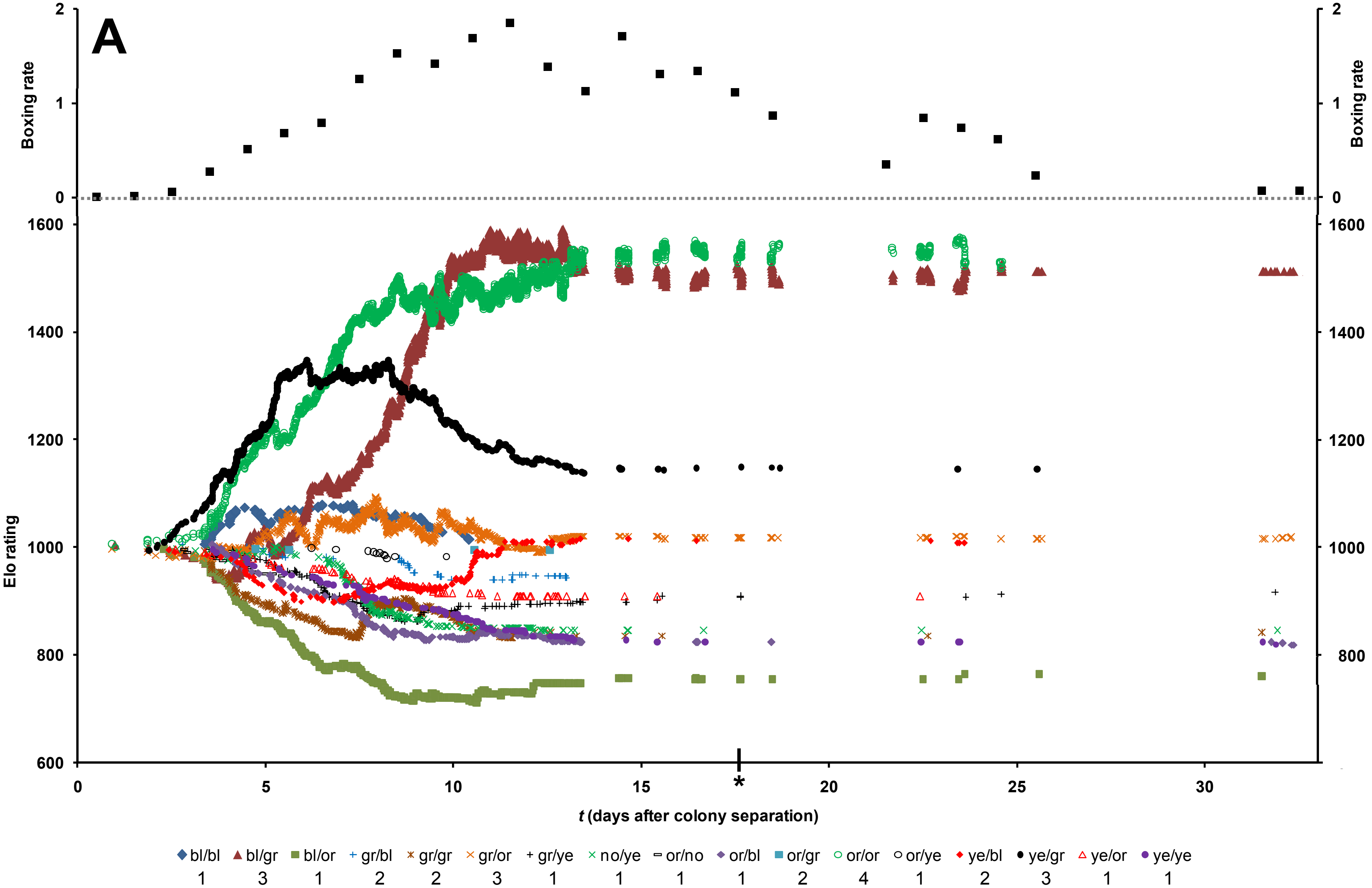

Figure 15 Elo ratings and boxing rates of colony PS-Fl1. **A:** Ratings for $k = 10$. Boxing rate = average number of observed boxing events per hour per individual. Below the individual names, ovary size scores are presented. * Discovery of first eggs. Individual or/or (green circles, single registered egg layer) was removed after 24 days of observation. Note that the Elo rating y-axis scaling does not begin with 0.

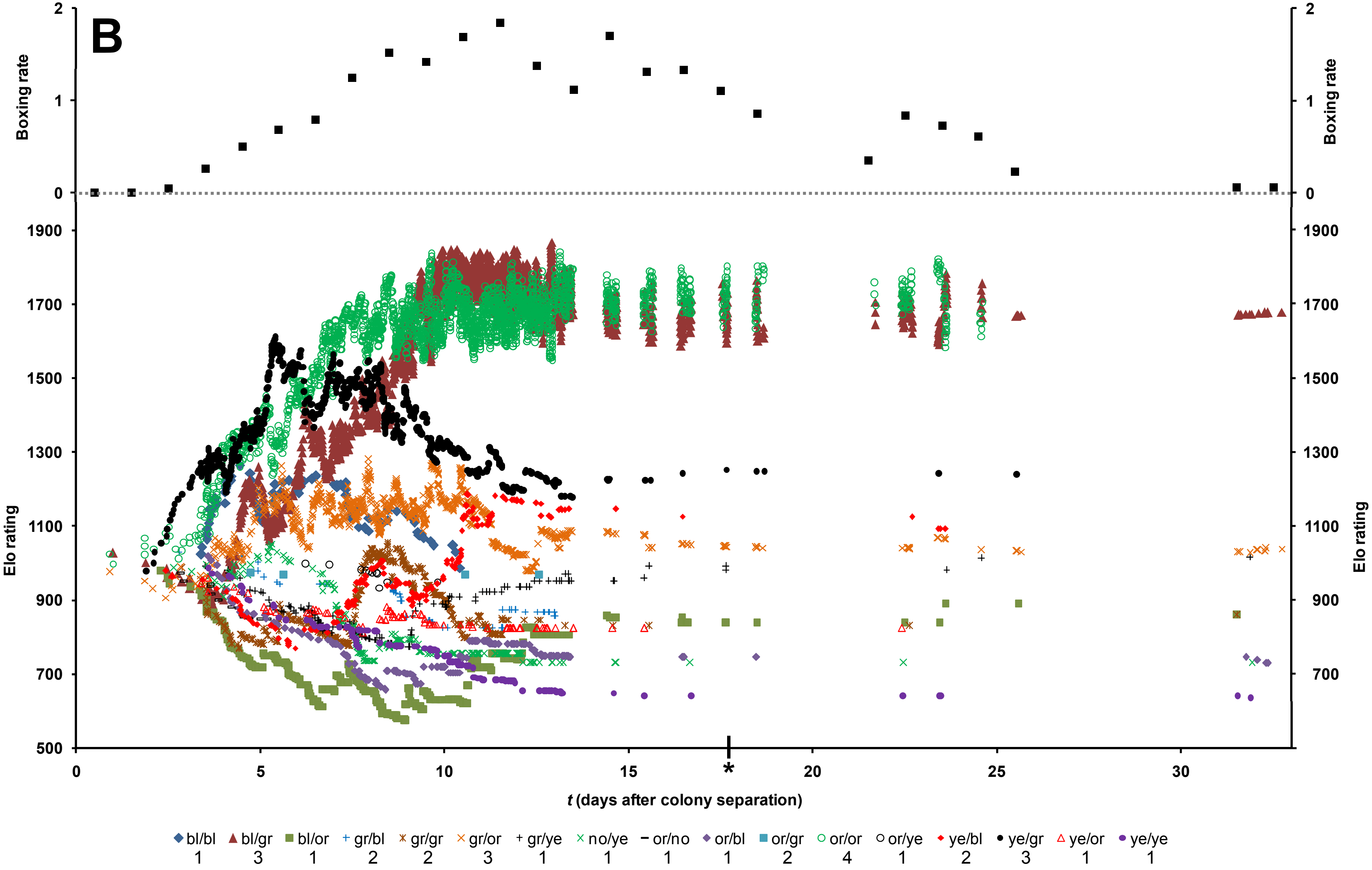

Figure 15 Elo ratings and boxing rates of colony PS-Fl1. **B:** Ratings for $k = 50$. Boxing rate = average number of observed boxing events per hour per individual. Below the individual names, ovary size scores are presented. * Discovery of first eggs. Individual or/or (green circles, single registered egg layer) was removed after 24 days of observation. Note that the Elo rating y-axis scaling does not begin with 0.

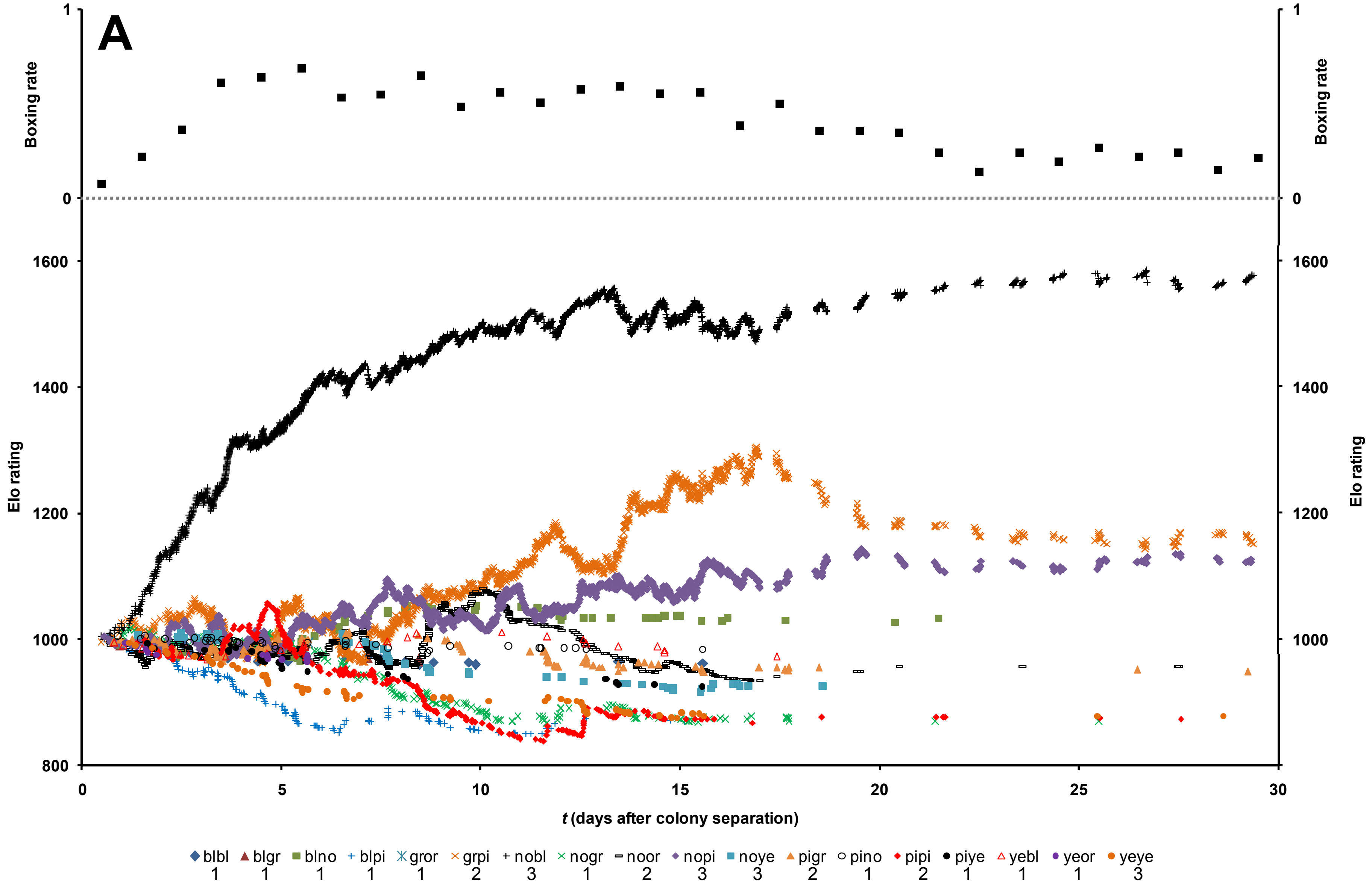

Figure 16 Elo ratings and boxing rates of colony PS-Fl2. **A:** Ratings for $k = 10$. Boxing rate = average number of observed boxing events per hour per individual. Below the individual names, ovary size scores are presented. Note that the Elo rating y-axis scaling does not begin with 0.

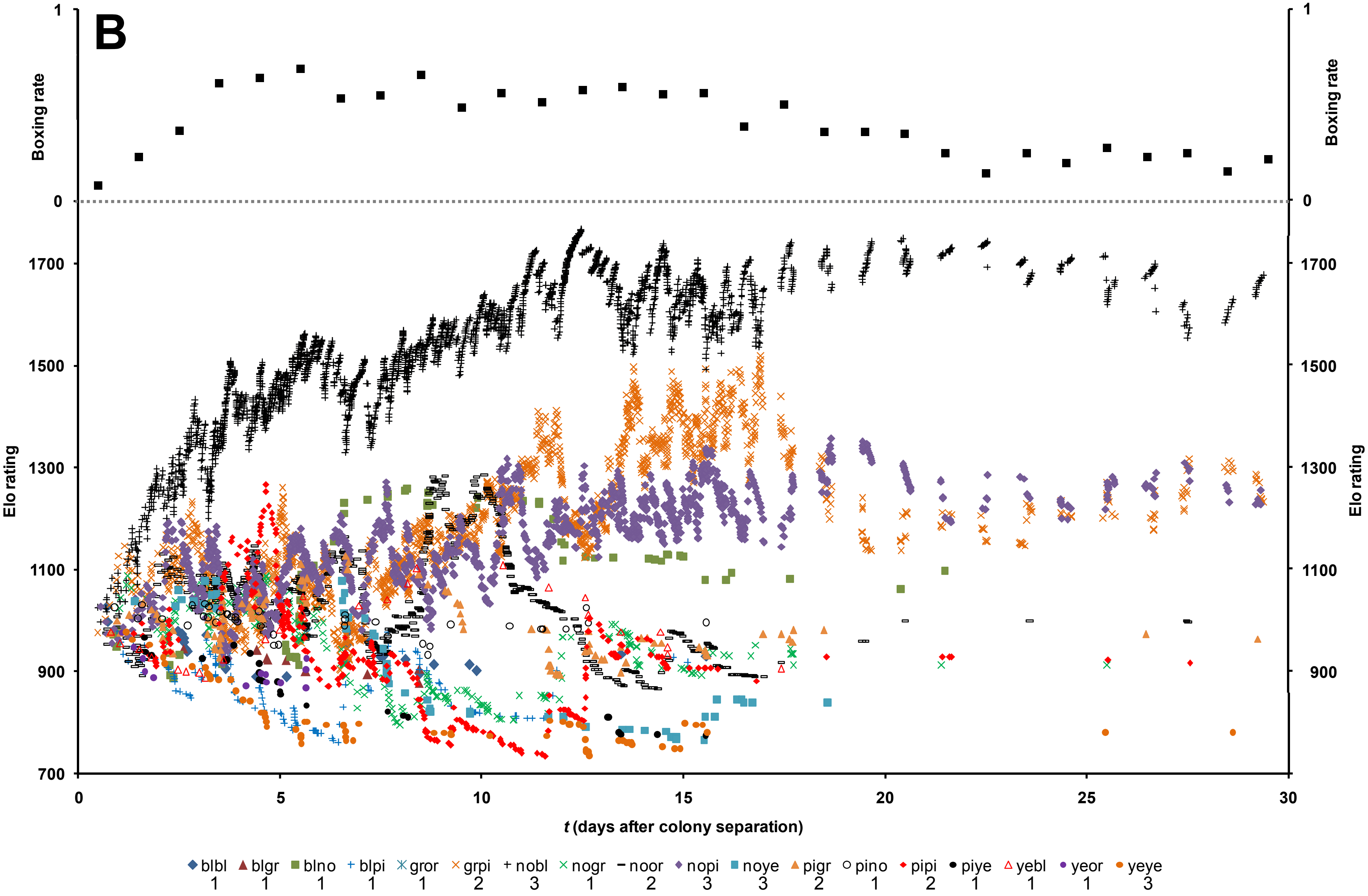

Figure 16 Elo ratings and boxing rates of colony PS-F12. **B:** Ratings for *k* = 50. Boxing rate = average number of observed boxing events per hour per individual. Below the individual names, ovary size scores are presented. Note that the Elo rating y-axis scaling does not begin with 0.

Table 7 Interaction matrices of colony PS-Fl1 in Experiment 2 for different time periods. Rows: boxing individuals; columns: boxed individuals; Ind.: individual name; Elo(10)/Elo(50): Elo ratings for *k* = 10 and *k* = 50, respectively; Ovary: ovary size score.

Whole observation period

	bl/bl	bl/gr	bl/or	gr/bl	gr/gr	gr/or	gr/ye	no/ye	or/no	or/bl	or/gr	or/or	or/ye	ye/bl	ye/gr	ye/or	ye/ye	Sums
bl/bl	x	1	1	0	13	12	1	3	0	1	0	2	0	1	2	2	3	42
bl/gr	11	x	121	6	80	213	14	45	2	79	2	774	4	38	94	17	35	1535
bl/or	0	0	x	1	0	4	0	3	0	0	0	4	0	1	0	1	2	16
gr/bl	0	0	0	x	0	0	0	0	0	0	0	2	0	0	0	0	0	2
gr/gr	0	1	8	0	x	7	3	5	0	3	0	1	0	3	4	1	1	37
gr/or	7	3	30	9	27	x	22	19	4	30	0	18	1	18	19	9	20	236
gr/ye	0	0	12	0	3	3	x	1	0	2	0	0	0	1	0	0	3	25
no/ye	0	0	2	1	4	1	3	x	1	3	1	0	0	0	0	1	1	18
or/no	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0	0	0
or/bl	1	0	0	0	1	2	0	0	0	x	0	1	0	0	0	0	0	5
or/gr	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0
or/or	15	787	243	21	99	435	55	122	4	113	1	x	0	63	149	45	80	2232
or/ye	0	0	0	0	0	0	1	0	0	0	0	0	x	0	0	0	0	1
ye/bl	0	1	20	1	10	5	2	2	0	6	0	3	0	x	0	2	5	57
ye/gr	20	94	126	7	125	63	21	25	4	17	0	48	4	20	x	8	6	588
ye/or	0	0	2	0	0	0	0	0	0	0	0	1	1	1	0	x	0	5
ye/ye	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	1
Sums	55	887	565	46	362	745	122	225	15	254	4	854	10	146	268	86	156	4800

Days 0-6

	bl/bl	bl/gr	bl/or	gr/bl	gr/gr	gr/or	gr/ye	no/ye	or/no	or/bl	or/gr	or/or	or/ye	ye/bl	ye/gr	ye/or	ye/ye	Sums
bl/bl	x	1	0	0	8	8	0	1	0	1	0	2	0	1	2	1	1	26
bl/gr	1	x	12	0	5	7	1	4	2	3	0	12	0	8	6	1	3	65
bl/or	0	0	x	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
gr/bl	0	0	0	x	0	0	0	0	0	0	0	1	0	0	0	0	0	1
gr/gr	0	0	0	0	x	1	1	0	0	0	0	1	0	1	0	0	1	5
gr/or	2	1	10	3	7	x	3	4	4	5	0	7	0	5	3	3	2	59
gr/ye	0	0	2	0	0	0	x	0	0	0	0	0	0	0	0	0	0	2
no/ye	0	0	1	1	3	1	2	x	1	2	1	0	0	0	0	1	0	13
or/no	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0	0	0
or/bl	0	0	0	0	1	0	0	0	0	x	0	0	0	0	0	0	0	1
or/gr	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0
or/or	2	52	36	2	6	33	6	6	4	7	1	x	0	12	19	4	5	195
or/ye	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0
ye/bl	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0
ye/gr	9	54	53	0	37	24	7	5	4	6	0	31	0	4	x	0	1	235
ye/or	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	x	0	1
ye/ye	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0
Sums	14	108	114	6	67	74	20	20	15	24	2	55	0	31	30	11	13	604

Ind.	Elo(10)	Elo(50)	Ovary
bl/bl	1022	986	1
bl/gr	1513	1678	3
bl/or	759	859	1
gr/bl	943	856	2
gr/gr	840	858	2
gr/or	1018	1038	3
gr/ye	917	1017	1
no/ye	844	729	1
or/no	946	838	1
or/bl	819	730	1
or/gr	993	967	2
or/or	1531	1704	4
or/ye	983	949	1
ye/bl	1007	1092	2
ye/gr	1145	1240	3
ye/or	908	823	1
ye/ye	820	636	1

Days 6-14

	bl/bl	bl/gr	bl/or	gr/bl	gr/gr	gr/or	gr/ye	no/ye	or/no	or/bl	or/gr	or/or	or/ye	ye/bl	ye/gr	ye/or	ye/ye	Sums
bl/bl	x	0	1	0	5	4	1	2	0	0	0	0	0	0	0	1	2	16
bl/gr	11	x	106	6	74	182	12	38	0	72	2	639	4	29	84	15	29	1303
bl/or	0	0	x	1	0	4	0	3	0	0	0	2	0	1	0	0	2	13
gr/bl	0	0	0	x	0	0	0	0	0	0	0	1	0	0	0	0	0	1
gr/gr	0	1	7	0	x	6	2	5	0	3	0	0	0	2	4	1	0	31
gr/or	5	2	19	6	20	x	19	15	0	23	0	11	1	12	16	6	18	173
gr/ye	0	0	10	0	3	1	x	1	0	2	0	0	0	1	0	0	1	19
no/ye	0	0	1	0	1	0	1	x	0	1	0	0	0	0	0	0	1	5
or/no	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0	0	0
or/bl	1	0	0	0	0	2	0	0	0	x	0	1	0	0	0	0	0	4
or/gr	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0
or/or	13	579	195	19	91	371	46	114	0	104	0	x	0	48	126	38	72	1816
or/ye	0	0	0	0	0	0	1	0	0	0	0	0	x	0	0	0	0	1
ye/bl	0	1	20	1	10	5	2	2	0	6	0	3	0	x	0	2	5	57
ye/gr	11	40	72	7	88	39	13	20	0	11	0	16	4	15	x	8	5	349
ye/or	0	0	2	0	0	0	0	0	0	0	0	0	1	1	0	x	0	4
ye/ye	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	1
Sums	42	623	433	40	292	614	97	200	0	222	2	673	10	109	230	71	135	3793

Days 14-25

	bl/bl	bl/gr	bl/or	gr/bl	gr/gr	gr/or	gr/ye	no/ye	or/no	or/bl	or/gr	or/or	or/ye	ye/bl	ye/gr	ye/or	ye/ye	Sums
bl/bl	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
bl/gr	0	x	2	0	1	13	1	2	0	2	0	123	0	1	3	1	2	151
bl/or	0	0	x	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2
gr/bl	0	0	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gr/gr	0	0	0	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0
gr/or	0	0	1	0	0	x	0	0	0	0	0	0	0	1	0	0	0	2
gr/ye	0	0	0	0	0	2	x	0	0	0	0	0	0	0	0	0	1	3
no/ye	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0	0	0	0
or/no	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0	0	0
or/bl	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0	0
or/gr	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0
or/or	0	156	12	0	2	32	3	2	0	2	0	x	0	3	4	3	3	222
or/ye	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0
ye/bl	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0
ye/gr	0	0	1	0	0	0	1	0	0	0	0	1	0	1	x	0	0	4
ye/or	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0
ye/ye	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0
Sums	0	156	16	0	3	47	5	4	0	4	0	126	0	6	7	4	6	384

Table 8 Interaction matrices of colony PS-Fl2 in Experiment 2 for different time periods. . Rows: boxing individuals; columns: boxed individuals; Ind.: individual name; Elo(10)/Elo(50): Elo ratings for *k* = 10 and *k* = 50, respectively; Ovary: ovary size score.

Whole observation period

	blbl	blgr	blno	blpi	gror	grpi	nobl	nogr	noor	nopi	noye	pigr	pino	pipi	piye	yebl	yeor	yeye	Sums
blbl	x	0	0	0	0	2	1	0	0	0	1	0	0	1	0	1	0	1	7
blgr	1	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
blno	1	1	x	1	0	5	5	2	4	3	0	1	0	0	1	1	0	1	26
blpi	0	0	0	x	0	5	2	2	0	3	0	0	2	1	1	2	2	0	20
gror	0	0	0	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
grpi	4	0	5	23	1	x	116	62	71	195	5	14	7	55	4	3	1	17	583
nobl	8	4	16	62	1	793	x	68	263	859	19	35	13	136	10	5	2	27	2321
nogr	0	1	4	5	0	13	4	x	0	8	0	1	0	8	0	1	2	3	50
noor	2	3	3	6	0	36	17	12	x	16	7	5	1	15	2	2	0	2	129
nopi	2	0	3	20	0	152	70	18	37	x	9	14	4	29	2	0	3	8	371
noye	0	0	1	0	0	2	2	0	2	1	x	0	0	1	0	0	0	0	9
pigr	1	0	1	1	0	7	7	2	2	4	0	x	1	3	0	0	0	1	30
pino	0	1	0	0	0	3	0	1	0	3	1	2	x	0	1	0	0	2	14
pipi	2	0	2	3	0	15	6	4	4	8	4	7	1	x	0	1	0	2	59
piye	0	0	0	0	0	1	0	0	0	0	0	0	0	0	x	0	0	0	1
yebl	0	0	0	0	0	4	0	0	0	1	0	2	0	1	2	x	0	0	10
yeor	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	x	1	3
yeye	0	0	0	0	0	1	0	2	0	0	0	0	0	0	1	1	0	x	5
Sums	21	10	35	121	2	1039	230	173	384	1101	46	81	29	250	25	17	10	65	3639

Days 0-3

	blbl	blgr	blno	blpi	gror	grpi	nobl	nogr	noor	nopi	noye	pigr	pino	pipi	piye	yebl	yeor	yeye	Sums
blbl	x	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
blgr	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
blno	0	0	x	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2
blpi	0	0	0	x	0	0	1	0	0	0	0	0	0	0	1	0	2	0	4
gror	0	0	0	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
grpi	2	0	1	2	0	x	14	7	4	8	0	1	2	5	0	1	1	2	50
nobl	4	1	1	13	0	32	x	6	31	37	2	7	0	6	1	4	0	2	147
nogr	0	0	2	1	0	1	2	x	0	1	0	1	0	0	0	1	1	2	12
noor	1	1	0	2	0	2	7	2	x	1	0	0	0	1	1	0	0	0	18
nopi	0	0	1	3	0	9	8	0	2	x	1	1	1	2	0	0	1	0	29
noye	0	0	0	0	0	1	1	0	0	0	x	0	0	0	0	0	0	0	2
pigr	0	0	1	0	0	1	1	0	0	0	0	x	0	1	0	0	0	0	4
pino	0	1	0	0	0	1	0	0	0	1	0	0	x	0	0	0	0	0	3
pipi	0	0	1	0	0	1	1	1	1	0	0	0	0	x	0	1	0	0	6
piye	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0
yebl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0
yeor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0
yeye	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0
Sums	7	3	7	21	0	48	38	16	38	48	3	10	3	15	3	7	5	6	278

Ind.	Elo(10)	Elo(50)	Ovary
blbl	962	930	1
blgr	970	905	1
blno	1034	1097	1
blpi	870	918	1
gror	994	976	1
grpi	1152	1232	2
nobl	1578	1677	3
nogr	870	911	1
noor	957	998	2
nopi	1128	1255	3
noye	925	838	3
pigr	949	964	2
pino	985	995	1
pipi	873	917	2
piye	926	775	1
yebl	973	905	1
yeor	975	926	1
yeye	879	781	3

Days 12-20

	blbl	blgr	blno	blpi	gror	grpi	nobl	nogr	noor	nopi	noye	pigr	pino	pipi	piye	yebl	yeor	yeye	Sums
blbl	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
blgr	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
blno	0	0	x	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	3
blpi	0	0	0	x	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
gror	0	0	0	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
grpi	0	0	2	0	0	x	53	12	27	99	1	4	0	8	0	1	0	7	214
nobl	0	0	9	0	0	288	x	19	48	311	10	6	3	43	3	1	0	7	748
nogr	0	0	1	0	0	0	0	x	0	1	0	0	0	3	0	0	0	0	5
noor	0	0	0	0	0	1	0	1	x	1	1	1	0	0	0	2	0	1	8
nopi	1	0	0	2	0	58	21	7	13	x	5	6	0	9	2	0	0	6	130
noye	0	0	1	0	0	0	0	0	1	0	x	0	0	0	0	0	0	0	2
pigr	0	0	0	0	0	2	1	1	0	2	0	x	0	0	0	0	0	0	6
pino	0	0	0	0	0	0	0	0	0	1	0	0	x	0	0	0	0	0	1
pipi	0	0	0	0	0	3	0	1	0	0	1	2	1	x	0	0	0	2	10
piye	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0
yebl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0
yeor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0
yeye	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	x	2
Sums	1	0	13	2	0	354	75	41	90	415	18	19	4	63	6	7	0	23	1131

Days 20-30

	blbl	blgr	blno	blpi	gror	grpi	nobl	nogr	noor	nopi	noye	pigr	pino	pipi	piye	yebl	yeor	yeye	Sums
blbl	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
blgr	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
blno	0	0	x	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
blpi	0	0	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gror	0	0	0	0	x	0	0	0	0	0	0	0	0	0	0	0	0	0	0
grpi	0	0	1	0	0	x	7	0	0	7	0	0	0	0	0	0	0	0	15
nobl	0	0	0	0	0	63	x	2	3	61	0	0	0	4	0	0	0	2	135
nogr	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0	0	0	0	0
noor	0	0	0	0	0	1	0	0	x	0	0	0	0	0	0	0	0	0	1
nopi	0	0	0	0	0	9	3	0	0	x	0	2	0	2	0	0	0	0	16
noye	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0	0
pigr	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0	0
pino	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0	0
pipi	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0	0
piye	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0	0
yebl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0	0
yeor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0	0
yeye	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	x	0
Sums	0	0	1	0	0	73	10	2	3	69	0	2	0	6	0	0	0	2	168

7.4 Additional results

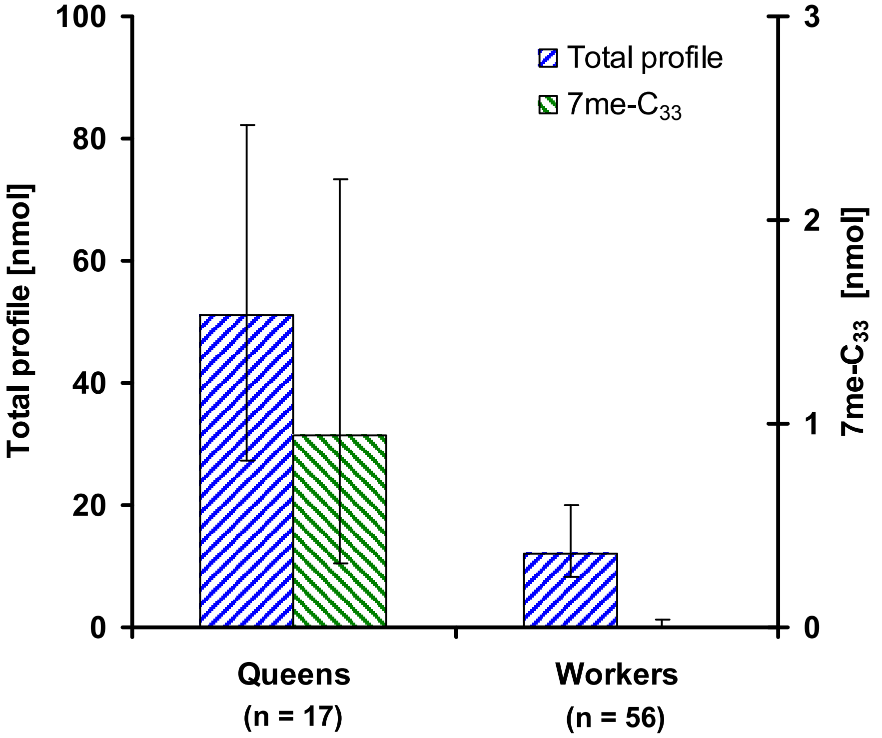

Figure 13 Substance amounts of cuticular hydrocarbons on individuals of *P. gracilis*. Given are medians (columns) and inter-quartile ranges (whiskers). Amounts of total profiles (left-right upwardly shaded columns) are scaled on the left y-axis, amounts of 7me-C_{33} (downwardly shaded columns) on the right y-axis.

Table 9 Results of MANOVAs with all 52 CHC compounds of the corresponding dataset as dependent variables. Effects on the single compound 7me-C_{33} are listed additionally. Factor: independent variable; *Wilk*: Wilk's Lambda; *df (H)*: degrees of freedom (hypothesis); *df (E)*: degrees of freedom (error); *Rank*: position in the compound list ordered by *p* value (lowest has rank 1), given only for factors caste and ovary size; *n*: number of samples (individuals).

		Whole CHC profile					**7me-C_{33}**			
Dataset	**Factor**	***Wilk***	***F***	***df (H)***	***df (E)***	***p***	***F***	***df***	***p***	***Rank***
Whole (*n* = 74)	Caste	0.057	0.637	52	2	0.782	16.81	1	<0.001	1
	Chemomorph	<0.001	107.8	52	2	0.009	1.334	1	0.253	-
	Colony[chemomorph]	<0.001	4.676	780	101	<0.001	1.895	15	0.045	-
	Ovary size	<0.001	1.079	156	7	0.513	2.441	3	0.074	5
Workers only (*n* = 57)	Chemomorph	<0.001	991.7	38	1	0.025	0.034	1	0.855	-
	Colony[chemomorph]	<0.001	6.330	570	79	<0.001	1.615	15	0.116	-
	Ovary size	0.002	0.501	76	2	0.857	4.866	2	0.013	1
Worker only, chemomorph 1 (*n* = 23)	Colony	<0.001	6.074	84	12	0.001	1.945	6	0.143	-
	Ovary size	0.015	0.504	28	2	0.843	12.24	2	0.001	1
Worker only, chemomorph 2 (*n* = 34)	Colony	<0.001	1.968	198	27	0.019	1.220	9	0.332	-
	Ovary size	0.009	0.424	44	2	0.894	0.812	2	0.457	28

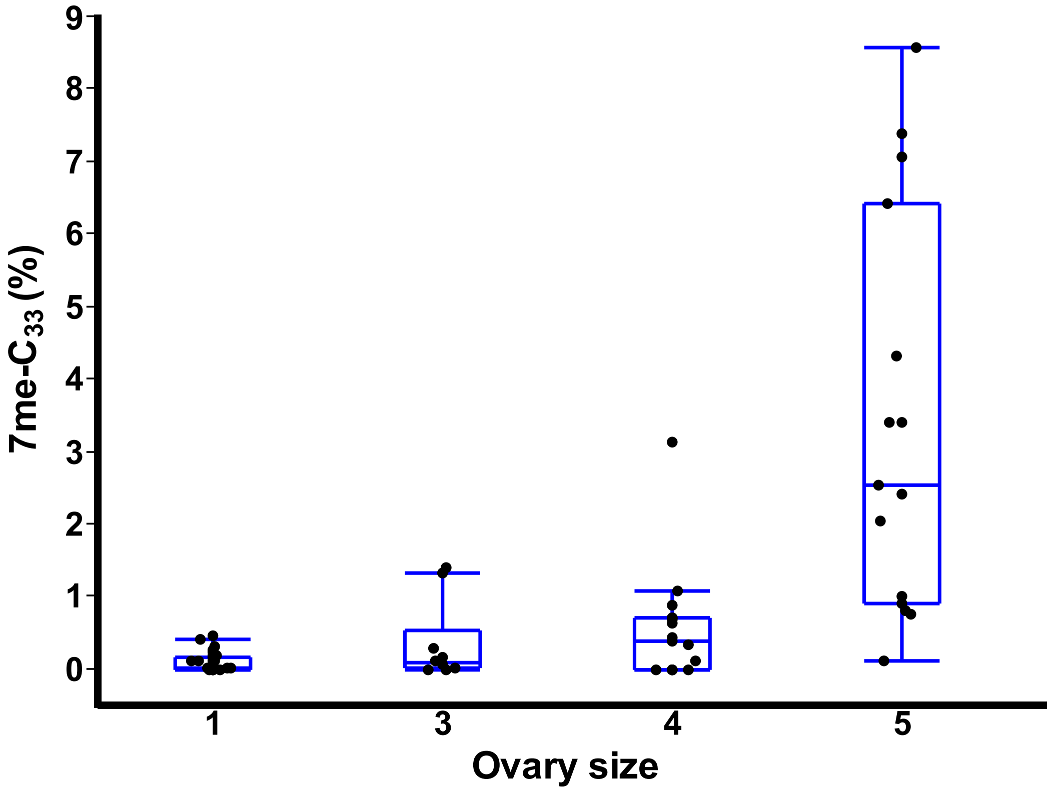

Figure 14 Relative (untransformed) amounts of the CHC compound 7-methyl-tritriacontane (7me-C_{33}) for each ovary size score in workers and queens of *Pseudomyrmex gracilis*. (100% = total amount of CHC profile.) Data points were jittered for better visibility. Points of score 5 all represent queens, as well as the three highest values of score 4. Box plots show median (middle line) and inter-quartile range (box); whiskers represent the range of all points that fall within the following limits: upper quartile + 1.5 • (interquartile range), lower quartile – 1.5 • (inter-quartile range).

Table 10. Survey of literature on mono- and polygyny in 34 pseudomyrmecine ant species. OPA = obligate plant ant; # queens = number of queens per queenright colony; *P.* = *Pseudomyrmex*; *T.* = *Tetraponera*; *n* = number of nests examined.

Note: Presumably, several references cited are redundant because sometimes a general statement about the number of queens is made without providing data or a reference, especially in the cases with unknown sample sizes. Exceptions are some species that were, on the one hand, extensively studied by Janzen (1966-1975) who, on the other hand, rarely presented sample sizes. Most of Janzen's publications might, though, be regarded as redundant among each other because he presumably referred (without explicitly stating this) to the entirety of his countless field observations.

Species	OPA?	# queens	n	Reference	Remarks and/or quotations
P. apache	no	≥1	13	Ward 1985	n = 5: no dealate females; n = 6: single queen; n = 1: two inseminated queens; n = 1: six dealate queens; polydomy: 0.625 queenless nests per queenright nest.
P. boopis	no	11 (?)	1	Kempf 1960	Referred to as *P. excavatus*; E. O. Wilson was named as collector of the single nest; the putative queens were referred to as females but it is unclear whether they were alate or not.
P. brunneus	no	1	unknown	Ward 1989a	Source of data not presented.
		1	1	Ward 1985	
P. concolor	yes	3.4 ± 2.4 (mean ± SD)	16	Fonseca 1993	Species found in two ant-plant species (n = 8 each) with slightly differing means of queen number per colony; "one to some queens" per leaf domatium; 25% of colonies monogynous; indications for functional polygyny with skewed contingents of reproduction.
P. ejectus	no	≥1	77	Klein 1987	>1 queens in "15.6% of all nests, up to 10 queens"; polydomy: 1.3 queenless nests per queenright nest.
		≥1	unknown	Ward 1989a	"Nests monogynous and polygynous"; source of data not presented.
		1	5	Ward 1985	Three monogynous and two queenless colonies; polydomy: 0.667 queenless nests per queenright nest. "In Florida some nests of this species are polygynous (R. W. Klein, pers. comm.)."
P. elongatus	no	≥1	7	Creighton 1955	"In most colonies a single female is present." Not clear whether dealate but it sounds as if dealate queens are meant.
P. ethicus	no	≥1	unknown	Ward 1989a	"Usually multiple-queened"; no data or reference presented.

Table 10 continued.

Species	OPA?	# queens	*n*	Reference	Remarks and/or quotations
P. ferrugineus	yes	1	unknown	Frumhoff & Ward 1992	"Characteristic number of queens" (more than one dealate queen in 0-24% of nests).
		1	unknown	Janzen 1966	
		1	1	Janzen 1967a	
		1	1	Janzen 1975	
		≥1	unknown	Clement 2005	Mostly monogynous, no data or reference presented.
		1	unknown	Janzen 1967b	Sample size appears to be large enough for confident conclusions.
		1	unknown	Janzen 1973	"Unpublished field notes" and a number of further papers are cited, so sample size appears to be large enough for confident conclusions.
		≥1	"a few"	Ward 1993	
P. flavicornis[1]	yes	1	unknown	Frumhoff & Ward 1992	"Characteristic number of queens" (more than one dealate queen in 0-24% of nests).
		1	unknown	Ward 1993	
		1	unknown	Janzen 1969	
		1	1	Janzen 1975	
P. gracilis	no	1	$9 \leq n \leq 20$	Kautz et al. 2009	
		≥1	75	present study	Mostly monogynus, 5 of 75 nests with >1 physogastric, dealate female; polydomy: 35 queenless nests, i.e. 0.875 queenless nests per queenright nest. Genetic evidence (*n* = 15) for occasional functional polygyny, see results section.
P. janzeni	yes	>1	unknown	Ward 1993	
		>1	unknown	Janzen 1969	*P. janzeni* is referred to as "an undescribed species of obligate acacia-ant (brown)" which was later described as *P. janzeni* (Ward 1993).
P. leptosus	no	≥1	unknown	Ward 1989a	"Nests monogynous and polygynous"; source of data not presented; "workerless social parasite".
		2	2	Ward 1985	Additional collection mentioned with "queens in nest" (number of queens not provided).
P. mixtecus	yes	1	unknown	Janzen 1973	"Unpublished field notes" and a number of further papers are cited, so sample size appears to be large enough for confident conclusions. Ward (1993) revealed that "*P. mixtecus* was mistaken for *P. flavicornis* (= *belti*) by Janzen".
		≥1	unknown	Janzen 1966	Queen adoption occurs; Ward (1993) revealed that "*P. mixtecus* was mistaken for *P. flavicornis* (= *belti*) by Janzen".
		1	unknown	Ward 1993	Author referring to "Janzen's field notes".
P. nigrescens	yes	1	5	Fonseca 1999	
		1	10	Fonseca & Benson 2003	

Table 10 continued.

Species	OPA?	# queens	*n*	Reference	Remarks and/or quotations
P. nigrocinctus	yes	1	unknown	Frumhoff & Ward 1992	“Usual number of queens”.
		2	1	Janzen 1975	
		1	unknown	Janzen 1967a	
		1	unknown	Janzen 1969	
		1	unknown	Janzen 1973	“Unpublished field notes” and a number of further papers are cited, so sample size appears to be large enough for confident conclusions. However, confusion with *P. peperi* possible (Ward 1993).
		1	unknown	Ward 1993	
P. nigropilosus	yes	11	1	Janzen 1975	
		1	$9 \leq n \leq 20$	Kautz et al. 2009	
P. oki	no	1	unknown	Ward 1989a	Source of data not presented.
P. pallidus	no	≥1	unknown	Ward 1989a	“Nests monogynous and polygynous”; source of data not presented.
		≥1	unknown	Ward 1985	“(…) majority of (…) nests (…) were queenless or monogynous, but sometimes larger numbers of mated, dealate queens cohabited (up to a maximum of 22). (…) colonies are often polydomous (…).”
		>1	unknown	Frumhoff & Ward 1992	“Characteristic number of queens” (more than one dealate queen in at least 25% of nests).
		≥1	46	Klein 1987	>1 queens in “32.6% of all nests, up to 23 queens”; polydomy: 0.9 queenless nests per queenright nest.
P. peperi	yes	42	2	Kautz et al. 2009	23 and 61 physogastric queens, respectively.
		≥1	unknown	Clement 2005	“Mostly polygynous”; no data or reference presented.
		≥1	?	Ward 1993	“Apparently polygynous over much of its range”.
P. phyllophilus	no	1	1	Kempf & Lenko 1976	
		1	1	personal observation	
P. salvini	no	1	$9 \leq n \leq 20$	Kautz et al. 2009	
P. satanicus	yes	≥1	unknown	Janzen 1973	
		>1	unknown	Janzen 1969	“Species with multiple queens”.
		“5-20 or more”	unknown	Ward 1993	
P. seminole	no	1	unknown	Ward 1985	“Some [nests] were queenless”.
		1	unknown	Ward 1989a	Source of data not presented.
		1	unknown	Frumhoff & Ward 1992	“Characteristic number of queens” (more than one dealate queen in 0-24% of nests).
		1	89	Klein 1987	Polydomy: 8.9 queenless nests per queenright nest.

Table 10 continued.

Species	OPA?	# queens	*n*	Reference	Remarks and/or quotations
P. simplex	no	≥1	unknown	Ward 1985	"Most *P. simplex* nests I dissected were queenless (indicating a high level of polydomy), some were monogynous, and one contained two functional (i.e. inseminated, with well-developed ovaries) dealate queens."
		≥1	unknown	Ward 1989a	"Nests monogynous and polygynous"; source of data not presented.
		1	unknown	Frumhoff & Ward 1992	"Characteristic number of queens" (more than one dealate queen in 0-24% of nests).
P. sp. PSW-06	no	1	$9 \leq n \leq 20$	Kautz et al. 2009	
P. spp.	yes	>1	unknown	Janzen 1967a	"Two undescribed species of *Pseudomyrmex*" one of which might be identical to *P. janzeni* because Janzen (1973) mentioned an undescribed *Pseudomyrmex* species that was later described as *P. janzeni* (Ward 1993).
P. spinicola	yes	1	unknown	Janzen 1969	
		1	unknown	Ward 1993	
P. tenuissimus	no	1	1	Ward 1989b	
P. veneficus	yes	>1	unknown	Janzen 1969	"Species with multiple queens".
		up to several 10000	unknown	Janzen 1973	Readoption of daughter queens occurs. "An old colony may occupy many hundreds of swollen-thorn acacias and have tens of thousands of egg-laying queens."
		>1	?	Frumhoff & Ward 1992	"Characteristic number of queens" (more than one dealate queen in at least 25% of nests).
T. aethiops	yes	1	1	Yumoto & Maruhashi 1999	
T. anthracina	no	one to nine	8	Terron 1977	Lives in dead twigs (Terron 1968).
T. binghami[2]	yes	1	unknown	Ward 2001	Refers to Buschinger et al. (1994).
		1	3	Buschinger et al. 1994	Highly polydomous.
T. penzigi	yes	≥1	unknown	Hocking 1970	"*T. penzigi* (…) may have several laying queens in one swelling".
T. tessmanni	yes	>1	unknown	Djiéto-Lordon et al. 2005	"Highly polygynous" ("numerous" queens per colony).

[1] *Pseudomyrmex flavicornis* has, in several early studies, been cited as *P. belti*.

[2] *Tetraponera binghami* has, in some early studies, been cited as *Tetraponera* sp. PSW-80.

8. Lists of figures and tables

List of figures

List of tables

9. Publications

Parts of this thesis have been published or prepared for publication as listed below.

Schmid, V. S.; Kautz, S.; Trindl, A.; Heinze, J. (2009). Polymerase chain reaction primers for polymorphic microsatellite loci in the ant *Pseudomyrmex gracilis* (Formicidae: Pseudomyrmecinae). *Molecular Ecology Resources* **9**(4): 1150-1152; doi: 10.1111/j.1755-0998.2009.02590.x

Schmid, V. S.; Kaltenpoth, M.; Strohm, E.; Heinze, J. (in prep.). Worker self-restraint and policing maintain the queen's reproductive monopoly in a pseudomyrmecine ant. In preparation for submission to *Behavioral Ecology and Sociobiology*.

10. Acknowledgements / Danksagung

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11. Declaration on lieu of oath / Eidesstattliche Erklärung

Eidesstattliche Erklärung

abzulegen bei der Fakultätsverwaltung

Declaration in lieu of oath – Declaration of originality

to render in the faculty administration

Name, Geb.Name **Name, Birth Name**	Schmid	**Vorname** **First Name**	Volker	**Geb.Datum** **Date of Birth**	22.11.1979

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitats gekennzeichnet.

Bei der Auswahl und Auswertung folgenden Materials haben mir die nachstehend aufgeführten Personen in der jeweils beschriebenen Weise entgeltlich/unentgeltlich geholfen:

I herewith declare in lieu of oath that I have composed this thesis without any inadmissible help of a third party and without the use of aids other than those listed. The data and concepts that have been taken directly or indirectly from other sources have been acknowledged and referenced.

The persons listed beneath have helped me to select and choose the following material gratuitously/for a consideration in the manner described in each case:

1. Stefanie Kautz (Mikrosatellitenentwicklung / microsatellite development)
2. Martin Kaltenpoth (Statistische Analyse der chemischen Profile und Identifizierung der Substanzen / statistical analysis of chemical profiles and substance identification)
3. Thomas Rössler, Daniela Wanke (Verhaltensbeobachtungen an den beiden Kolonien aus Florida / behavioural observations of the two colonies from Florida)

Weitere Personen waren an der inhaltlich-materiellen Herstellung der vorliegenden Arbeit nicht beteiligt. Insbesondere habe ich hierfür nicht die entgeltliche Hilfe eines Promotionsberaters oder anderer Personen in Anspruch genommen. Niemand hat von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Die Arbeit wurde bisher weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Ich versichere an Eides statt, dass ich nach bestem Wissen die reine Wahrheit gesagt und nichts verschwiegen habe.

Vor Aufnahme der obigen Versicherung an Eides statt wurde ich über die Bedeutung der eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unrichtigen oder unvollständigen eidesstattlichen Versicherung belehrt.

Other persons have not helped to produce this work as regards to its content or making. In particular, I have not used the services of any professional agencies in return for payment or those of other persons. Nobody has received payment in kind - neither directly nor indirectly - from me for any work that is connected with the content of this doctoral dissertation.

This thesis has not been submitted, wholly or substantially, neither in this country nor abroad for another degree or diploma at any university or institute.

I declare in lieu of oath that I have said nothing but the truth to the best of my knowledge and that I have not withheld any information.

Before the above declaration in lieu of oath had been taken down, I was advised about the significance of a declaration in lieu of oath as well as the legal consequences of an incorrect or incomplete declaration.

Ort, Datum **Place, Date**	**Unterschrift Doktorand/in** **Signature doctoral candidate**	**Unterschrift Beamtin/er** **Signature civil servant**

12. Curriculum vitae / Lebenslauf

Curriculum vitae of Volker Schmid, born on 22.11.1979 in Nürtingen

	Secondary school
10/1990 – 07/1999	Attendance of the „Max-Planck-Gymnasium Nürtingen“ (Baden-Württemberg, Germany). School leaving examination: mark 1.4
	Community service
08/1999 – 08/2000	Community service as alternative to military service in the central laboratory of the city hospital of Esslingen (Germany, Baden-Württemberg, district of Esslingen).
	Course of studies
10/2000 – 07/2006	Course of studies of biology at the University of Tübingen (Germany, Baden-Württemberg). Main subject: animal physiology; further subjects: zoology, organic chemistry. Diploma thesis („Tier-Pflanze-Interaktionen in der Mata Atlântica Südbrasiliens: Diversität und Verhalten von Ameisen an Bromelienblüten“) with six-month stay in Brazil for data collection Graduation as biologist (mark: very good)
	Research
07/2005 – 03/2007	Conduction of research within the project „Internal dynamics of rain forests: specificity of animal-plant interactions“ within the bi-national program „Mata Atlântica“
	PhD thesis
Since 10/2006	PhD thesis at the University of Regensburg: „Reproductive conflict among workers of the ant species *Pseudomyrmex gracilis* (Hymenoptera: Formicidae)”

Lebenslauf von Volker Schmid, geboren am 22.11.1979 in Nürtingen

	Gymnasium
10/1990 – 07/1999	Besuch des Max-Planck-Gymnasiums Nürtingen (Baden-Württemberg). Abitur-Abschlussnote: 1,4
	Zivildienst
08/1999 – 08/2000	Zivildienst im Zentrallabor der Städtischen Kliniken Esslingen (Baden-Württemberg, Esslingen am Neckar).
	Studium
10/2000 – 07/2006	Diplomstudiengang Biologie an der Eberhard-Karls-Universität Tübingen Hauptfach: Tierphysiologie Nebenfächer: Zoologie, Organische Chemie Diplomarbeit („Tier-Pflanze-Interaktionen in der Mata Atlântica Südbrasiliens: Diversität und Verhalten von Ameisen an Bromelienblüten“) mit halbjährigem Brasilienaufenthalt zur Erhebung der Daten Abschluss als Diplombiologe (Note: sehr gut)
	Forschungsarbeit
07/2005 – 03/2007	Mitarbeit im Projekt „Interne Dynamik des Regenwaldes: spezifische Tier-Pflanze-Interaktionen“ im Rahmen des BMBF-Forschungsprogramms „Mata Atlântica“
	Doktorarbeit
Seit 10/2006	Doktorarbeit an der Universität Regensburg: „Reproductive conflict among workers of the ant species *Pseudomyrmex gracilis* (Hymenoptera: Formicidae)”