Wingless ant males adjust mate-guarding behaviour to the competitive situation in the nest

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We investigated whether wingless sexuals of the ant *Hypoponera opacior* adjust mate-guarding behaviour to the level of competition in the nest. Males mate with young nestmate females shortly before these emerge from the cocoon. Aggressive interactions among adult males have never been observed, but males embrace and guard the cocoons of their mating partners for up to 2 days. In laboratory experiments, the duration of pupal guarding increased with the number of adult males in the nest, but decreased with an increasing number of mating partners per male. These findings demonstrate that males are aware of the competitive situation in the nest and adjust their mating behaviour in an adaptive manner. Males also guarded and attempted to copulate with sterile worker and male pupae. These misdirected behaviours might be the result of identification errors, as we found that the cuticular hydrocarbon profiles of young individuals of the different castes were very similar. Copulatory behaviour towards sterile workers is certainly maladaptive, whereas interactions with young males may provide a fitness benefit: We found a high mortality rate of young males that were embraced and guarded by adult males. Adult male–male pupae interactions predominantly occurred when only a single male was present in the nest. In addition, single-male nests were found at unusually high frequencies. These findings suggest that wingless males try to kill their pupal rivals through embracing when there are few adult competitors in the nest, but switch to mate-guarding behaviour when intramale competition is high.

Sexual selection can select for diverse morphological, sensory or behavioural traits, such as ornamentation, weapons, sensory acuity and courtship or aggressive behaviour. The two main processes of sexual selection, female choice and male–male competition, select for different characters that either make a male more attractive to females or help in fights among males (e.g. Wiley & Poston 1996; Sullivan-Beckers & Cocroft 2010). In some animal species, males do not openly fight for access to females, but guard their mating partner to prevent copulations of the female with other males. Mate guarding is especially common if the last male that copulates with a female fertilizes most of her eggs (last-male sperm precedence) and/or if the opportunities to find a second receptive female are low (e.g. Birkhead & Hunter 1990; Jormalainen 1998). The latter is the case if females have a short receptive period, are widely scattered or difficult to find. Mate-guarding behaviour is widespread in the animal kingdom and has been described in both vertebrates and invertebrates (e.g. Gilbert 1976; Watts 1998; Conner & Itagaki 1984;

Fahey & Elgar 1997; Jormalainen 1998; Yamauchi et al. 2001; Morbey 2002; Stephenson & Verrell 2003; Parker & Vahed 2010; Nichols et al. 2010; Parga 2010; Estrada et al. 2010).

We can distinguish between noncontact mate guarding, where males stay close to the females, but are not attached to them, and contact mate guarding, also described as 'passive phases' of matings. During these phases, males are attached to females without genital insertion and either guard them until they become receptive (precopulatory passive phases) or stay associated with them after the copulation is terminated (postcopulatory passive phase) to reduce the chance of sperm competition (Parker 1974). Time investment during mate guarding is costly, and therefore males should adjust their mate-guarding behaviour to the current social environment. For example, males are expected to guard their mating partner for longer if male–male competition is high (Parker 1974). Indeed, mate-guarding duration has been shown to be affected by the number of competitors and potential mating partners in several animal taxa (e.g. Ward 1983; Cuadrado 2000; Komdeur 2001; García-González & Gomendio 2006; Takeshita & Henmi 2010).

In contrast to the diverse sexual strategies found in many insect taxa, social Hymenoptera show little diversity and usually mate in large swarms during nuptial flights. Under these conditions, both

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male–male competition and active mate choice are difficult and social Hymenopteran males have not developed weapons, ornamentations or fighting/courtship behaviour. Instead, scramble competition occurs in mating flights which selected for sensory acuity (large eyes and sensitive antennae) and good flying abilities (Foitzik et al. 2002). However, some social Hymenoptera, such as bees of the genus *Perdita* or ants of the genera *Formicoxenus, Cardiocondyla, Technomyrmex* and *Hypoponera*, also produce wingless worker-like males that mate within the mother nest (Michener 1974; Hölldobler & Wilson 1990; Danforth 1991). Some of these male morphs, driven by local mate competition, developed strong mandibles, which they use in deadly fights (Hamilton 1979; Danforth 1991; Heinze & Hölldobler 1993; Yamauchi et al. 1996) or to defend territories within the colony (Frohschammer & Heinze 2009). Yet, wingless males of other *Hypoponera* ant species do not fight, but guard their mating partner for hours. They embrace pupae (Fig. 1) and mate with very young queens before these emerge from the cocoon (Yamauchi et al. 2001; Foitzik et al. 2002). This guarding of pupal females has also been observed in butterflies (Gilbert 1976; Estrada et al. 2010) and mosquitos (Conner & Itagaki 1984) and allows the male to be the first mating partner of an emerging female.

We investigated matings and mate-guarding behaviour in wingless sexuals of the ant *Hypoponera opacior*. Ant colonies from a population in the Chiricahua Mountains, Arizona, U.S.A., raise winged sexuals in early summer that mate during nuptial flights in July. During a second reproductive season in August–September only wingless sexuals are produced, which mate in the mother nest. Wingless males copulate with queens during the last stages of pupal development and mate-guard their partners (Foitzik et al. 2010). Male genitalia are inserted at the rear end of the cocoons, which have been opened to facilitate the emergence of the young ants. The duration of mate guarding with or without genital contact varies between a few minutes and several hours (up to 2 days), although sperm can be transferred within minutes (Foitzik et al. 2002). Copulations are usually terminated by the emergence of wingless queens, which are invariably inseminated. Emerged queens have been observed to resist further copulations, so that the first mating partner of a young queen is in most cases also the last one. Our goal was to determine whether males adjust their guarding behaviour to the competitive situation in the nest as proposed by Parker (1974). We predicted that males should guard mating partners for longer, if more competitors are in the nest. However, in nests with many pupae per adult male, they should shorten their guarding behaviour to take advantage of additional mating opportunities.

Second, we investigated whether the caste of pupae influences male behaviour. *Hypoponera* males have been observed attempting to mate with worker and male pupae and we aimed at determining how common this behaviour is (Yamauchi et al. 2001; Foitzik et al. 2002). We were interested in whether males guard queens more often than expected by chance. Furthermore, we studied whether wingless males show different behaviours towards queen and worker pupae, such as different frequencies of genital insertion. There are only slight differences in body size between the wingless castes of *H. opacior* (Foitzik et al. 2010) and consequently cocoons of the different castes do not vary in size or shape. Hence, we assumed that males use chemical cues to identify the caste of pupae and we therefore analysed the cuticular hydrocarbon profiles of young wingless queens, males and workers.

Third, it was previously observed that young *Hypoponera* males sometimes do not survive the embrace of other males (Yamauchi et al. 2001; Foitzik et al. 2002). If true, embracing of male pupae might be a form of male–male competition. We found support for this idea in data from a sex ratio analysis of *H. opacior* nests (Foitzik et al. 2010), which showed a strong variance in the number of wingless males per nest. While some nests contained many males, we found a high number of nests with only a single adult male. This could be the outcome of adult males killing young emerging competitors and thereby lengthening their reproductive monopoly. We recorded the survival rate of embraced pupal males and investigated whether single-male nests occur more often than expected. Furthermore, we expected the killing of male pupae to occur predominantly in nests with few adult competitors. In multimale colonies all males would benefit from the removal of new rivals, but the killer male would bear the costs of this behaviour.

METHODS

Ant Collection and Maintenance

Nests of the ant *H. opacior* were collected in the Chiricahua Mountains in Arizona, U.S.A., close to the Southwestern Research Station (31°52.000′N, 109°12.609′W) in August and early September 2010. Ants occur in the upper soil layers, preferentially under stones. Therefore we turned over stones and collected complete nests with an aspirator. Nests were then transferred to our laboratory at the Southwestern Research Station where we kept them in three-chamber boxes (10 × 10 cm and 3 cm high) with a moistened plaster floor. A circular cavity in the floor, of approximately 3 mm depth and a radius of 1.5 cm, was covered with a microscope slide. This cavity served as a nest chamber. Ants were fed with water and dead insects every 1–2 days.

Mate Guarding and Pupal Development

We checked our laboratory nests one to three times per day for mate-guarding behaviour. Males were recorded as showing mate-
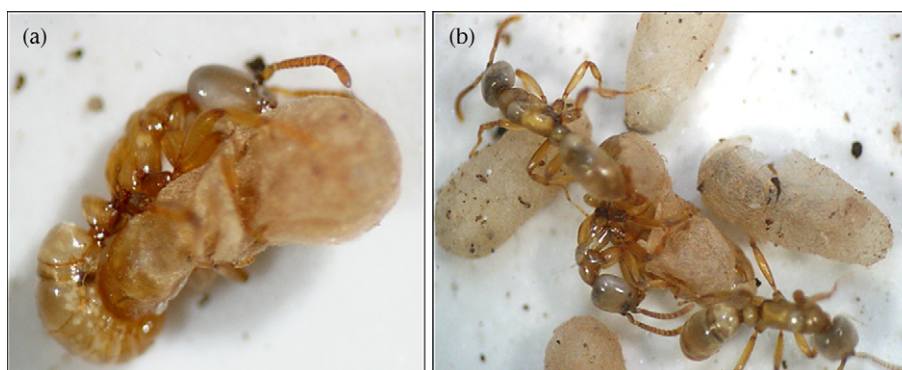

Figure 1. (a) Male embraces a pupa that is still enclosed in the cocoon. (b) Local mate competition: two males are still searching for mating partners while the third is already guarding a pupa.

guarding behaviour if they embraced cocoons (Fig. 1) and/or inserted their genitalia into the genital opening of the mating partner. Matings often ended when queens emerged from the cocoon and started walking around in the nest. They then dragged copulating males around until they detached. Occasionally males left guarded pupae after a while, without having established genital contact. As soon as guarding behaviour was detected, we recorded the time and counted the males, females and potential mating opportunities (cocoons) in the nest. We added together the numbers of adult workers and wingless queens, hereafter named 'females', because it is difficult to discriminate between older individuals of these two castes. Wingless queens closely resemble workers in external morphology, and can only be recognized by comparing eye size, which is larger in queens (Foitzik et al. 2010), or by dissection, because only queens have ovaries (Foitzik et al. 2002). For our analysis, however, an exact caste determination of adult females was unnecessary, because males only mate with newly emerging individuals. Potential mating opportunities were recorded as the number of cocoons in the nest, because the determination of caste of young pupae is impossible. We furthermore noted whether the mating partner had already emerged from the cocoon and, if distinguishable, if there was insertion of genitalia or not.

After termination of mate guarding, we isolated the mating pairs together with three adult workers and recorded both the caste of the emerging individuals and their survival rate within the first 2 days after being embraced. The isolated individuals were kept in the same type of three-chamber boxes and under the same conditions as the colonies. Workers were added because they occasionally help emerging individuals by removing parts of the cocoons. In cases in which we could not clearly discriminate between workers and queens by eye size, we dissected their ovaries at the end of our observation.

To determine which proportions of cocoons contained male, queen or worker pupae, we carefully studied the emergence of pupae in a subsample of 42 nests from the same season. Each day we counted the newly emerged individuals and determined their caste. It is easy to identify the caste of freshly emerged ants (callows) as these have a lighter cuticle than older individuals, so that the difference in eye size between queens and workers is more distinct. After caste determination we removed the callows from the nest. The proportions obtained were used to analyse whether males preferentially mated with queen pupae.

Statistical analyses were conducted using the program STATISTICA version 6.0 (StatSoft, Tulsa, OK, U.S.A.).

Chemical Analysis

We extracted cuticular hydrocarbons from *Hypoponera* ants by submerging individuals in 30 µl of hexane for 5 min. We removed the ant from the hexane with a sterile glass pipette and stored our samples at $-20\,^{\circ}$C. Samples were then analysed using coupled gas chromatography and mass spectrometry (GC–MS) on an Agilent Technologies 6890N GC and 5975 MSD which was equipped with a Restek Rxi-5MS column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness). Analyses were conducted as in Foitzik et al. (2011). We only included cuticular hydrocarbons in our analysis, which were identified by retention time and their mass spectra with the Wiley 7N spectral database. We standardized by the maximum peak area in order to detect differences in relative proportions.

Chemical data were analysed using the software PRIMER 6 version 6.1.12 (Primer-E Ltd., Ivybridge, U.K.) with the PERMANOVA+ add-in version 1.0.2 (Anderson et al. 2008). Nonparametric, permutational multivariate analyses of variance (PERMANOVA) were conducted with 9999 permutations. To test for differences in profiles between castes we conducted pairwise tests for the three possible combinations of wingless castes (males, queens and workers). Bray–Curtis similarities were calculated for each analysis using the zero-adjusted Bray–Curtis index (Bray & Curtis 1957). For the graphic illustration of multivariate profile differences (see Results) we used multidimensional scaling (MDS).

In total, we extracted and analysed cuticular profiles from 23 wingless males, 61 workers and 51 wingless queens. We grouped individuals into three age classes: age class 1 contained individuals up to 6 h after their emergence from the cocoon ($N_{males} = 14$; $N_{workers} = 19$; $N_{queens} = 6$), age class 2 contained individuals from 6 h to 2 weeks after emergence ($N_{males} = 9$; $N_{workers} = 24$; $N_{queens} = 17$) and age class 3 contained individuals that were older than 1 month ($N_{males} = 0$; $N_{workers} = 18$; $N_{queens} = 28$). The reason for the absence of males in age class 3 is that none of the males in our artificial nests survived long enough to be grouped into this age class. Additionally, we extracted hydrocarbons from 28 pupae that were still enclosed in cocoons. The samples stemmed from 48 nests with between one and 23 analysed individuals per nest.

Analysis of the Number of Males within Nests

We noticed in the field that most *H. opacior* nests with males contained only a single male. To determine whether nests with a single male are indeed more common than expected, we counted the males in 127 *H. opacior* nests at the time of collection. All nests contained at least one male and 6–255 adult females (mean number of adult females = 32) and were collected in the Chiricahua Mountains in late summer of 2004, 2005, 2009 and 2010. We then compared the distribution of the number of males per nest to the distributions of five other ant species with similarly small nest sizes: *Temnothorax longispinosus* ($N_{nests} = 58$, number of adult females 7–159, mean = 44), *Leptothorax acervorum* ($N_{nests} = 160$, number of adult females 6–328, mean = 84), *Leptothorax muscorum* ($N_{nests} = 59$, number of adult females 7–201, mean = 63), *Temnothorax crassispinus* ($N_{nests} = 53$, number of adult females 8–181, mean = 70) and *Temnothorax curvispinosus* ($N_{nests} = 34$, number of adult females 8–64, mean = 30). Only nests with at least one male were included in the analysis. All of these ant species have winged males, which mate in nuptial flights and we therefore did not expect competitive fights among nestmate males in these species. We admit that our selection is taxonomically biased, as the comparative species all belong to the subfamily Myrmicinae, but these data were available and can give a first indication of whether single-male nests are more common in *H. opacior* than in other ant species.

RESULTS

Mate Guarding and Pupal Development

We observed mate guarding and measured the guarding duration of 119 males from 41 nests. In 103 of the 119 interactions we recorded the caste of the mating partner and in 95 cases we noted the presence or absence of genital contact. Of the 103 mating partners, 89 were still in the cocoon at the beginning of our observation. The other 14 individuals were newly emerged callows. We presumably observed only the end of the interaction in those 14 cases and mate guarding, as in most other observations, might have started before these individuals had fully emerged from the cocoon. Our estimates of mate-guarding duration are therefore always lower bound, but since we made this error systematically for all guarding interactions, meaningful comparisons should be possible. Additionally, we might have missed very short interactions, as we

did not observe the ant nests continuously. However, we did record 10 interactions that were shorter than 10 min.

To investigate whether males adjust their mate-guarding behaviour to the competitive situation in the nest, we tested whether the number of adult males in the nest (hence the competition) and the number of available cocoons divided by the number of males (potential mating partners per male) were correlated with the length of the guarding behaviour. We found that guarding duration increased with the number of adult males in the nest (Spearman rank correlation: $r_S = 0.45$, $N = 119$, $P < 0.000001$; Fig. 2a), but did not change with the nest size (i.e. number of females; Spearman rank correlation: $r_S = -0.09$, $N = 119$, $P = 0.30$). Yet, the length of guarding behaviour decreased with increasing mating opportunities, that is the number of cocoons per male (Spearman rank correlation: $r_S = -0.49$, $N = 119$, $P < 0.000001$; Fig. 2b).

Caste determination of guarded individuals showed that only 29 (28%) of the mating partners were queens, whereas 61 (59%) were workers and 13 (13%) were males (Fig. 3a). Even among the 14 already emerged mating partners, we found seven queens, six workers and one male. Taking only matings with queens into account, we found the same effects of an increase in guarding duration with the number of males and a decrease in guarding duration with increased mating opportunities (Spearman rank correlations: number of males: $r_S = -0.54$, $N = 29$, $P < 0.003$; number of females: $r_S = -0.29$, $N = 29$,
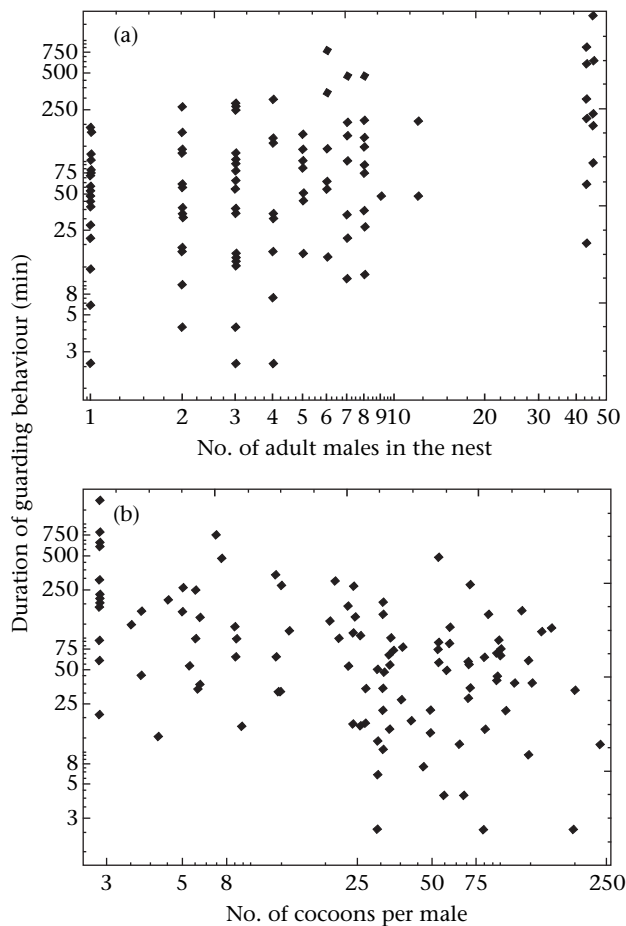

Figure 2. Association between (a) the number of adult males in the nest and (b) the number of cocoons per male in the nest and the duration of mate-guarding behaviour (min). Axes are given in a logarithmic scale, because there were a few outliers. Excluding outliers did not change the significance of our findings.
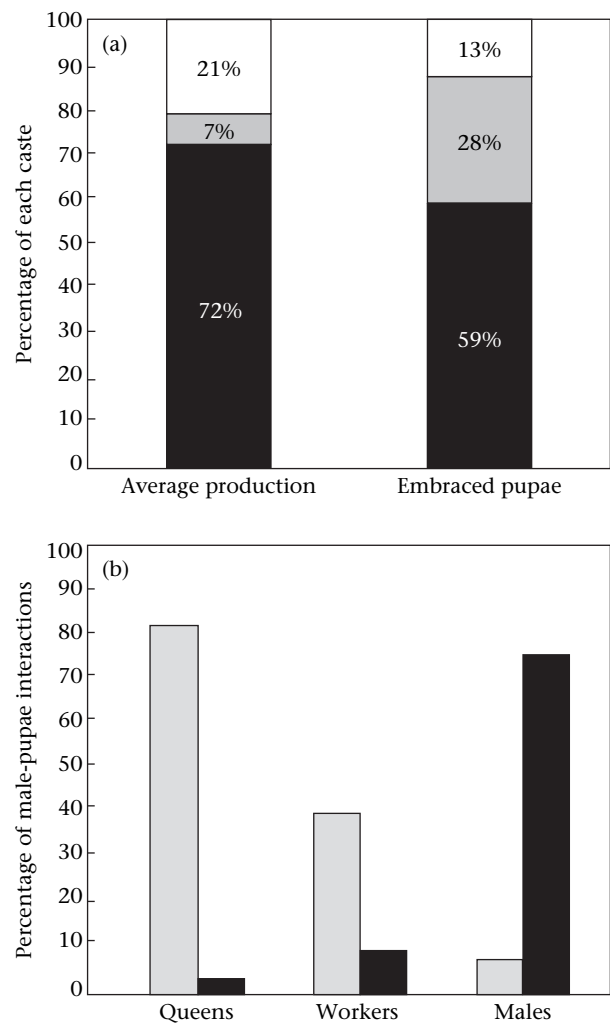

Figure 3. (a) Percentage of males (white), queens (grey) and workers (black) among newly produced individuals. Average production: the percentage of each caste among pupae in a subsample of 42 nests ($N = 1204$). Embraced pupae: the percentage of castes among pupae that were embraced by adult males ($N = 103$). (b) Percentage of male–pupa interactions with genital contact (grey bars; separated by caste; $N_{queens} = 24$; $N_{workers} = 59$; $N_{males} = 12$) and percentage of the three castes dying after being embraced by a male (black bars; $N_{queens} = 25$; $N_{workers} = 50$; $N_{males} = 11$).

$P = 0.13$; number of cocoons per male: $r_S = -0.58$, $N = 29$, $P < 0.001$). Interactions (guarding, embracing and copulating) of males with pupae lasted longer than male interactions with emerged adult queens (Mann–Whitney U test: $U = 312$, $N_1 = 15$, $N_2 = 104$, $P < 0.0002$).

In the subsample of nests ($N = 42$) for which we determined the caste of all newly emerged individuals, we found that, on average, 72% of the cocoons contained worker pupae, 21% male pupae and only 7% queen pupae (Fig. 3a). Next, we tested the observed distribution of castes among mating partners against the expected distribution of mating partners if males guarded pupae randomly. We found that males guarded queens more often than expected compared to males (chi-square test: $\chi_1^2 = 13.84$, $P < 0.002$) or workers ($\chi_1^2 = 14.26$, $P < 0.002$). We did not find a difference in the relative frequency with which males and workers were guarded ($\chi_1^2 = 0.73$, $P = 0.39$). *Hypoponera opacior* males therefore guarded and copulated with young queens more often than expected by chance, that is if they showed no choice, but they still guarded queens in less than 30% of cases. Moreover, we did not find a difference in the length of the guarding behaviour between

interactions of males with queen pupae, male pupae or worker pupae (Kruskal–Wallis test: $H_{2,103} = 0.06$, $P = 0.97$). Genital contact was observed not only in interactions between males and queens, but also between males and workers or other males (Fig. 3b). However, genital insertions were more frequent in male–queen interactions ($\chi^2_1 = 12.37$, $P < 0.004$) or male–male interactions ($\chi^2_1 = 16.97$, $P < 0.0001$). Males inserted their genitalia more frequently into worker pupae than into male pupae ($\chi^2_1 = 3.95$, $P < 0.05$). The length of the mate-guarding behaviour did not vary with whether or not we observed a male inserting its genitalia (Mann–Whitney U test: $U = 1173$, $N_1 = 64$, $N_2 = 45$, $P = 0.10$).

Additionally, we analysed the demography of the ant colonies during pupal guarding and studied whether the interactions of males with different pupal castes depended on the composition of the nest. We found that embracing between males and male pupae took place in nests with fewer males than interactions with either queen or worker pupae (Kruskal–Wallis test: $H_{2,89} = 7.63$, $P = 0.022$; Mann–Whitney U tests: queen–male: $U = 61.5$, $P < 0.01$; worker–male: $U = 197$, $P = 0.03$, queen–worker: $U = 490$, $P = 0.20$). The median number of males present in nests during male–male pupae interactions was one, while male–queen and male–worker interactions occurred at a median of 5.5 and 4 adult males, respectively.

Of the 103 guarded individuals for which we identified the caste, 17 could not be further observed after males detached, because they either got lost within the nest by walking away or, if still partly enclosed in the cocoon, by being carried away before we could separate them from the nest. Yet, for 86 of the pupae we recorded not only the caste, but also their survival within the first 2 days after being guarded by a male including the typical embracing behaviour (Fig. 1). Of these cocoons, 11 contained males, 25 queens and 50 workers. Of the 11 males, eight (73%) died within the first 2 days after being guarded and embraced, whereas only five callow workers (10%) and one of the queens (4%) died (Fig. 3b). Therefore, males survived guarding and embracing less often than workers ($\chi^2_1 = 21.16$, $P < 0.0001$) and

queens ($\chi^2_1 = 19.24$, $P < 0.0001$). Almost all of these individuals died while still enclosed in the cocoon, but one male and two workers managed to emerge from the cocoon and died shortly afterwards. Genital insertion did not appear to be the cause of male death as we observed genital contact between a male and a male pupa only once and in this case the male survived the interaction.

Chemical Analysis

We detected the same cuticular hydrocarbons as in a previous analysis of older individuals of *H. opacior* (Foitzik et al. 2011), with the exception of methyl-heptacosadiene, 2-methyl-octacosane, nonacosene C and hentriacontene C. Instead, we found two additional hydrocarbons (dimethyl-hentriacontene and dimethyl-heptacosadiene), which were not previously detected on the cuticle of *H. opacior*.

Chemical profiles varied with age ($P < 0.001$) and caste ($P < 0.001$). Furthermore, the interaction between these factors was significant ($P < 0.001$). We detected only traces of cuticular hydrocarbons on extracted cocoons, which made caste differentiation at this developmental stage unlikely (Fig. 4). Therefore, we included only emerged individuals in our statistical analysis (Table 1). Pairwise tests revealed differences in profiles between all three castes, although those between workers and males were not significant after correction for type 1 error. This was done because the same data were used in the overall analyses and the separate analyses for each age class. For the young individuals of age class 1, which represent mating partners of pupal mating males at the time when copulations are usually terminated, we found no significant difference between castes. Caste difference approached significance for comparisons between workers and queens, and workers and males, in age class 2 and were highly significant in workers and queens that were at least 1 month old. The amount of cuticular hydrocarbons clearly increased in the hours after emergence from the cocoon (Fig. 4). This has been shown in other ants as well, in which freshly emerged callow
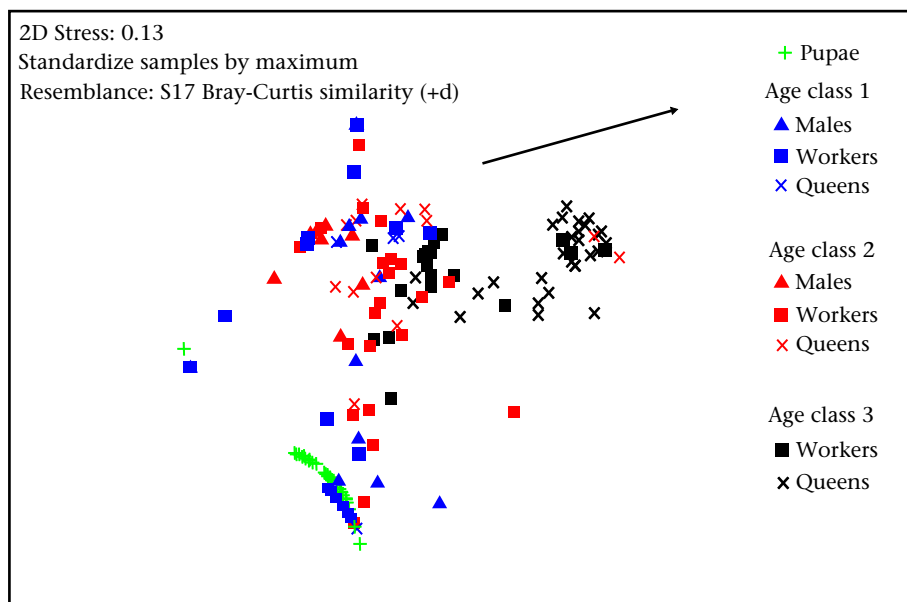

Figure 4. Multidimensional scaling (MDS) of cuticular hydrocarbon profiles of males, workers and queens from age class 1 (up to 6 h after emergence), age class 2 (6 h to 2 weeks after emergence) and age class 3 (older than 1 month). The direction of the black arrow indicates the increase in the total amount of cuticular hydrocarbons. Comparison of total amount of hydrocarbons: $P < 0.001$ for each pairwise comparison of age classes based on log-transformed data; ANOVA followed by post hoc least significant difference test: $F_{2,135} = 62.72$.

Table 1
Differences in the hydrocarbon profiles of ants of different castes

	Comparisons	t	P	Permutations
All	Males, workers	1.77	0.03	9949
	Males, queens	3.96	**0.0001**	9951
	Workers, queens	4.02	**0.0001**	9957
Age class 1	Males, workers	0.87	0.45	9954
	Males, queens	0.65	0.70	5042
	Workers, queens	1.32	0.17	8533
Age class 2	Males, workers	1.66	0.05	9959
	Males, queens	1.17	0.25	9921
	Workers, queens	1.47	0.07	9935
Age class 3	Workers, queens	5.11	**0.0001**	9944

Age class 1 contained individuals up to 6 h after their emergence from the cocoon ($N_{males} = 14$; $N_{workers} = 19$; $N_{queens} = 6$), age class 2 contained individuals from 6 h to 2 weeks after emergence ($N_{males} = 9$; $N_{workers} = 24$; $N_{queens} = 17$) and age class 3 contained individuals that were older than 1 month ($N_{males} = 0$; $N_{workers} = 18$; $N_{queens} = 28$). Pairwise PERMANOVA were conducted with the factor 'caste'. Significant P values after correction for type 1 error are given in bold.

individuals were described as chemically insignificant (Lenoir et al. 1999).

Analysis of Number of Males per Nest

We found that about half of the 127 investigated *H. opacior* nests contained only one male while the percentage of single-male nests varied in the five other species between 27% and 34% (Fig. 5a). The distribution of the number of males per nest differed significantly between *H. opacior* and all other species (Kolmogorov–Smirnov tests: $P < 0.05$), with the exception of *T. curvispinosus*, where we found only a trend in the same direction ($P < 0.10$). Moreover, the distributions did not differ between the other species (Kolmogorov–Smirnov tests: $P < 0.10$ for *T. crassispinus–L. acervorum*; $P > 0.10$ for all other comparisons). These analyses demonstrate that single-male nests occur in *H. opacior* at unusually high frequencies (Fig. 5b).
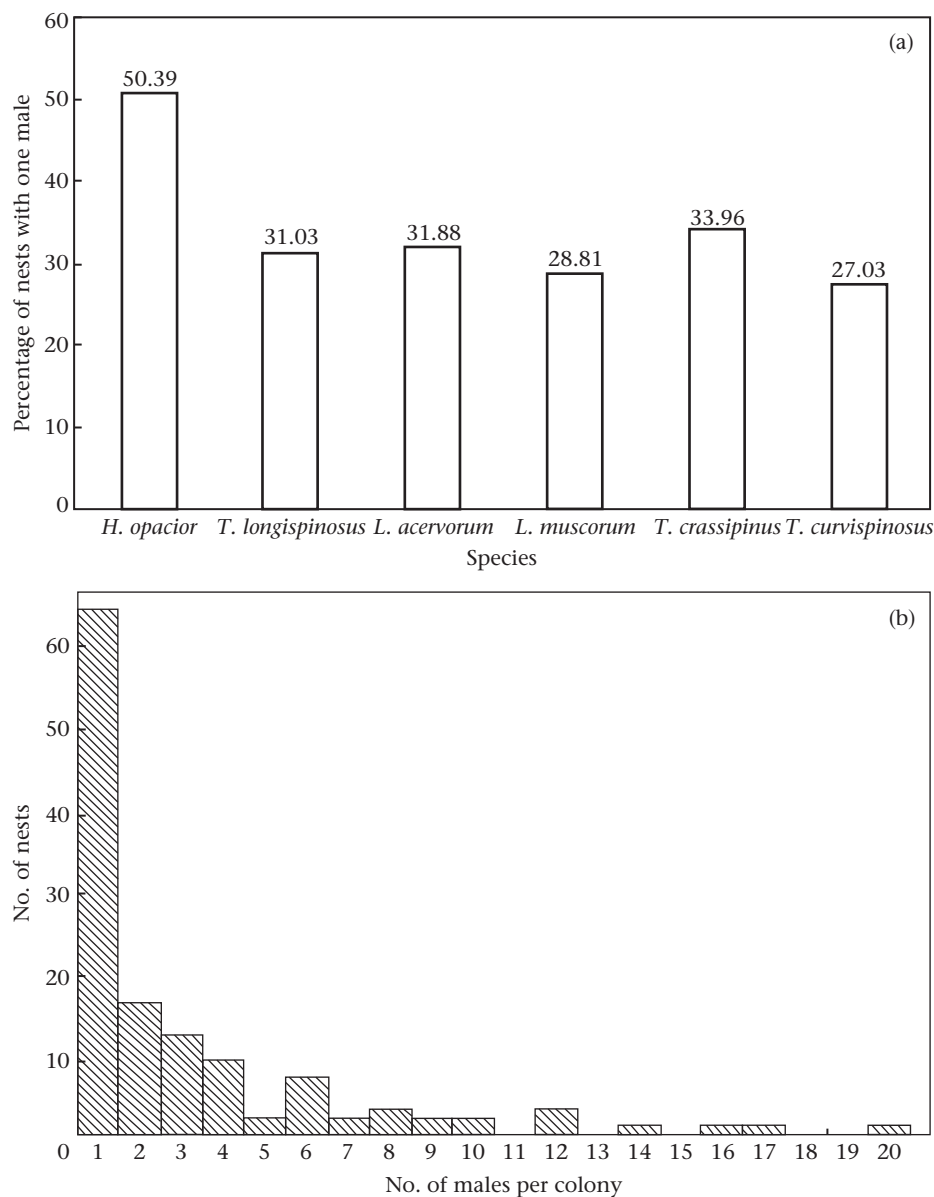

Figure 5. (a) Percentages of nests containing only one male for the species *H. opacior, T. longispinosus, L. acervorum, L. muscorum, T. crassispinus, T. curvispinosus* (nests without males were excluded from the data set). (b) Number of males per nest in *H. opacior* nests with males ($N = 126$) directly after collection. One nest with 45 males was left out of this figure.

DISCUSSION

The results of this study suggest that *H. opacior* males increase the length of mate guarding with the number of rivals in the nest. Moreover, they guard their partners for less time if there are more mating opportunities, that is, more pupae available per male. These findings are in line with Parker's (1974) prediction and similar observations in crustaceans, reptiles, birds and other insects (Ward 1983; Cuadrado 2000; Komdeur 2001; García-González & Gomendio 2006; Takeshita & Henmi 2010). They demonstrate that wingless males correctly assess the competitive situation in the nest and that they adjust their mating behaviour in an adaptive manner. In a different *Hypoponera* species, males were already shown to lengthen their guarding behaviour if they detected rival males (Yamauchi et al. 2001). Yet, we have shown here that males not only correctly detect the presence or absence of rivals, but also alter their mating behaviour in response to the number of competitors in the nest and to the potential mating opportunities.

The mate-guarding behaviour of *H. opacior* males can be regarded as a combination of pre- and postcopulatory mate guarding, because males start to guard pupae before making genital contact and often stay attached to them until the latter emerge from the cocoon. This behaviour is probably adaptive. First, if a young queen is guarded before she becomes receptive, the guarding male ensures he is her first mating partner. Second, adult queens are very reluctant to mate after emergence and most copulations end when young queens fully emerge from the cocoon. Hence a male that is still with a queen until this point will most likely remain her only mating partner. On the other hand, it has been shown that *H. opacior* males can mate several times (Foitzik et al. 2002), which explains why mate-guarding durations are shorter when there are more mating opportunities available per male.

Although the adaptive adjustment of mate guarding indicated that mating behaviour is under selection, we found that males did not exclusively guard queens. Males preferentially guarded young queens, but there was an unexpectedly high number of males that guarded worker or male pupae. The guarding of workers in *H. opacior* can be regarded as maladaptive because workers have no ovaries and are completely sterile (Rüger et al. 2008). As mentioned before, cocoons of the three wingless castes do not differ in size or outer appearance and we therefore expected caste discrimination to be based mainly on olfactory cues. Our chemical analyses showed that caste differences in chemical profiles develop during early adulthood and that profiles of young individuals are still very similar. These findings explain the high rate of caste recognition errors by sexually active males. Males that are better able to single out queen pupae should have a higher reproductive success, and indeed we found that males did not guard pupae at random, but more often attended queen pupae than expected by the relative frequency of queen and worker pupae in the nest. This indicates that slight chemical or morphological differences are detectable at least by some males. The guarding duration did not vary with caste of the pupae.

Homosexual mountings have been described for several animal taxa (Aiken 1981; Bagemihl 1999; Harari et al. 2000; Maklakov & Bondurianksy 2009) and have been interpreted as practice for later heterosexual interactions, as dominance interactions caused by rivalry (Dagg 1984; Vasey 1995) or, as they mainly occur in insects, as a result of perception errors (e.g. Parker 1968; Aiken 1981; Serrano et al. 2000; Estrada et al. 2010; Dukas 2010). In contrast to the mate guarding of worker pupae, wingless *H. opacior* males might directly benefit from the guarding and embracing of male pupae. Similar to observations in *Hypoponera nubatama* (Yamauchi et al. 2001), we found that a high percentage (73%) of

male pupae died after being embraced. Regarding the chemical similarity of young individuals, one could argue that the guarding and embracing of male pupae is also simply due to recognition errors. Behavioural observations, however, suggest that this might not be the case. Males embracing male pupae more rarely inserted their genitalia than those interacting with queen or worker pupae. This suggests that at some point males recognize that they are currently guarding a male pupa. Nevertheless, they continue to embrace these pupae, and the guarding duration did not differ from that of interactions with female pupae. *Hypoponera opacior* males are incapable of openly fighting against each other, in contrast to the wingless ant males of other *Hypoponera* or *Cardiocondyla* species, which bear strong and sharp mandibles (Hamilton 1979; Heinze & Hölldobler 1993; Yamauchi et al. 1996). Moreover, adult *H. opacior* males invariably behaved peacefully towards each other. Most of the time, they were either occupied with guarding and embracing of pupae or with investigating pupae with their antennae. Squeezing male pupae with a very soft cuticle might be the only possibility to influence competition in the nest actively.

The killing of young males that are still enclosed in the cocoon could thus be a second strategy, besides mate guarding, to deal with male–male competition. Besides the high mortality rate of previously embraced male pupae, this hypothesis is further supported by the analysis of the distribution of males within *H. opacior* nests: We found an unusually high fraction of nests containing just a single male compared to other ant species with similar nest sizes. For example, four times as many nests contained a single male as contained two males. On the other hand, several nests contained multiple males with up to 45 males per nest. We interpret these findings as the first males to emerge trying to lengthen their reproductive monopoly by killing pupal rivals, but when many males emerge at once, they give up and tolerate each other. Especially in larger nests with many pupae, it is impossible for a male to eliminate all young competitors. Once several adult males have emerged, the time-consuming deadly embrace of other males is expected to be a less beneficial competitive strategy than mate guarding. All males would benefit from the death of their rivals, while only males that invest time and energy in killing male pupae would lose mating opportunities. In support of these predictions, we found male–male pupae interactions in nests with few adult males, while guarding of queen and worker pupae occurred more often in nests with many males. Furthermore, the guarding durations increased in nests with many males, pointing to a switch to a mate-guarding tactic under strong male–male competition. These behavioural variations of wingless *H. opacior* males can be regarded as two tactics of a conditional strategy (Gross 1996). Conditional strategies are usually status dependent, but a switch-point adjustment to the demographic environment, including operational sex ratio, has also been shown in other insect species (e.g. Crespi 1988; Carroll & Corneli, 1995).

To verify this last assumption of rival killing through embracing of male pupae, further studies are planned. We intend to compare survival rates of embraced and nonembraced pupae, as it is still possible that the survival rate of male pupae is generally lower than that of queens and workers. Furthermore, we intend to study male–male interactions by carefully recording the adult males' behaviour before and during the interaction with male pupae. If males embrace male pupae to harm or kill them, we would expect their behaviour to differ from mating behaviour towards queens. For example, males might squeeze male pupae more tightly to indent the soft cuticle of male pupae. Indeed, we found males with indented heads; however, whether these injuries were caused by other males is currently unclear.

In summary, our study illustrates a complex and adaptive mating behaviour of wingless ant males of the species *H. opacior*.

They were shown to adapt their mate-guarding behaviour precisely to the competitive situation in their local environment as proposed by Parker (1974). The high fraction of mate-guarding behaviour towards sterile workers is most likely caused by perception errors, while embracing of male pupae could reduce male–male competition and thereby carry a fitness benefit.

Acknowledgments

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