

Research article

Hammering, mauling, and kissing: stereotyped courtship behavior in *Cardiocondyla* ants

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Abstract. Sex appears to be a rather prosaic and casual event in the life of most social Hymenoptera. In contrast, mating in the ant genus *Cardiocondyla* is regularly preceded by a prolonged and stereotypic courtship display. Pummeling the head of the female with mandibles and / or antennae and vibrations of the gaster, presumably stridulation, are essential parts of male courtship. The overall structure of the mating pattern is conserved throughout species and between winged and wingless, “ergatoid” males, but exhibits species-specific idiosyncrasies. For example, *C. elegans* males regularly end the interaction with a female with a short mouth-to-mouth contact. Variation in the duration of the pre-copulatory phase and the copulation itself might reflect different degrees of inter- and intrasexual selection. More information on the dynamics of sperm transfer and the risk and intensity of sperm competition are needed to better understand the evolution of the complex mating behavior in this genus.

Keywords: *Cardiocondyla*, mating behavior, male polymorphism, sexual selection.

Introduction

Males of many animal species compete for access to females by overt or ritualized fighting or by attracting their mates by colorful ornaments and elaborate displays (Andersson, 1994). Mating is therefore often preceded by

time-consuming and complex courtship behavior. In contrast, sex appears to be strikingly unspectacular in the social Hymenoptera (Hölldobler and Bartz, 1985; Baer, 2003; Boomsma et al., 2005). In ants, sexual behavior often consists merely of locating and approaching the partner, several short, investigative antennation strokes, and a few seconds of sperm transfer. There is little evidence for male-male fighting other than scramble competition, and even less for female choice (Davidson, 1982; Wiernasz et al., 1995, 2001; Abell et al., 1999). Instead, females and males often appear to mate indiscriminately with the first or the first few partners they encounter (Woyciechowski, 1990). The apparent simplicity of the sexual life is presumably explained by the extremely short duration of the mating period, which in many ant species lasts only for a few hours per year and during which thousands of sexuals have to find a mate. Most ant males die after one or a few matings, and young queens use their sperm to fertilize eggs for over 25 years without re-mating. This results in the quite unique partner commitment for life, or, in the case of males, even beyond death (Hölldobler and Bartz, 1985; Boomsma et al., 2005).

Males of the ant genus *Cardiocondyla* have a considerably more exciting sex life. *Cardiocondyla* is characterized by a peculiar polymorphism of winged and wingless (“ergatoid”) males (Kugler, 1983; Seifert, 2003). Winged males are docile; they first mate with female nestmates and later emigrate from the nest to locate female sexuals outside. Ergatoid males, in contrast, are often territorial and engage in lethal fighting over the harem of female sexuals in their nests (Kinomura and Yamauchi, 1987; Stuart et al., 1987). Though ergatoid males should benefit also from killing their winged rivals

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(Anderson et al., 2003), the latter appear to be protected through chemically mimicking female sexuals at least in one species (Cremer et al., 2002). Colonies therefore often contain a single ergatoid male plus multiple winged competitors.

However, not all species of *Cardiocondyla* show this ancestral pattern. Several species have secondarily lost winged males, and their colonies usually contain single, fighting ergatoid males (Heinze et al., 1993, 2005). Furthermore, in some species with single-queen colonies, colonies may contain multiple, mutually tolerant ergatoid males (Lenoir et al., 2007). As queens mate multiply, this variation in the number of competing winged and ergatoid males might affect the degree of intersexual and intrasexual selection, which again might be reflected in the details of male courtship and mating behavior. For example, assuming that the amount of sperm transferred during a copulation increases with time, as is the case in numerous insects (e.g., Sauer et al., 1997; Danielsson, 1998; Arnqvist and Danielsson, 1999), copulations might last longer in those species in which ergatoid males compete with winged males. Pre-copulatory behavior might be shorter in species with single ergatoid males, because females cannot choose among co-existing males.

According to previous studies, the mating behavior of *Cardiocondyla* is more complex than that of other ants (Kinomura and Yamauchi, 1987; Stuart et al., 1987; Heinze et al., 1993; Yamauchi and Kinomura, 1993), but it has not yet been described in detail. The aim of the present study is, firstly, to thoroughly analyze courtship and mating behavior of one exemplary species, *C. elegans*, and, secondly, to compare it to the courtship behavior in species with different reproductive structures. Specifically, we examine whether variation in the reproductive life history of species, i.e., the presence of winged or wingless rivals, affects crucial parameters, such as the duration of pre-mating interactions and copulation.

Material and methods

Ant collection and maintenance

Colonies or fragments of colonies of different *Cardiocondyla* species were collected from their nests in soil (*C. batesii* (Forel, 1894): S^m. Elvira and Guadix, Spain; *C. elegans* (Emery, 1869): Montlouis sur Loire, France; *C. emeryi* (Forel, 1881), Arno's Vale, Tobago; *C. mauritanica* (Forel, 1890): Motril, Spain; *C. minutior* (Forel, 1899): Itabuna, Brazil; *C. kagutsuchi* (Terayama, 1999): Nualolo and Awa'awapuhi Trailheads, Kaua'i, USA; *C. venustula* (Wheeler, 1908): Nualolo Trailhead, Kau' ai, USA) or rolled lemon leaves and aborted coconuts (*C. obscurior* (Wheeler, 1929): Itabuna and Una, Brazil) and housed in 9.8 x 9.8 x 2.8 cm³ plastic boxes with a layer of plaster in incubators at 26–30°C or at room temperature (appr. 22–24°C; *C. elegans*) as described before (e.g., Heinze et al., 1998). Colonies were provided with honey and pieces of cockroaches or crickets every 3–4 days.

Phylogeny of the studied species

According to a phylogeny based on mitochondrial genes (Heinze et al., 2005), male polymorphic *C. obscurior*, *C. emeryi*, and *C. minutior* are rather basal groups. *C. mauritanica* and *C. elegans* / *C. batesii* are derived clades that convergently lost winged males. *C. kagutsuchi* appears to be related to *C. mauritanica* and is a species complex, in which some taxa have both male morphs, others have lost winged males, and still others are characterized by a peculiar intermorphic male, resembling ergatoids with more or less well developed wings (Yamauchi et al., 2005). The studied material belongs to a clade in which as yet only ergatoid males have been found. While ergatoid males fight in all facultatively polygynous species, male tolerance in *C. elegans* and *C. batesii* is derived and appears to be associated with the secondary evolution of monogyny (Schrempf and Heinze, 2007).

C. venustula was not included in the earlier phylogenetic studies. In a neighbor-joining tree based on 1427 base pairs of CO I / CO II from material collected on Kaua'i and in three sites in Ethiopia (Weliso, Debre Markos, Senbete) it clusters in the same monophylum as *C. elegans* / *C. batesii* and other monogynous species without winged males (A. Trindl, unpublished), albeit with low bootstrap support. Multiple-queening and only fighting ergatoid males were observed in a single colony from Kaua'i (J. Heinze and S. Frohschammer, unpublished).

The low resolution of the branching patterns of *C. emeryi*, *C. minutior* and *C. obscurior* does not yet allow rigid testing of hypotheses on the evolution of mating behavior, but it was suggested that fighting among ergatoid males with saber-shaped mandibles, as in *C. obscurior*, constitutes the ancestral state, whereas attacking only callows is derived (Heinze et al., 2005). For the social structure of colonies of the study species see Table 1.

Description of courtship behavior

Male and female sexual pupae were separated into different nests to prevent unnoticed mating. To observe matings, one or two males and two to eight female sexuals were placed in a nest box with a few workers. Courtship and mating behavior was observed at room temperature under a binocular microscope and was recorded using a Panasonic AG 6730 time lapse video recorder. Observation sessions lasted from 30 min to 3 hrs.

For the more detailed observations on *C. elegans*, sexuals from different laboratory colonies were individually introduced into a circular arena (5 mm in diameter) and allowed to acclimatize to the unfamiliar environment in isolation from each other for 10 min. Thereafter, the pairs were filmed under red light for 30 min (in total 19 pairs). We noted the duration of each of the courtship displays performed by the male during this 30 min period of observation, and in detail described the sequence of various discernible behaviors occurring during such displays (see the list in Table 2). The occurrence of transitions from one type of behavior to another was noted in order to determine potential stereotypies. In a similar way, we recorded and analyzed the courtship and mating behavior in the other species, examining similarities and differences to *C. elegans* and focusing on species-specific elements, which are described in the results.

In addition, we noted the time interval from first antennal contact to copulation and the duration of the copulation itself for males of either morph from each available species. In those species, in which multiple copulations per male were observed, individual males occasionally differed considerably in the duration of pre-copulatory behavior and copulation (e.g., *C. obscurior*, 21 copulations of five winged males, Kruskal-Wallis H-test, $H=9.741$, $p=0.045$, but 94 copulations of seven ergatoid males, Kruskal-Wallis H-test, $H=1.756$, $p=0.940$). To avoid dependence of the data we therefore always considered only the first copulation of a given male. Though we attempted to observe copulations of at least 10 different males per species, the data base is occasionally much smaller due to the lack of males and occasional difficulties in defining the beginning of an interaction. For *C. emeryi* and *C. venustula*, only one ergatoid male each was available.

Table 1. Colony characteristics, median duration of the pre-copulatory phase and the copulation in seconds (and range) in ergatoid and winged males of various species of the ant genus *Cardiocondyla*. *C. batesii*, *C. elegans*, and *C. mauritanica* have exclusively ergatoid males, *C. emeryi*, *C. minutior*, and *C. obscurior* have both ergatoid and winged males. In the studied colonies of *C. kagutsuchi* and *C. venustula*, only ergatoid males have eclosed. The number of observed males is indicated after the name of each species (ergatoid/winged).

	Colony characteristics	Ergatoid males Pre-copulation	Winged males Copulation	Pre-copulation	Copulation
<i>C. batesii</i> (10)	1 ♀, ≥ e ♂ ♂	11 (6–59)	7 (5–15)		
<i>C. elegans</i> (10)	1 ♀, ≥ e ♂ ♂	10 (5–23)	5.75 (3–55)		
<i>C. kagutsuchi</i> (3)	≥ 1 ♀ ♀ fighting e ♂ ♂	83 (64–172)	18 (13–26)		
<i>C. mauritanica</i> (10)	≥ 1 ♀ ♀, fighting e ♂ ♂	55.5 (16–106)	5.75 (1–10)		
<i>C. venustula</i> (1)	≥ 1 ♀ ♀? fighting e ♂ ♂	120	17		
<i>C. emeryi</i> (1 / 10)	≥ 1 ♀ ♀ fighting e ♂ ♂, w ♂ ♂		15	52.5 (31–144)	14 (2–34)
<i>C. minutior</i> (10 / 10)	≥ 1 ♀ ♀ fighting e ♂ ♂, w ♂ ♂	21.5 (11–114)	15.5 (3–24)	11.5 (5–48)	3 (3–7)
<i>C. obscurior</i> (7 / 4)	≥ 1 ♀ ♀, fighting e ♂ ♂, w ♂ ♂	5 (4–9)	32	3 (0–4)	32
			25.4 ¹		11.1 ¹

¹ data from Kinomura and Yamauchi, 1987 and Yamauchi and Kinomura, 1993.

Table 2. Behavioral components of the courtship display exhibited by winged and ergatoid males of *Cardiocondyla* spp.

Abbreviation	Name	Description
AC	Antennal contact	Male contacts female sexual with its antennae from the ground
CT	Climb onto thorax	Male climbs onto the thorax of the female sexual
St	Stridulation	Male stridulates
AB	Antennal boxing	Male vigorously antennates the female sexual
Cp	Copulation	Male curves its abdomen and inserts its genitalia into the female's genital chamber
MC	Mandible contact	Male initiates mandible contact with the body of the female (except for <i>C. elegans</i>); mandible hammering on female's head in <i>C. mauritanica</i>
MM	Mouth-Mouth contact	Male initiates mouth-to-mouth contact with female (only in <i>C. elegans</i>)

Female sexuals were dissected after the encounter to verify if they were mated or not (except in *C. venustula* and *C. kagutsuchi*). Results were statistically analyzed by ANOVA and Mann-Whitney U-tests using the software package STATISTICA.

Results

We could dissect interactions between male and female sexuals of *Cardiocondyla* into four different phases: recognition, pre-copulatory behavior, copulation, and post-copulatory behavior. In *C. elegans*, after a first antennal contact, the male climbed onto the female's thorax from the side, then orientated its head towards the female's head. Pre-copulatory behavior in addition involved heavy stridulation and antennal boxing of the female's head and thorax (Table 2). Although no sound could be heard or recorded, the rapid vertical vibrations

of the male's gaster closely resembled stridulation known from workers and queens of other formicoxenine ants, such as *Leptothorax* or *Formicoxenus*, during fighting or trophallaxis (e.g., Wilson, 1975; Stuart and Bell, 1980; Lenoir et al., 1992). A well-developed stridulatory apparatus suggests that males indeed stridulate during courtship. Thereafter, the male attempted to copulate by extending and curving its gaster around the female's wings towards its genitalia; successful copulations resulted in the male slightly tilting backwards with its genitalia inserted in the female's genital chamber (see also Heinze et al., 1993). Copulations were very brief and in this series of experiments with *C. elegans* lasted for about 3 sec (median; quartiles 1.5 sec and 4.9 sec; n=32). Post-copulatory behavior consisted of a peculiar "kiss", i.e., the male moved forwards on the female's back and brought its mandibles in close contact with those of the

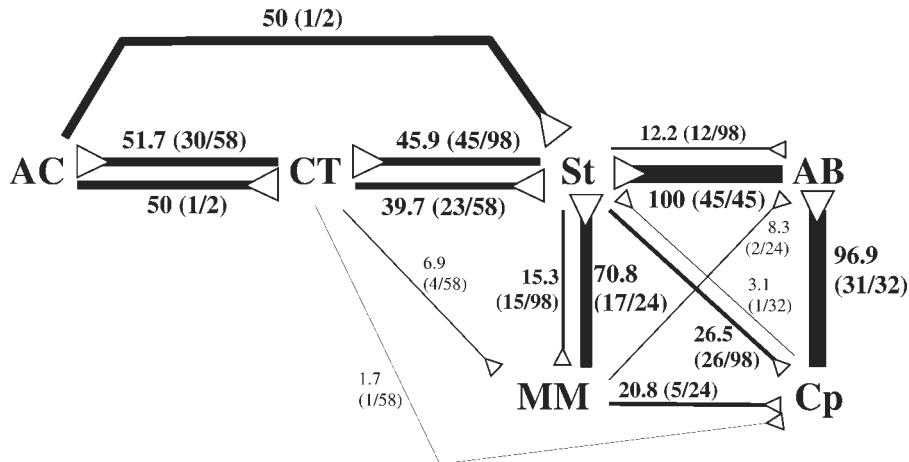


Figure 1. Flow diagram depicting transitions between the various behavioral components of courtship display and mating of ergatoid males of the ant *Cardiocondyla elegans* (32 copulations, 8 pairs). For abbreviations of different behaviors see Table 2. Arrows indicate the direction of transitions, however, the numbers above and below the arrows indicate the percentage (and proportion) of a certain type of behavior “B” being preceded by a behavioral display “A”, e.g., 45.9% of all stridulations (45 out of the 98 transitions leading to stridulation) were preceded by climbing onto the female’s thorax. The thickness of arrows also represents the frequency of transitions.

female. The time males allocated to the different behavioral elements differed considerably between interactions that finally did or did not result in a successful copulation. The median time spent with stridulation (successful: median 523.3 sec, quartiles 256.2, 696.3 vs. without success: 44.2, quartiles 0, 67.7; $U = 13$; $p = 0.01$), antennal boxing (median 66.1, quartiles 53.3, 79.9 vs. median 1.8, quartiles 0, 5.8; $U = 10$; $p < 0.005$) and mouth to mouth contact (median 8.7, quartiles 3.9, 21.6 vs. median 35.9, quartiles 17.7, 49.9; $U = 15$; $p = 0.016$) was significantly longer when at least one successful copulation occurred than during interactions without any copulation. Stridulation seems to be the predominant display in the sequence (Table 3). Fig. 1 documents the behavioral

sequences in *C. elegans*. In a classical flow diagram, the arrows represent the direction and the number of transitions occurring between behavior “A” and an immediately following behavior “B”. However, the numbers above and below the arrows in Fig. 1 represent the percentage (and proportion) of transitions between behavioral element “B” and an immediately preceding behavior “A”. This was necessary to avoid extremely small percentages, as of the 1024 interaction sequences observed in the 19 pairs of *C. elegans*, only 26 resulted in one and three resulted in two copulations, whereas the remaining interactions remained without success. Using the unusual notion with preceding behaviors allowed us to show, for example, that all but one copulation were preceded by antennal boxing and that antennal boxing was always preceded by stridulation.

The overall structure of the mating pattern was conserved throughout morphs and species of *Cardiocondyla*, but at the same time exhibited species-specific idiosyncrasies (Table 4). For example, following an initial antennal contact or mauling with the mandibles, males of *C. obscurior* either directly mounted the female sexuals or first approached with an anterior-posterior orientation and then mounted. Pre-copulatory behavior consisted again of mauling (MC) and antennal boxing of various parts of the female sexual. Stridulation was rare. Finally, the male gripped the female with its saber-shaped mandibles and attempted copulation (Table 4b,d). In *C. minutior*, most of the behavioral transitions occurred during the complex pre-copulatory phase (Table 4a,c). In *C. mauritanica* (referred to as *C. nuda* by Heinze et al., 1993), pre-copulatory behavior was characterized by the male rapidly “hammering” with its mandibles on the head of the female sexual; in *C. venustula*, the male slowly opened and closed its mandibles in front of the female’s mouthparts apparently without touching them, and in *C.*

Table 3. Exemplary sequences of behavioral components in the courtship display of *Cardiocondyla* spp. n indicates multiple repetitions of behavioral sequences.

Species	Male	Typical courtship sequence
<i>C. batesii</i>	ergatoid	AC-CT-Cp-AB-Cp-AB
<i>C. elegans</i>	ergatoid	AC-CT-St-AB-Cp-St
<i>C. kagutsuchi</i>	ergatoid	AC-CT-(MC-St)n-Cp
<i>C. mauritanica</i>	ergatoid	AC-CT-AB-(MC-Cp)n
<i>C. venustula</i>	ergatoid	AC-CT-MC-St-Cp
<i>C. emeryi</i>	winged	AC-CT-AB-Cp-(St-AB)n
<i>C. minutior</i>	ergatoid	AC-CT-AB-(Cp-AB)n
	winged	AC-MC-CT-AB-(Cp-AB)n
<i>C. obscurior</i>	ergatoid	CT-AB-(Cp-AB)n
	winged	AC-CT-AB-MC-AB-(Cp-AB)n

Table 4. Frequency of transitions between behavioral components of the courtship displays of
a) ergatoid males of *C. minutior* (98 displays, 22 copulations, 13 pairs)
b) ergatoid males of *C. obscurior* (135 displays, 46 copulations, 18 pairs)
c) winged males of *C. minutior* (317 displays, 79 copulations, 34 pairs)
d) winged males of *C. obscurior* (76 displays, 27 copulations, 11 pairs).
For abbreviations of behaviors see Table 2.

	Preceding behavior	Following behavior					
		AC	CT	St	AB	Cp	MC
a)	AC		5	0	0	0	0
	CT	0		2	8	0	3
	St	1	0		17	1	0
	AB	0	0	11		21	4
	Cp	1	0	4	14		0
	MC	0	0	2	4	0	
b)	AC		7	0	1	0	0
	CT	0		0	14	0	4
	St	0	0		1	0	1
	AB	0	1	1		46	6
	Cp	1	0	0	41		0
	MC	0	2	1	8	0	
c)	AC		7	0	1	0	24
	CT	5		2	27	0	5
	St	0	0		9	2	1
	AB	0	5	5		74	24
	Cp	4	0	2	59		5
	MC	1	18	5	29	3	
d)	AC		4	0	0	0	1
	CT	0		0	8	0	3
	St	0	0		0	0	0
	AB	0	1	0		26	3
	Cp	1	1	0	21		2
	MC	0	0	0	4	1	

kagutsuchi, the male rubbed its mandibles over the female's head.

A comparative analysis of the durations of pre-copulatory phase and copulation did not reveal a clear pattern associated with the different types of male behavior (Table 1). The time interval between first antennal contact between the sexuals and the beginning of a copulation varied considerably among species (Table 1, median test, $\chi^2=41.04$, d.f.=9, $p < 0.0001$). Within polymorphic species, ergatoid males showed longer pre-copulatory behavior than winged males (data not available for all individuals; *C. minutior*: $U_{6,10}=12$, $p=0.051$; *C. obscurior*: $U_{7,4}=3$, $p=0.037$). Ergatoid males of species with callow-killing ergatoid males (*C. mauritanica*, *C. kagutsuchi*, *C. venustula*, and *C. minutior*) had a

particularly long pre-copulation period compared to ergatoid males from the two species with mutually tolerant ergatoid males ($U_{20,20}=28$, $p < 0.0001$) and *C. obscurior*, in which adult ergatoid males fight ($U_{20,7}=0$, $p=0.0001$; no data available for *C. emeryi*). Pre-copulation period did not differ between ergatoid males from species with and without winged males ($U_{30,13}=132.5$, $p=0.099$; *C. venustula* and *C. kagutsuchi* excluded, as it is at present not known whether winged males might occur during a certain phase of colony life history).

Similarly, the duration of the copulation exhibited significant variation between species (median test, $df=10$, $\chi^2=41.61$, $p < 0.0001$), and again, ergatoid and winged males did not differ when species were pooled ($U_{52,24}=585.5$, $p=0.667$). Copulation duration in ergatoid

males did not differ with male tolerance ($U_{21,25}=240.5$, $p=0.628$), but ergatoid males from polymorphic species copulated significantly longer than those from species without winged males ($U_{31,18}=155.5$, $p=0.010$). A comparison of the unweighted median durations of copulations per species corroborates this result ($U_{3,3}=0$, $p=0.046$). Again, *C. venustula* and *C. kagutsuchi* were excluded, but it should be noted that ergatoid males of these species copulate for much longer than those of *C. elegans*, *C. batesii*, and *C. mauritanica*.

Ergatoid males copulated significantly longer than winged males in *C. minutior* ($U_{10,10}=10$, $p=0.001$), but not so in *C. obscurior* ($U_{7,4}=11$, $p=0.569$, but see Yamauchi and Kinomura 1993).

Discussion

Our study showed that mating in *Cardiocondyla* is regularly preceded by a prolonged and more or less stereotypic courtship display, which shows species-specific elements. Though individual phases of pre-copulatory behavior recorded in our study did rarely surpass 3 min, males that did not succeed with mating may repeat pre-copulatory behavior for much longer periods of time, occasionally interrupted by self-grooming or resting (Heinze et al., 1993, 1998).

Pummeling the head of the female with mandibles and antennae and / or stridulation are essential parts of the courtship behavior in all investigated species. Acoustical or vibrational signals play an important role in courtship and mating in many species. For example, both male and female mutillids, *Dasymutilla foxi*, produce coordinated sound sequences while attempting to mate (Spangler and Manley, 1978). Stridulatory organs have been described for males of several myrmicine ants (Janet, 1902; Kermarrec et al., 1976; Hernández et al., 2002), but while stridulatory signals of female ants seem to have a broad function in alarm, recruitment communication or trophallaxis (e.g., Markl, 1965; Stuart and Bell, 1980; Le Roux, 1976, 1977, 1993; Hölldobler, 1999; Lenoir, 2000), male stridulation has to our knowledge not been reported previously. At least in *C. elegans*, stridulation and antennal boxing are absolutely necessary before copulation and the transitions from stridulation and antennal boxing to copulation are highly ritualized. Ritualization, “an evolutionary process by which behavioral patterns become modified to serve communication” (McFarland, 1981), has previously been described in ants in the context of intra- or interspecific aggression or food recruitment (reviewed by Hölldobler and Wilson, 1990; Yamaguchi, 1995; Mercier and Dejean, 1996; Mercier et al., 1997, 1998), but never in the context of mating. Stridulation may express the level of stimulation of the male, whereas antennal boxing may be a signal to prevent the female from moving and disturbing the copulation. Though antennation of the female’s body by the male appears to be of general importance in ants, intensive contact

between the male’s mouthparts and the head of the female has as yet rarely been mentioned explicitly. In *Pogonomyrmex*, males may grasp the queens with their mandibles before mating (Nagel and Rettenmeyer, 1973; Hölldobler, 1976), and Abell et al. (1999) found that mating males had shorter and, because of the smaller moment, presumably also stronger mandibles than males collected at random from *P. occidentalis* leks.

With the prolonged interactions and the repeated transitions between different types of behavior, the pre-courtship display of *Cardiocondyla* appears to be far more complex than that of most other hitherto investigated ants and, in its duration, is surpassed only by the pre-copulatory mate guarding of wingless *Hypoponera* males (Yamauchi et al., 2001; Foitzik et al., 2002). The duration of the copulation itself, during which the male inserts its genital appendages into the genital chamber of the female and slightly tilts backwards, ranged from approximately 1 to 30 sec and is thus shorter than that reported for most other ant species. For example, copulation durations range from approximately 12 sec in *Rossomyrmex minuchae* (Ruano and Tinaut, 1995), 25 sec in *Meranoplus peringueyi* (Robertson and Villet, 1989), 30 sec in *Gnamptogenys menadensis* (Gobin et al., 2001), 35 sec in *Formica subpolita* (O’Neill, 1994), 30–60 sec in *Myrmica americana* (Kannowski and Kannowski, 1957) and *Monomorium pharaonis* (Allard et al., 2006), and 40–60 sec in *Myrmecina graminicola* (Buschinger, 2003), to several minutes in *Pogonomyrmex* (Hölldobler, 1976) and *Carebara vidua* (Robertson and Villet, 1989) and even longer in species, in which mating is suicidal for the male (e.g., army ants, Gotwald, 1995; *Diacamma*, Fukumoto et al., 1989; *Dinoponera*, Monnin and Peeters, 1998). Copulation duration is probably shorter in *Cardiocondyla* because males transfer only a comparatively small amount of sperm during copulation. The spermathecae of queens of *C. emeryi* and *C. obscurior*, inseminated by one male, contained only a few hundred sperm cells (Heinze et al., 1998; A. Schrenpf, pers. comm.), which, however, are sufficient to fertilize all the eggs queens can lay throughout their lives. Similarly, after mating with an average of 4.5 ± 1.6 ergatoid males (Lenoir et al., 2007), spermathecae of *C. elegans* queens contained a median of 4695 sperm cells (quartiles 2543, 6600; E. Darrouzet, pers. comm.), i.e., presumably less than 1000 sperm cells are transferred during each copulation. Thanks to their continuous spermatogenesis (Heinze and Hölldobler, 1993), an individual *Cardiocondyla* male can inseminate large numbers of queens—more than 75 in *C. elegans*—during its whole life.

Whereas in several insects, including the ant *Hypoponera nubatama* (Yamauchi et al., 2001) the (potential) presence of a competitor leads to prolonged copulation and increased ejaculate size (e.g., Gage and Baker, 1991; Schaus and Sakaluk, 2001), our data on copulation duration in various species of *Cardiocondyla* do not reveal a clear and simple pattern. This is probably not

surprising, given that the correlation of optimal sperm expenditure and sperm competition intensity is not linear (e.g., Parker et al., 1996; Schaus and Sakaluk, 2001; Engqvist and Reinhold, 2005), and that the mean sperm competition risk in nature to which male behavior is adapted is difficult to determine in *Cardiocondyla*. Furthermore, it is presently unknown whether increased duration of copulation indeed reliably reflects increased ejaculate size in ants. It is suggestive that pre-copulatory behavior was shortest and copulations were longest in *C. obscurior* in which ergatoid males usually can monopolize mating, as winged males are not regularly produced and adult ergatoid males not only attack callows but also adult ergatoid rivals. In contrast, copulations were shortest in *C. batesii* and *C. elegans*, in which ergatoid males are mutually tolerant and therefore multiple males are regularly present in the nest (Schrempf et al., 2005; Lenoir et al., 2007) and *C. mauritanica*, in which large laboratory colonies often contained multiple males despite male attacks against callows (Heinze et al., 1993). Ergatoid males copulated longer than winged males in two of three polymorphic species, but our result of similar mating duration in the two morphs of *C. obscurior* stands at odds with data published by Yamauchi and Kinomura (1993). Like in other social insects (e.g., Davidson, 1982; Oberstadt and Heinze, 2003), queens appear to be capable of rejecting a male and preventing copulation, suggesting a certain degree of female choice. All these differences in the durations of pre-copulatory behavior and copulation might reflect different degrees of sperm competition and female choice. For example, prolonged courtship in those species of *Cardiocondyla*, in which several males co-exist, might help females to choose the right partner. However, at present it cannot be excluded that the variation simply reflects selectively neutral species idiosyncrasies.

Ritualization and the evolution of male courtship behavior

Studies on agonistic interactions in ants generally consider the following behaviors to be increasingly aggressive: antennal contact; mandible opening; biting; curling the gaster and stinging (Errard et al., 2005). “Antennal contact” means that the opponent has been discriminated, while “mandible opening” is considered to be a threat display and “biting” and “stinging” to be very aggressive displays. Two pre-copulatory displays (mandibular and antennal contacts) seem to play an important role during courtship behavior in *Cardiocondyla* species. As they usually express aggressiveness in male-male interactions, they can be considered as stereotyped ritualization of aggressive displays. The very strong and highly stereotyped “mandibular contact” may result from a shift in “biting” display, with a change in function (from hurting to strong intimidation, like hammering, pummeling, mauling). “Antennal boxing” is a highly repeated “antennal contact”, which has changed in its function

(from discrimination to intimidation). Therefore, antennal boxing can be considered as a less aggressive ritualized behavior than mandibular contact.

Male courtship behavior of the presently studied species appears to have evolved from an ancestral courtship with multiple violent mandibular and antennal contacts in species with lethal fighting among ergatoid males (*C. obscurior*, *C. emeryi*, and *C. minutior*), to a less aggressive courtship with predominantly multiple antennal contacts in species with mutually tolerant ergatoid males (*C. batesii*, *C. elegans*, *C. venustula*). In *C. venustula*, a highly ritualized version of “biting” display occurs when the male slowly opens and closes his mandibles in front of the female’s head without touching her. *C. mauritanica* and *C. kagutsuchi*, where callow males are usually killed, may represent a second evolutionary route, in which antennal contacts progressively disappear first and mandibular contacts secondarily become less aggressive (“hammering” in *C. mauritanica*, then “rubbing” in *C. kagutsuchi*). In contrast, stridulatory behavior seems to have progressively replaced mandibular contacts. Thus, stridulations are rare in *C. obscurior* and *C. minutior*, more frequent in *C. emeryi*, *C. kagutsuchi* and *C. venustula*, and necessary in *C. elegans*.

Previous research has shown that male-male interactions are extraordinarily diverse in *Cardiocondyla* compared to most other social Hymenoptera (Kinomura and Yamauchi, 1987; Stuart et al., 1987; Heinze et al., 1993, 1998; Schrempf et al., 2005; Lenoir et al., 2007). Our study indicates that male-female interactions show similar complexity. Given that first steps have been made to completely resolve the phylogeny of this genus and to trace the evolution of male reproductive tactics (Heinze et al., 2005), we expect that in the near future it will be possible to elucidate the evolution and the meaning of the different facets of male courtship display in *Cardiocondyla* in more detail.

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References

- Abell A.J., Cole B.J., Reyes R. and Wiernasz D.C. 1999. Sexual selection on body size and shape in the western harvester ant, *Pogonomyrmex occidentalis* Cresson. *Evolution* **53**: 535–545
- Allard D., Børgesen L., van Hulle M., Bobbaers A., Billen J. and Gobin B. 2006. Sperm transfer during mating in the pharaoh’s ant, *Monomorium pharaonis*. *Physiol. Entomol.* **31**: 294–298
- Anderson C., Cremer S. and Heinze J. 2003. Live and let die: why fighter males of the ant *Cardiocondyla* kill each other but tolerate their winged rivals. *Behav. Ecol.* **14**: 54–62

- Andersson M. 1994. *Sexual Selection*. Princeton University Press, Princeton, N.J. 599 pp
- Arnqvist G. and Danielsson I. 1999. Postmating sexual selection: the effects of male body size and recovery period on paternity and egg production in a water strider. *Behav. Ecol.* **10**: 358–365
- Baer B. 2003. Bumblebees as model organisms to study male sexual selection in social insects. *Behav. Ecol. Sociobiol.* **54**: 521–533
- Boomsma J.J., Baer B.C. and Heinze J. 2005. The evolution of male traits in social insects. *Annu. Rev. Entomol.* **50**: 395–42
- Buschinger A. 2003. Mating behavior in the ant, *Myrmecina gramminicola* (Myrmicinae). *Insect. Soc.* **50**: 293–296
- Cremer S., Sledge M. and Heinze J. 2002. Male ants disguised by the queens' bouquet. *Nature* **419**: 897
- Danielsson I. 1998. Mechanisms of sperm competition in insects. *Ann. Zool. Fennici* **35**: 241–257
- Davidson D. 1982. Sexual selection in harvester ants (Hymenoptera: Formicidae: *Pogonomyrmex*). *Behav. Ecol. Sociobiol.* **10**: 245–250.
- Engqvist L. and Reinhold K. 2005. Pitfalls in experiments testing predictions from sperm competition theory. *J. evol. Biol.* **18**: 116–123
- Errard C., Hefetz A. and Jaisson P. 2006. Social discrimination tuning in ants: template formation and chemical similarity. *Behav. Ecol. Sociobiol.* **59**: 353–363
- Foitzik S., Heinze J., Oberstadt B. and Herbers J.M. 2002. Mate guarding and alternative reproductive tactics in the ant *Hypoponera opacior*. *Anim. Behav.* **63**: 597–604
- Fukumoto Y., Abe T., and Taki A. 1989. A novel form of colony organization in the "queenless" ant *Diacamma rugosum*. *Physiol. Ecol. Japan* **26**: 55–61
- Gage M.J.G. and Baker R.R. 1991. Ejaculate size varies with socio-sexual situation in an insect. *Ecol. Entomol.* **16**: 331–337
- Gobin B., Billen J. and Peeters C. 2001. Dominance interactions regulate worker mating in the polygynous ponerine ant *Gnamptogenys menadensis*. *Ethology* **107**: 495–508
- Gotwald W.H. jr. 1995. *Army Ants. The Biology of Social Predation*. Cornell University Press, Ithaca London. 302 pp
- Heinze J. and Hölldobler B. 1993. Fighting for a harem of queens: physiology of reproduction in *Cardiocondyla* male ants. *Proc. Natl. Acad. Sci. USA* **90**: 8412–8414
- Heinze J., Kühnholz S., Schilder K. and Hölldobler B. 1993. Behavior of ergatoid males in the ant, *Cardiocondyla nuda*. *Insect. Soc.* **40**: 273–282
- Heinze J., Hölldobler B. and Yamauchi K. 1998. Male competition in *Cardiocondyla* ants. *Behav. Ecol. Sociobiol.* **42**: 239–246
- Heinze J., Trindl A., Seifert B. and Yamauchi K. 2005. Evolution of male morphology in the ant genus *Cardiocondyla*. *Mol. Phylog. Evol.* **37**: 278–28
- Hernández J.M., Martínez M.D. and Ruiz E. 2002. Descripción del órgano estridulador en *Messor barbarus* (Linneo, 1767) (Hymenoptera, Formicidae). *An. Biol.* **24**: 167–174
- Hölldobler B. 1976. The behavioral ecology of mating in harvester ants (Hymenoptera: Formicidae: *Pogonomyrmex*). *Behav. Ecol. Sociobiol.* **1**: 405–423
- Hölldobler B. 1999. Multimodal signals in ant communication. *J. comp. Physiol. A* **184**: 129–141
- Hölldobler B. and Bartz S.H. 1985. Sociobiology of reproduction in ants. In: *Experimental Behavioral Ecology and Sociobiology* (Hölldobler B. and Lindauer M., Eds), Fischer Verlag, Stuttgart. pp 237–257
- Hölldobler B. and Wilson E.O. 1990. *The Ants*. Harvard University Press, Cambridge, Mass. 732 pp
- Janet C. 1902. *Anatomie du gaster de la Myrmica rubra*. Carré et Naud, Paris. 68 pp + viii
- Kannowski, P.B. and Kannowski, P.M. 1957. The mating activities of the ant *Myrmica americana* Weber. *Ohio J. Sci.* **57**: 371–374
- Kermarrec A., Mauléon H. and Abud Antun A. 1976. La stridulation de *Acromyrmex octospinosus* Reich. (Formicidae, Attini): Biométrie de l'appareil stridulateur et analyse du signal produit. *Insect. Soc.* **23**: 29–47
- Kinomura K. and Yamauchi K. 1987. Fighting and mating behaviors of dimorphic males in the ant *Cardiocondyla wroughtoni*. *J. Ethol.* **5**: 75–81
- Kugler J. 1983. The males of *Cardiocondyla* Emery (Hymenoptera: Formicidae) with the description of the winged male of *Cardiocondyla wroughtoni* (Forel). *Isr. J. Entomol.* **17**: 1–21
- Lenoir A., Errard C., Francoeur A. and Loiseau R. 1992. Relations entre la fourmi parasite *Formicoxenus provancheri* et son hôte *Myrmica incompleta*. Données biologiques et éthologiques (Hym. Formicidae). *Insect. Soc.* **39**: 81–97
- Lenoir J.C. 2000. Stridulation lors des échanges alimentaires chez deux espèces de *Crematogaster* (Myrmicinae). *Master's thesis, University of Tours, France*. 23 pp
- Lenoir J.C., Schrempf A., Lenoir A., Heinze J. and Mercier J.-L. 2007. Genetic structure and reproductive strategy of the ant *Cardiocondyla elegans*: strictly monogynous nests invaded by unrelated sexuals. *Mol. Ecol.* **16**: 345–354
- Le Roux G. 1976. Etude des stridulations produites par *Myrmica laevinodis* (Hym. Formicidae). *Ann. Soc. Ent. Fr.* **12**: 615–620
- Le Roux G. 1977. Note sur l'organisation temporelle des séquences de stridulation produites par *Myrmica laevinodis* Nyl. (Hymenoptera, Formicidae). *C.R. Acad. Sc. Paris* **285**: 415–418
- Le Roux G., Le Roux A. and Berton F. 1993. Les émissions de stridulation, signaux de modulation chez les Myrmicines (Formicidae). *Actes Coll. SFECA*, Rennes, 7–9 avril 1993. p 46
- Markl H. 1965. Stridulation in leaf-cutting ants. *Science* **149**: 1392–1393
- McFarland D. (Ed.) 1981. *The Oxford Companion to Animal Behavior*. Oxford University Press, Oxford. 657 pp
- Mercier J.L. and Dejean A. 1996. Ritualized behavior during competition for food between two Formicinae. *Insect. Soc.* **43**: 17–29
- Mercier J.L., Lenoir A. and Dejean A. 1997. Ritualised versus aggressive behaviors displayed by *Polyrhachis laboriosa* (F. Smith) during intraspecific competition. *Behav. Proc.* **41**: 39–50
- Mercier J.L., Dejean A. and Lenoir A. 1998. Limited aggressiveness among African arboreal ants (Hymenoptera: Formicidae) sharing the same territories: the result of a co-evolutionary process. *Sociobiology* **32**: 139–150
- Monnin T. and Peeters C. 1998. Monogyny and regulation of worker mating in the queenless ant *Dinoponera quadriceps*. *Anim. Behav.* **55**: 299–306
- Nagel H.G. and Rettenmeyer C.W. 1973. Nuptial flights, reproductive behavior and colony founding of the western harvester ant, *Pogonomyrmex occidentalis* (Hymenoptera: Formicidae). *J. Kansas Entomol. Soc.* **46**: 82–101
- Oberstadt B. and Heinze J. 2003. Mating biology and population structure of the ant, *Leptothorax gredleri*. *Insect. Soc.* **50**: 340–345
- O'Neill K.M. 1994. The male mating strategy of *Formica subpolita* Mayr (Hymenoptera: Formicidae): swarming, mating, and predation risk. *Psyche* **101**: 93–108
- Parker G.A., Ball M.A., Stockley P. and Gage M.J.G. 1996. Sperm competition games: individual assessment of sperm competition intensity by group spawners. *Proc. R. Soc. Lond. B* **263**: 1291–1297
- Robertson H.G. and Villet M.H. 1989. Mating behavior in three species of myrmicine ants (Hymenoptera: Formicidae). *J. Nat. Hist.* **23**: 767–773
- Ruano F. and Tinaut A. 2005. Mating behaviour in a slave-making ant, *Rossomyrmex minuchae* (Hymenoptera, Formicidae). *Naturwissenschaften* **92**: 328–331
- Sauer K.P., Sindern J. and Kall N. 1997. Nutritional status of males and sperm transfer in the scorpionfly *Panorpa vulgaris* (Mecoptera: Panorpidae). *Entomol. Gener.* **21**: 189–204
- Schaus J.M. and Sakaluk S.K. 2001. Ejaculate expenditures of male crickets in response to varying risk and intensity of sperm competition: not all species play games. *Behav. Ecol.* **12**: 740–745
- Schrempf A. and Heinze J. 2007. Back to one: consequences of derived monogyny in an ant with polygynous ancestors. *J. evol. Biol.* **20**: 792–799
- Schrempf A., Reber C., Tinaut A. and Heinze J. 2005. Inbreeding and local mate competition in the ant *Cardiocondyla batesii*. *Behav. Ecol. Sociobiol.* **57**: 502–510

- Seifert B. 2003. The ant genus *Cardiocondyla* (Insecta: Hymenoptera: Formicidae) – a taxonomic revision of the *C. elegans*, *C. bulgarica*, *C. batesii*, *C. nuda*, *C. shuckardi*, *C. stambuloffii*, *C. wroughtonii*, *C. emeryi*, and *C. minutior* species groups. *Ann. Naturhist. Mus. Wien* **104B**: 203–338
- Spangler H.G. and Manley D.G. 1978. Sounds associated with the mating behavior of a mutillid wasp. *Ann. Entomol. Soc. Am.* **71**: 389–392
- Stuart R.J. and Bell P.D. 1980. Stridulation by workers of the ant *Leptothorax muscorum* (Nylander) (Hymenoptera: Formicidae). *Psyche* **87**: 199–210
- Stuart R.J., Francoeur A. and Loiselle R. 1987. Lethal fighting among dimorphic males of the ant, *Cardiocondyla wroughtonii*. *Naturwissenschaften* **74**: 548–549
- Wiernasz D.C., Yencharis J. and Cole B.J. 1995. Size and mating success in males of the western harvester ant, *Pogonomyrmex occidentalis* (Hymenoptera: Formicidae). *J. Insect Behav.* **8**: 523–531
- Wiernasz D.C., Sater A.K., Abell A.J. and Cole B.J. 2001. Male size, sperm transfer, and colony fitness in the western harvester ant, *Pogonomyrmex occidentalis*. *Evolution* **55**: 324–329
- Wilson E.O. 1975. *Leptothorax duloticus* and the beginnings of slavery in ants. *Evolution* **29**: 108–119
- Woyciechowski M. 1990. Mating behaviour in the ant *Myrmica rubra* (Hymenoptera, Formicidae). *Acta Zool. Cracov.* **33**: 565–574
- Yamaguchi T. 1995. Intraspecific competition through food robbing in the harvester ant, *Messor aciculatus* (Fr. Smith) and its consequences on colony survival. *Insect. Soc.* **42**: 89–101
- Yamauchi K. and Kinomura K. 1993. Lethal fighting and reproductive strategies of dimorphic males in *Cardiocondyla* ants (Hymenoptera: Formicidae) In: *Evolution of Insect Societies* (Inoue T. and Yamane S., Eds.), Hakuhinsha, Tokyo. pp 372–402 (in Japanese).
- Yamauchi K., Oguchi S., Nakamura Y., Suetake H., Kawada N. and Kinomura K. 2001. Mating behavior of dimorphic reproductives of the ponerine ant, *Hypoponera nubatama*. *Insect. Soc.* **48**: 83–87
- Yamauchi, K., Asano, Y., Lautenschläger, B., Trindl, A. and Heinze, J. 2005. A new type of male dimorphism with ergatoid and short-winged males in *Cardiocondyla* cf. *kagutsuchi*. *Insect. Soc.* **52**: 274–281

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