Insectes Sociaux

RESEARCH ARTICLE

Queen–worker caste ratio depends on colony size in the pharaoh ant (*Monomorium pharaonis*)

A. M. Schmidt · T. A. Linksvayer · J. J. Boomsma · J. S. Pedersen

Abstract The success of an ant colony depends on the simultaneous presence of reproducing queens and non-reproducing workers in a ratio that will maximize colony growth and reproduction. Despite its presumably crucial role, queen–worker caste ratios (the ratio of adult queens to workers) and the factors affecting this variable remain scarcely studied. Maintaining polygynous pharaoh ant (*Monomorium pharaonis*) colonies in the laboratory has provided us with the opportunity to experimentally manipulate colony size, one of the key factors that can be expected to affect colony level queen–worker caste ratios and body size of eclosing workers, gynes and males. We found that smaller colonies produced more new queens relative to workers, and that these queens and workers both tended to be larger. However, colony size had no effect on the size of males or on the sex ratio of the individuals reared. Furthermore, for the first time in a social insect, we confirmed the general life history prediction by Smith and Fretwell (Am Nat 108:499–506, 1974) that offspring number varies more than offspring size. Our findings document a high level of plasticity in energy allocation toward female castes and suggest that polygynous species with budding colonies may adaptively adjust caste ratios to ensure rapid growth.

Keywords Caste · Colony size · Ergonomics · Resource allocation · Polygyny

A. M. Schmidt (✉) · T. A. Linksvayer ·
J. J. Boomsma · J. S. Pedersen
Department of Biology, Centre for Social Evolution,
University of Copenhagen, Universitetsparken 15,
2100 Copenhagen, Denmark
e-mail: amschmidt@bio.ku.dk

T. A. Linksvayer
e-mail: tlinksvayer@gmail.com

J. J. Boomsma
e-mail: jjboomsma@bio.ku.dk

J. S. Pedersen
e-mail: jspedersen@bio.ku.dk

Introduction

Ant colonies are typically founded by a single queen and go through different stages of resource allocation to optimize growth and reproduction. During the founding stage, queens rear the first batch of workers (e.g. Oster and Wilson, 1978), after which the colony enters the ergonomic stage, characterized by rapid exponential growth. Once the colony has reached a certain worker number, it enters the reproductive stage, during which excess resources are periodically invested in new reproductives (males and gynes) rather than more workers (Oster and Wilson, 1978; Hölldobler and Wilson, 1990; Bourke and Franks, 1995). However, there are also many polygynous ant species that have variable numbers of queens per colony and recurrently readopt newly fertilized daughter queens (Glancey and Lofgren, 1988; Hölldobler and Wilson, 1990; Crozier and Pamilo, 1996).

Although both queen and worker numbers vary in polygynous species, systematic studies of queen–worker caste ratio allocation have rarely been done, likely due to logistic difficulties usually associated with such colony level studies. Instead, studies have focused on phenotypic plasticity in allocation to different worker castes. Several studies have emphasized the importance of specific environmental components in determining allocation toward

workers and soldiers as a function of competition (Passera et al., 1996) or food availability (McGlynn and Owen, 2002). In recent years, other studies have shown that caste determination may have a significant genetic component (Jaffe et al., 2007; Hughes and Boomsma, 2008; Schwander et al., 2008). Such studies also often indicate a strong effect of the social environment, i.e. of the workers rearing the new brood and actively influencing resource allocation during larval development (reviewed by e.g. Anderson et al., 2008; Schwander et al., 2010).

Pharaoh ants (*Monomorium pharaonis*) are perhaps one of only few ant species that can be mass-reared in the laboratory for many generations, because the species is highly polygynous (Wheeler, 1986), produces new broods of reproductives at intervals of down to only a few months, and has intranidal mating (i.e. mating in the nest without dispersal of either sex). New queens thus become reproductively active and contribute to colony growth shortly after eclosing. In addition, the species exclusively disperses by colony budding of worker groups and brood that either contain queens or rapidly rear a new cohort of queens and males (Berndt and Eichler, 1987). Finally, pharaoh ants have genetically highly structured populations, but can nonetheless be freely mixed in laboratory cultures without any significant aggression between unrelated workers or queens (Schmidt et al., 2010). This implies that both queen number and worker number in pharaoh ants vary continuously, providing unique opportunities for studying queen–worker caste allocation under experimentally controlled conditions, i.e. allocation toward egg-laying potential on one hand, and "somatic" support by completely sterile workers on the other.

The objective of our study was to experimentally create queenless colonies of three size classes, provide them with a constant amount of brood relative to workers, and measure the queen–worker caste ratio, body sizes and sex ratio of the brood that these colonies reared. This design was meant to provide insight into the extent to which colony level selection may have produced phenotypically plastic responses that are likely to maximize colony survival across a range of different colony sizes, consistent with earlier correlative evidence in the leafcutter ant, *Atta cephalotes* (Wilson, 1983) and experimental evidence in the red imported fire ant, *Solenopsis invicta* (Porter and Tschinkel, 1985a). In addition, this experimental setup further allowed us to test the basic life history principle formulated by Smith and Fretwell (1974) that offspring number is expected to vary more than offspring size. The rationale of this idea is that offspring size should normally be under balancing (optimizing) selection, so that different parental resource levels translate primarily into varying offspring number. To our knowledge, this hypothesis has never been tested in social insects.

Methods

Study species

Pharaoh ants (*M. pharaonis*) are commonly found as massive pest infestations in houses (Edwards, 1986; Berndt and Eichler, 1987). There does not appear to be any seasonality in their reproductive cycles as new sexuals are produced every 3–8 months (Petersen-Braun, 1975, AMS pers. obs.), which may be due to their tropical origin. Caste determination has been shown to depend on the social environment, i.e. on whether fertile queens are present in the colonies (Edwards, 1987; 1991), and on the age of queens (Petersen-Braun, 1977). As in the Argentine ant (*Linepithema humile*) and the red imported fire ant, pharaoh ant workers have been shown to be able to discriminate and kill sexual larvae and thereby potentially control colony caste ratios (Vargo and Fletcher, 1986; Edwards, 1991; Vargo and Passera, 1991; Klobuchar and Deslippe, 2002). Eggs laid by queens older than 4 weeks are bipotent (Petersen-Braun, 1977), meaning that they can develop into either gynes or workers. Eggs are always queen-laid, as workers have no ovaries and are thus completely sterile (Berndt and Eichler, 1987), and experimental removal of queens from a colony results in the almost immediate rearing of new sexuals. New queens and males mate within their natal nest so that pharaoh ants can potentially be kept in the laboratory indefinitely (Peacock and Baxter, 1949; Berndt and Eichler, 1987). Pharaoh ants generally display only low levels of intraspecific aggression, which means that it is usually possible to combine worker ants from different colonies without agonistic interactions (Schmidt et al., 2010).

Experimental setup

Brood and workers from ten genetically different laboratory colonies were mixed to form one large queenless experimental colony. Three different experimental colony sizes (small, medium and large) were subsequently created by transferring either ca. 1, 2, or 3 ml brood and workers from the experimental colony to 15-cm diameter Petri plates using a metal measuring spoon. This approach provided sub-colonies of approximately the same worker–brood composition, but did not enable us to determine the exact numbers of individuals in each colony. Water tubes plugged with cotton wool and food ad libitum (a diet consisting of cooked liver, boiled egg yolk, honey, mealworms and almonds) were added, and colonies were kept in climate rooms at 26–28°C. Starting 23 days after the creation of the experimental colonies, all pupae produced were continuously removed and the number of offspring workers, gynes and males counted every 4–5 days for the next 13–18 days until no brood was left. To avoid matings and enable

verification of caste identifications, gynes and males collected from each colony were reared separately in 10-cm diameter Petri plates, which were also supplied with food and water ad libitum. A few adult workers were added to the gyne and male plates to facilitate eclosure of the pupae (Edwards, 1986). This experimental setup gives several replicate samples that are not independent in the sense of representing different source colonies, but which enable testing of whether sub-colonies derived from the same experimental source colony have a consistent response to colony size.

Once eclosed, the new workers, gynes, and males were placed in 95% EtOH and subsequently the head widths of individuals from each caste and experimental colony were measured at $50\times$ magnification using a Leica MZ125 microscope with attached Leica DFC420 camera and analyzing data using Leica Application Suite. Head width was measured directly above the eyes, and chosen as proxy for overall body size, as head width is known to correlate strongly with overall size of ants (Hölldobler and Wilson, 1990). Fifteen workers and gynes were measured from each colony and, when possible, 15 males as well. The number of males measured varied from 4 to 15 as male pupae appear to be particularly fragile and have high pre-eclosure mortality, so that a large number of the male pupae did not survive till eclosure. We do not know whether our handling had any role in causing the mortality, so we chose to calculate sex ratios based on the number of pupae that were originally removed from the colonies.

Statistics

Queen–worker caste ratios of the reared brood were calculated as the ratio of gynes produced to the total number of females (gynes + workers) produced. The data were analyzed in R version 2.10.1 (R Development Core Team, 2009) using a Generalized Linear Model (GLM function) on the queen–worker caste ratios of colonies with group size category (small, medium or large) or colony productivity (measured as the total number of individuals reared by the workers of a given colony) as fixed effect and a quasibinomial error structure. Logit regressions were used to predict changes in queen–worker caste ratio relative to experimental colony size or total offspring production, using also an analysis of deviance to compare the three colony size categories. In addition, we used a GLM with a binomial error structure to analyze queen–worker caste ratios as a function of colony productivity as fixed effect, i.e. offspring number adjusted for differences in the mean fresh weight of workers, males and gynes: unpublished data for workers 0.247 mg ($n = 714$), gynes 1.359 mg ($n = 247$), males 0.955 mg ($n = 235$). Sex ratios were calculated as the ratio of gynes to the total number of males and gynes

produced, and the data were analyzed by the same approach as for the queen–worker caste ratio data, using a binomial error structure in the GLM. Linear Mixed Effect models (LME function) were applied to the individual size measurement data using group size category (small, medium, large) or productivity as a fixed effect and colony ID as random effect. The group size effect analyses were also combined with analyses of deviance.

Results

The colonies produced between 52 and 157 gynes, 15 and 71 males and 196 and 1,280 workers and the total number of individuals ranged from 263 to 1,436. Queen–worker caste ratios varied from 0.076 to 0.238 gynes/(gynes + workers): median = 0.155, interquartile range = 0.048 (Fig. 1). Sex ratios were female biased at 0.60–0.85 gyne/(gynes + males): median = 0.739, interquartile range = 0.028. Our analyses showed a significant effect of colony size category (small, medium, large) on caste ratio (GLM, analysis of deviance, $P < 0.001$) as size change from large to medium caused an increase in queen–worker caste ratio by a factor of 1.21 ($t_{36} = 2.09$, $P = 0.04$) and large to small caused an increase by a factor of 1.79 ($t_{36} = 5.54$, $P < 0.001$). We also found a significant negative correlation between queen–worker caste ratio and total number of offspring reared, as the queen–worker caste ratio decreased by a factor of 0.37 for every unit of increase in offspring number (Fig. 1a; $t_{37} = -8.93$, $P < 0.001$). Likewise, a significant correlation was found between queen–worker caste ratio and total productivity measured as adjusted fresh weight (Fig. 1b; $z_{37} = 4.55$, $P < 0.001$). Expressed as ratios rather than proportions, the small colonies had queen–worker caste ratios of approximately 1:3 (number of queens to number of workers), whereas the large colonies had queen–worker caste ratios of around 1:12 (right vertical axis in Fig. 1).

Colony size category had a small, but statistically significant effect on mean gynes size ($F_{2,36} = 3.2$, $P = 0.050$) as the medium and small groups produced larger gynes (large to medium: $t_{36} = 2.27$, $P = 0.029$; large to small: $t_{36} = 2.12$, $P = 0.040$), and although not significant overall ($F_{2,36} = 2.33$, $P = 0.111$), it appeared that worker size was also slightly affected as the small groups produced slightly larger workers than the large groups (large to medium: $t_{36} = 1.68$, $P = 0.100$; large to small: $t_{36} = 2.01$, $P = 0.051$). Colony size category had no effect on the size of males ($F_{2,36} = 1.40$, $P = 0.36$) nor on the sex ratio produced (analysis of deviance, $P = 0.33$). There was no significant effect of the total number of offspring produced on the size of any of the castes (Fig. 2; workers: $t_{36} = -0.80$, $P = 0.43$; gynes: $t_{36} = -1.18$, $P = 0.24$; males: $t_{36} = -1.78$, $P = 0.083$), nor on the sex ratio of the brood produced ($t_{37} = 0.72$, $P = 0.48$).
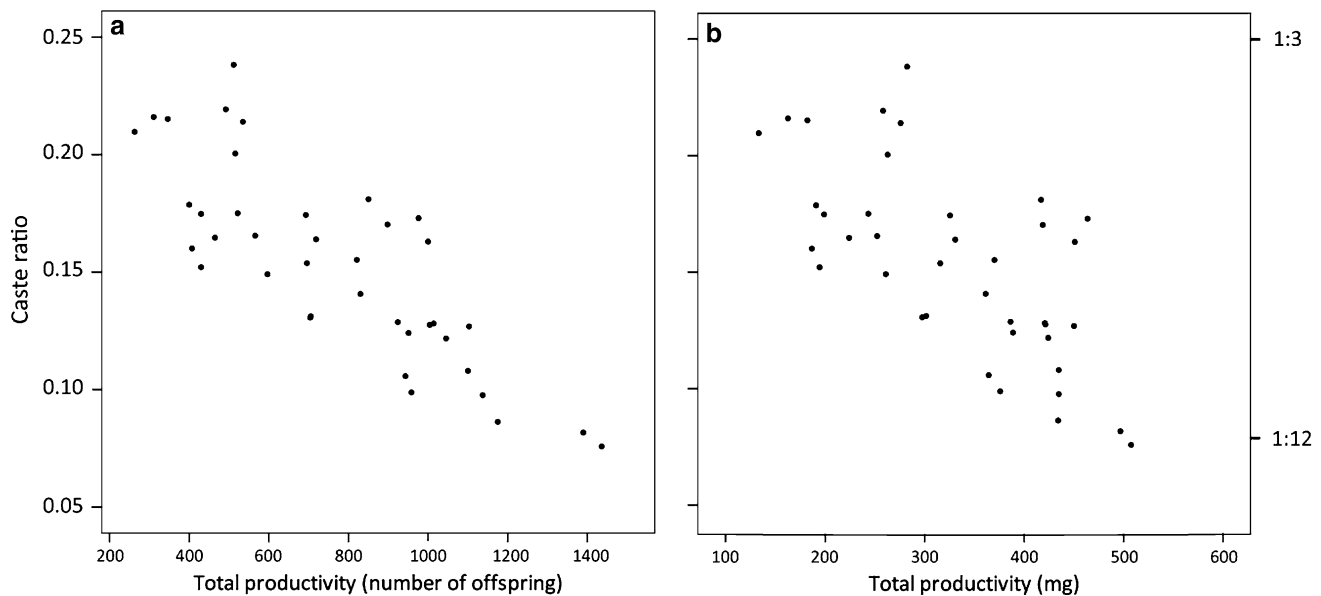
Fig. 1 Queen–worker caste ratio (ratio of gynes produced relative to total production of females) as a function of colony productivity, expressed as **a** total number of offspring and **b** total fresh weight of offspring ($n = 39$ colonies). The gyne:worker ratios corresponding to the proportion of gynes are given on the *right-hand side*

Discussion

Although many polygynous ants reproduce by colony budding (Hölldobler and Wilson, 1990; Bourke and Franks, 1995), the pharaoh ant breeding system has taken this mode of reproduction to extremes because worker groups that bud off from the parental nest do not need to contain queens, and often rear them from bipotent female brood (Petersen-Braun, 1977; Berndt and Eichler, 1987). This implies that flexible caste-ratio responses likely have evolved in response to selection for maximizing colony growth. As constraints on either queen or worker production appear to be negligible in pharaoh ants, our data show that the kind of plasticity colony level selection has resulted in when it comes to the effect of group size on queen–worker caste ratio. It further allows an assessment of the degree to which such selection regime has affected how individual body size varies relative to variation on offspring number.

In ants where queens and males disperse, reproduction normally occurs when the marginal fitness returns from further worker production have fallen below a certain threshold (Tschinkel, 1993; Crozier and Pamilo, 1996; Bourke and Ratnieks, 1999). However, when sexuals do not disperse but remain in the colony (either as new queens or as sperm stored by these queens), the overall ergonomic principles for queen–worker caste allocation should be more straightforward because intra-colony conflict has disappeared (Nonacs, 1988; Pamilo, 1991; Nonacs, 1993): workers are completely sterile and queens can be produced and culled at almost any time when this serves the collective

interest. Our results indicate that under such circumstances there is likely no single optimal proportion of individuals reared as gynes versus workers, but instead we see a graded response producing four times as many gynes in small relative to large experimental colonies (Fig. 1). This high level of plasticity likely enables colonies to respond rapidly to the environmental disturbances that naturally tend to create colonies of similar small size as our experimental colonies (Buczkowski and Bennett, 2009, AMS, pers. obs.). We hypothesize that the observed plasticity of the queen–worker caste ratio is adaptive as it prioritizes egg-laying potential in the smallest colony fragments and foraging potential in larger fragments where enough queens are present so that eggs are no longer a limiting factor for colony growth.

The colonies used to create the mixed experimental colony had all been established from colony fragments at least 1 year prior to the experiment, which meant that they should be considered mature in the sense of having reached a rather large size and a balanced queen–worker caste ratio. None of the source colonies were producing sexuals at the time, so we assumed that all diploid broods in the experimental colonies that we created were developmentally bipotent (i.e. able to develop into both gynes and workers). The queen–worker caste ratios found in our experimental colonies should therefore reflect the typical allocation of "colony buds" as our experimental colony sizes were small compared to the mature source colonies used to create the overall stock. Because we mixed ants and brood from a number of genetically different colonies when creating our experimental
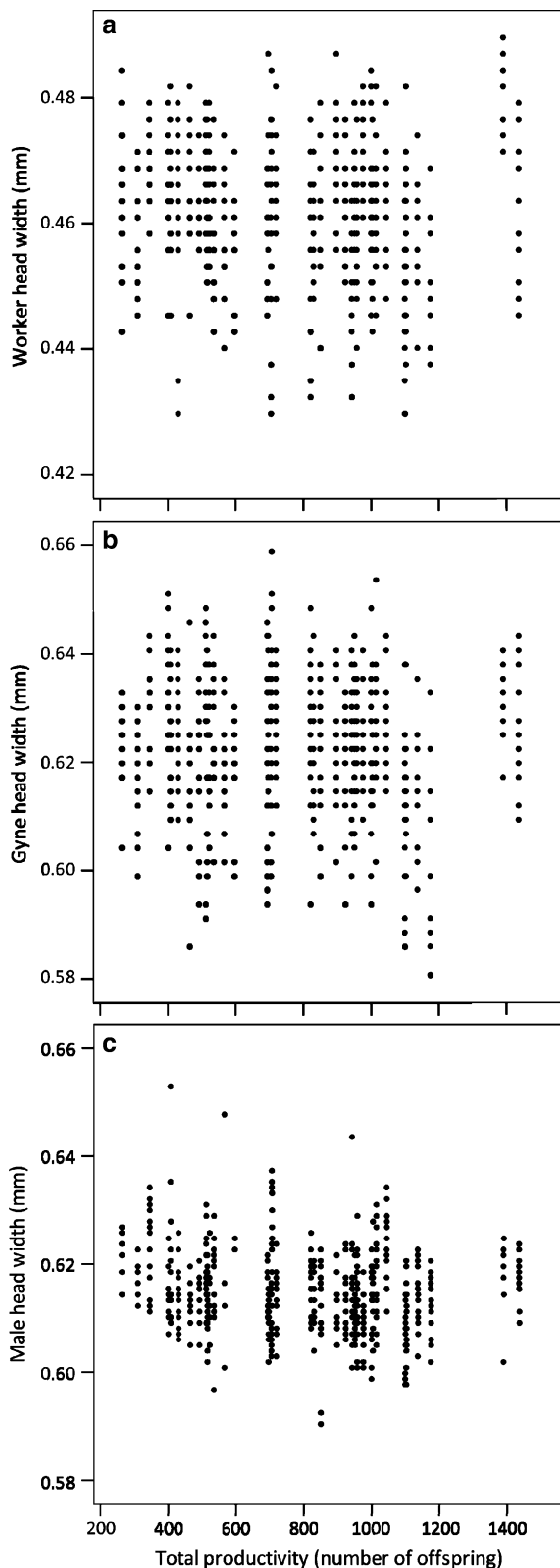
Fig. 2 Relationship between colony productivity (total number of offspring reared) in a given colony and the size (head width) of **a** workers, **b** gynes and **c** males (*n* = 39 colonies)

colonies, we expect that our results reflect a general average relationship between colony size and caste ratio in pharaoh ants. However, this does not preclude that variation around this average may exist across different genetic lineages of pharaoh ants, as preliminary data from non-mixed colonies seem to indicate (AMS and TAL, pers. obs.).

The observed effect of colony size on gyne and worker body size provides a test of the Smith and Fretwell (1974) model predicting that in non-social animals offspring number should be much more variable than offspring size. Our results indicate that this expectation was largely met as gyne size varied by 2.01% around the overall mean and worker size by 2.37%, whereas gyne and worker numbers varied by 25.4 and 43.8%, respectively. This difference of about an order of magnitude suggests that group selection on collective performance per se is not sufficient to create more variation in body size. This may be because there is only a single way of being a good egg layer in pharaoh ants where new colonies are only produced by budding, in contrast to other polygynous ants where trade-offs from alternative dispersal tactics have led to the evolution of a bimodal queen caste (Rüppell and Heinze, 1999).

Previous studies on the red imported fire ant by Porter and Tschinkel (1985a; 1985b) have shown that the size of the brood reared was strongly affected by the composition of workers in the colony and that increasing brood density leads to a decrease in the average weight of reared pupae. Although the worker to brood ratio in our colonies is constant, the brood is kept in piles, and it is thus conceivable that the observed differences in individual sizes may also in part reflect consequences of crowding, which, if similar to those found in fire ant colonies, could also result in slightly smaller brood in the larger colonies.

Body size of gynes and workers varied with experimental colony size, but male body size and sex ratio remained constant across the three colony size categories. This indicates that, with exclusive intranidal mating and no dispersal, male size and number hardly represent an independent fitness component. All that apparently matters is that male number increases proportionally with gyne number (hence a constant sex ratio), so that gynes can be inseminated at a constant rate to initiate their reproductive roles. This suggests that there has been no selection for phenotypic plasticity in male function, neither in terms of body size nor in relative numbers per gyne to mate with.

References

Anderson K.E., Linksvayer T.A. and Smith C.R. 2008. The causes and consequences of genetic caste determination in ants (Hymenoptera: Formicidae). *Myrmecol. News* **11**: 119-132

Berndt K.P. and Eichler W. 1987. Die Pharaoameise, *Monomorium pharaonis* (L.) (Hym., Myrmicidae). *Mitt. Zool. Mus. Berl.* **63**: 3-186

Bourke A.F.G. and Franks N.R. 1995. *Social Evolution in Ants*. Princeton University Press, Princeton, New Jersey. 529 pp

Bourke A.F.G. and Ratnieks F.L.W. 1999. Kin conflict over caste determination in social Hymenoptera. *Behav. Ecol. Sociobiol.* **46**: 287-297

Buczkowski G. and Bennett G. 2009. Colony budding and its effects on food allocation in the highly polygynous ant, *Monomorium pharaonis*. *Ethology* **115**: 1091-1099

Crozier R.H. and Pamilo P. 1996. *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection*. Oxford University Press, New York. 306 pp

Edwards J.P. 1986. The biology, economic importance, and control of the Pharaoh's Ant. In: *Economic Impact and Control of Social Insects* (S.B. Vinson, Ed). Praeger Publishers, New York, pp 257-271

Edwards J.P. 1987. Caste regulation in the pharaoh's ant *Monomorium pharaonis*: the influence of queens on the production of new sexual forms. *Physiol. Entomol.* **12**: 31-39

Edwards J.P. 1991. Caste regulation in the pharaoh's ant *Monomorium pharaonis*: recognition and cannibalism of sexual brood by workers. *Physiol. Entomol.* **16**: 263-271

Glancey B.M. and Lofgren C.S. 1988. Adoption of newly-mated queens; a mechanism for proliferation and perpetuation of polygynous red imported fire ants, *Solenopsis invicta*, Buren. *Florida Entomol.* **71**: 581-587

Hughes W.O.H. and Boomsma J.J. 2008. Genetic royal cheats in leaf-cutting ant societies. *Proc. Natl. Acad. Sci. U. S. A.* **105**: 5150-5153

Hölldobler B. and Wilson E.O. 1990. *The Ants*. Springer, New York. 732 pp

Jaffe R., Kronauer D.J.C., Kraus F.B., Boomsma J.J. and Moritz R.F.A. 2007. Worker caste determination in the army ant *Eciton burchellii*. *Biol. Lett.* **3**: 513-516

Klobuchar E.A. and Deslippe R.J. 2002. A queen pheromone induces workers to kill sexual larvae in colonies of the red imported fire ant (*Solenopsis invicta*). *Naturwissenschaften* **89**: 302-304

McGlynn T.P. and Owen J.P. 2002. Food supplementation alters caste allocation in a natural population of *Pheidole flavens*, a dimorphic leaf-litter dwelling ant. *Insect. Soc.* **49**: 8-14

Nonacs P. 1988. Queen number in colonies of social Hymenoptera as kin-selected adaptation. *Evolution* **42**: 566-580

Nonacs P. 1993. The effects of polygyny and colony life history on optimal sex investment. In: *Queen Number and Sociality in Insects* (L. Keller, Ed). Oxford University Press, New York, pp 110-131

Oster G.F. and Wilson E.O. 1978. *Caste and Ecology in the Social Insects*, vol 12. Princeton University Press, Princeton, New Jersey. 352 pp

Pamilo P. 1991. Evolution of colony characteristics in social insects .1. sex allocation. *Am. Nat.* **137**: 83-107

Passera L., Roncin E., Kaufmann B. and Keller L. 1996. Increased soldier production in ant colonies exposed to intraspecific competition. *Nature* **379**: 630-631

Peacock A.D. and Baxter A.T. 1949. Studies in Pharaoh's ant, *Monomorium pharaonis* (L.). 1. The rearing of artificial colonies. *Entomol. Mon. Mag.* **85**: 256-260

Petersen-Braun M. 1975. Investigations on social organization in Pharaohs ant, *Monomorium pharaonis* L (Hym Formicidae) 1. Regulation of brood cycle. *Insect. Soc.* **22**: 269-291

Petersen-Braun M. 1977. Investigations on social organization of Pharaohs ant *Monomorium pharaonis* L (Hymenoptera, Formicidae) 2. Caste determination. *Insect. Soc.* **24**: 303-318

Porter S.D. and Tschinkel W.R. 1985a. Fire ant polymorphism (Hymenoptera: Formicidae): factors affecting worker size. *Ann. Entomol. Soc. Am.* **78**: 381-386

Porter S.D. and Tschinkel W.R. 1985b. Fire ant polymorphism: the ergonomics of brood production. *Behav. Ecol. Sociobiol.* **16**: 323-336

R Development Core Team. 2009. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rüppell O. and Heinze J. 1999. Alternative reproductive tactics in females: the case of size polymorphism in winged ant queens. *Insect. Soc.* **46**: 6-17

Schmidt A.M., d'Ettorre P. and Pedersen J.S. 2010. Low levels of nestmate discrimination despite high genetic differentiation in the invasive pharaoh ant. *Front. Zool.* **7**: 20

Schwander T., Humbert J.-Y., Brent C.S., Cahan S.H., Chapuis L., Renai E. and Keller L. 2008. Maternal effect on female caste determination in a social insect. *Curr. Biol.* **18**: 265-269

Schwander T., Lo N., Beekman M., Oldroyd B.P. and Keller L. 2010. Nature versus nurture in social insect caste differentiation. *Trends Ecol. Evol.* **25**: 275-282

Smith C.C. and Fretwell S.D. 1974. Optimal balance between size and number of offspring. *Am. Nat.* **108**: 499-506

Tschinkel W.R. 1993. Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle *Ecol. Monogr.* **63**: 425-457

Vargo E.L. and Fletcher D.J.C. 1986. Evidence of pheromonal queen control over the production of male and female sexuals in the fire ant, *Solenopsis invicta*. *J. Comp. Physiol. A-Sens. Neural Behav. Physiol.* **159**: 741-749

Vargo E.L. and Passera L. 1991. Pheromonal and behavioral queen control over the production of gynes in the Argentine ant *Iridomyrmex humilis* (Mayr). *Behav. Ecol. Sociobiol.* **28**: 161-169

Wheeler D.E. 1986. Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *Am. Nat.* **128**: 13-34

Wilson E.O. 1983. Caste and division of labor in leaf-cutter ants (Hymenoptera, Formicidae, *Atta*). 4. Colony ontogeny of *Atta cephalotes*. *Behav. Ecol. Sociobiol.* **14**: 55-60