



RESEARCH ARTICLE - ANTS

Polygyny, Inbreeding and Wingless Males in the Malagasy Ant *Cardiocondyla shuckardi* Forel (Hymenoptera, Formicidae)

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Abstract

The ant genus *Cardiocondyla* exhibits a fascinating diversity of its reproductive biology, with winged and wingless males, long-winged and short-winged queens, strict monogyny and facultative polygyny with or without queen fighting. Here we report on the previously unstudied Malagasy ant *C. shuckardi*. We describe the nesting habits, male morphology and colony structure of this species. Furthermore, based on the genotypes from three microsatellite loci we document a very high incidence of sib-mating.

Introduction

The myrmicine ant genus *Cardiocondyla* comprises about 100 species of minute to small ants that are widely distributed throughout Africa, Europe, and Asia (Seifert, 2003). Colony composition and the reproductive behavior of queens and males vary tremendously among species, making *Cardiocondyla* an ideal system to investigate the evolution of social structures and alternative reproductive tactics in ants.

Because of its ancestral male diphenism with winged disperser males and wingless, “ergatoid” males (Kugler, 1983), *Cardiocondyla* has become a model system for the investigation of alternative reproductive tactics in male ants (e.g., Oettler et al., 2010; Schwander & Leimar, 2011). Winged males resemble typical males of other ant species in morphology, reproductive physiology, and behavior: they are peaceful, disperse shortly after eclosion, and use their limited sperm supply to inseminate young queens from other colonies. Wingless males, in contrast have strong shear- or sickle-shaped mandibles, are relatively long-lived (several

weeks to one year, Yamauchi et al., 2006) and, uniquely among social Hymenoptera, have lifelong spermatogenesis (Heinze & Hölldobler, 1993). Wingless males rarely if ever disperse, and in many species engage in lethal fighting with rivals and attempt to monopolize mating with all female sexuals emerging from their natal nests (Stuart et al., 1987; Kinomura & Yamauchi, 1987; Heinze et al., 1998). Within *Cardiocondyla*, winged disperser males have been lost convergently in at least two clades, and in several species fighting among wingless males has been replaced by territoriality or mutual tolerance (Froeschammer & Heinze, 2009; Lenoir et al., 2007). Queen behavior is similarly variable. Facultative polygyny, i.e., the peaceful coexistence of several fertile queens per nest, appears to be the ancestral state, from which queen-queen fighting (Yamauchi et al., 2007; Heinze & Weber, 2011) and obligate monogyny (a single queen per nest) have evolved (Schrempf & Heinze, 2007).

Previous investigations have focused on the social organization and life history of species of Southeast Asian or Central European origin (Kugler, 1983; Kinomura & Ya-



mauchi, 1987; Heinze et al., 1998; Lenoir et al., 2007), but little is known about the African “*C. shuckardi* group” (sensu Seifert, 2003). This group is of particular interest as it is phylogenetically situated between the *C. nuda* clade (with facultative polygyny and male fighting at least in *C. mauritanica* and *C. kagutsuchi*) and a clade consisting of several monogynous species with only wingless, mutually tolerant males (Oettler et al., 2010).

One of the six presently recognized species of the *C. shuckardi* group, *C. venustula* Wheeler, 1908 has recently been studied in populations introduced to Kaua’i (Hawai’i; Frohschammer & Heinze, 2009) and Puerto Rico (J. Heinze and Susanne Jacobs, unpubl.), and in a native population in South Africa (Heinze et al., 2013). As yet, only wingless males have been found, with some particularly large males from South Africa combining the typical morphology of wingless males with the presence of ocelli and vestigial wings without otherwise approaching the morphology of typical winged ant males. *C. venustula* males appear to defend small territories and fight against males that intrude into their home range. Colonies were at least temporarily polygynous, in contrast to Wheeler’s report on monogyny (Wheeler, 1908).

C. shuckardi Forel, 1891 was described from Imerina, the central highlands of Madagascar. According to B. Seifert (pers. comm.) it appears to be restricted to this island. Records of “*C. shuckardi*” from other parts of Africa (Samways, 1983; van Hamburg et al., 2004; Hita Garcia et al., 2009; Kone et al., 2012), the Arab peninsula (Collingwood & Agosti, 1996) and Iran (Ghahari & Collingwood, 2011) are probably misidentifications of other species of the *C. shuckardi* group owing to the often extremely close morphological similarities among species of *Cardiocondyla* (e.g., Seifert, 2003, 2008). Here we describe the results of a field study on the occurrence and colony composition of *C. shuckardi* on the outskirts of Antananarivo, Madagascar, and describe the wingless males of this species.

Material and Methods

Study area

We identified potentially suitable collecting sites from previous records of *C. shuckardi* captured in Malaise and pitfall traps as listed on www.antweb.org. In March 2012, we visited four sites: the garden of the Madagascar Biodiversity Centre at Tsimbazaza, Antananarivo (18° 55’ 57” S, 47° 31’ 31.8” E, 1284 m), a sandy threshing floor and paths in front of the palace of Ilafy (18° 51’ 14.5” S, 47° 33’ 56.8” E, 1356 m), the edge of a eucalypt plantation at Ambohidrabiby (18° 45’ 55” S, 47° 36’ 37” E, 1381 m), and a gravel path branching off Route Nationale 2 at Mandraka Park (18° 54’ 20” S, 47° 53’ 35” E, 1369 m). We followed foragers of *C. shuckardi* to their nest entrances in the soil. Nests were carefully excavated, adults and brood were collected into plastic vials with an aspirator. All specimens were transferred to the Madagascar Biodiver-

sity Centre in Antananarivo, and censused under a binocular microscope. Ovaries of queens were dissected following Buschinger and Alloway (1978).

Population genetics

We investigated the suitability of primers previously developed to amplify seven microsatellite loci in other species of *Cardiocondyla* (CE2-3A, CE2-4A, CE2-4E, CE2-5D, CE2-12D, Lenoir et al., 2005; CARD8 and CARD21, Schrempf et al., 2005) for the determination of the genetic structure of *C. shuckardi* colonies. Though only three loci exhibited some variability, we analyzed the genotypes of 4 to 20 workers from each of 14 colonies (total 143 workers) and 1 to 6 dealate queens from 11 colonies (total 31 queens) from Ilavy, 10 workers and 4 or 5 queens from each of two colonies from Mandraka, and 10 workers and 5 queens each from one colony from Ambohidrabiby and one colony from Tsimbazaza to obtain an estimate of colony and population structure of this ant (233 total individuals). Specimens included both old and young queens, most of which still had wings when collected. We could not determine the genotypes at all loci in some individuals, and three individuals were removed because their CE2-12D genotypes appeared to have alleles not found anywhere else. We used Relatedness 4.02 (Goodnight & Queller, 1999) to estimate inbreeding coefficients and nestmate relatedness in colonies from Ilafy. Confidence intervals were obtained by jackknifing by loci. From the inbreeding coefficient we calculated the frequency of sib-mating following Suzuki and Iwasa (1980).

Morphology of males

Six males of *C. shuckardi* were mounted on points and inspected under a Wild M10 binocular microscope with a Wild 1.6x planapochromatic objective at a magnification of 160-200x. We measured head width, scape length, eye diameter, mesonotum width and length (Weber’s length), petiole width, and postpetiole width. In addition, we counted the number of funicular segments.

Results

Occurrence and colony composition

C. shuckardi appears to be a common ant in degraded, open patches of grassland, in sandy areas along the edges of unpaved paths, and in parks and gardens in the central highlands of Madagascar. Nests were particularly dense and easy to locate in the regularly watered gardens of Madagascar Biodiversity Centre and near a parched ditch at Ilafy. Solitary workers were seen foraging over distances of more than two meters, and in a few cases we also observed pairs of workers engaged in tandem running, a typical behavior for *Cardiocondyla* (Wilson, 1959; Heinze et al., 2006). Colony nests

consisted of pea-sized chambers in sandy soil, under stones, roots, or between pebbles in the upper 15 cm of the ground. Most nest entrances were surrounded by conspicuous middens of corpses of other ants, predominantly *Pheidole*.

In total, we censused 62 colonies (32 from Ilafy, 16 from Tsimbazaza, 10 from Ambohidrabiby, 4 from Mandraka). Individual nests contained between one and seven dealate queens and up to 85 workers. In the most intensively studied site, Ilafy, six nests were queenless, 11 had one queen, and the remaining 15 nests had two to seven queens (26 queen-right colonies: median, quartiles 1, 2, 4). Nests contained 10 to 80 workers (median, quartiles, 30.5, 22, 40). Colony composition was similar in the other localities. Upon collection, 14 colonies contained 1 to 10 winged female sexuals, seven colonies contained a single wingless male each, and one colony contained two wingless males.

We observed numerous solitary, wingless queens moving outside the nest, suggesting that at least a fraction of young queens disperse after mating and shedding their wings in their natal nests. Dissection showed that the ovaries of all queens in two multi-queen colonies (all five queens from a colony from Tsimbazaza, five of 12 queens from a colony from Ambohidrabiby) contained developing and mature eggs and had a filled spermatheca. From ovarian status it appeared that three or four of these 10 queens had only recently begun to produce eggs. The ovaries of the other queens contained clearly visible corpora lutea and/or two or three mature eggs, suggesting that they had been fertile for a longer period. Multi-queen colonies therefore appear to be truly polygynous.

Population genetics

Only three of the seven tested *Cardiocondyla* primers were suitable for the genetic analysis of colony structure. In 183 workers and 50 queens from 18 colonies, we found seven alleles at CE2-3A (82–94bp), 5 alleles at CE2-12D (143, 159, 163, 165, 167) and two alleles at CARD8 (128 and 130bp). The number and variability of loci allows for only a crude analysis of colony structure. Nevertheless, our analysis clearly suggests a high frequency of inbreeding (Table 1). In the most intensively studied population, Ilafy, observed heterozygosities of workers and queens were much lower than expected heterozygosities at all three loci, resulting in inbreeding coefficients F_{IS} of 1.000 at Card 8, 0.808 at CE2-3A, and 0.559 at CE2-12D (overall F_{IS} $0.685 \pm SE$ 0.133, corresponding to an average percentage of sib-mating of 89.7%). The presence of null alleles cannot be ruled out for Card 8. Ignoring this locus, the inbreeding coefficients at CE2-3A and CE-12D alone give an average percentage of sib-mating of 94.4% and 83.5%, respectively. A similar excess of homozygotes was observed in colonies from the other collection sites (Table 1).

Eleven of 18 colonies contained worker genotypes that were not compatible with single mating by a single queen.

Several genotype combinations suggest the coexistence of several matrilineal lines rather than multiple queen matings. For example, one worker and one queen from the Mandraka 2 colony had a genotype at all three loci that did not overlap with those of other workers and queens.

Average nestmate relatedness in the 14 colonies from Ilafy was 0.784 ± 0.112 , ranging in individual colonies from 0.487 ± 0.079 to 1 ± 0 in colonies in which all workers had the same genotype at all loci. Excluding the almost invariable locus Card8 relatedness did not change the result (0.787 ± 0.098 , range 0.475 ± 0.069 to 1 ± 0). Relatedness among workers was 0.766 ± 0.108 , ranging from 0.356 ± 0.082 to 1 ± 0 (excluding Card 8 0.771 ± 0.095 ; range 0.353 ± 0.069 to 1 ± 0). The high inbreeding coefficient and the frequent occurrence of more than two worker genotypes per colony suggests that this value does not reflect monogyny and monandry. Instead, many colonies appear to be composed of several inbred lineages of workers. In contrast, queens showed much less variation than workers: all but two queens from Ilafy had the same homozygous genotype at loci Card8 and CE2-3A and nestmate queens had one or two different genotypes at CE2-12D. Queen relatedness in the five multi-queen colonies was 0.949 ± 0.034 (excluding Card 8 0.848 ± 0.076).

Morphology of males

Wingless males closely resemble those of *C. venustula* in morphology, coloration, and size (Fig 1). Compared to wingless males of other *Cardiocondyla* species, but similar to those of *C. venustula*, wingless males of *C. shuckardi* are relatively large (Weber's length 0.62–0.67 mm; thorax width 0.35–0.37 mm). They have large heads (head width 0.49–0.53mm) with strongly sclerotized mandibles and relatively small eyes (eye diameter 0.10–0.11 mm). The antennae resemble those of workers (scape length 0.39–0.41) and the funiculus consists of 10 to 11 segments (Fig. 2), with the typical fusion of segments previously reported from other wingless *Cardiocondyla* males (Seifert, 2003). The pronotal shoulders are well-developed but appear to be less angular than in *C. venustula* from South Africa (Fig. 1). Petiole and postpetiole width ranged from 0.16–0.19 mm and 0.25–0.28 mm, respectively, with a median ratio between petiole and postpetiole width of 0.65. Several males showed injuries such as missing legs (as in the *C. venustula* male in Fig. 1), and one male was found decapitated. This suggests that wingless males of *C. shuckardi* engage in fights for the monopolization of mating with nestmate female sexuals similar to those engaged in by their counterparts in other species.

Discussion

Cardiocondyla shuckardi appears to be rather common in the Madagascar highlands. Nest density was particularly high in places with regularly high humidity, i.e., in gardens or along dry ditches. This matches findings from other



Figure 1 - Dorsal view of the alitrunk of wingless males of the ants *Cardiocondyla shuckardi* (left, from Ilafy) and *C. venustula* (right, from uThukela valley, South Africa; same ant as in Heinze et al., 2013) (photos by Christiana Klingenberg).

Cardiocondyla species, which build nests in humid patches in xeric environments, i.e., on sandy river banks or beaches (Seifert, 2003; Lenoir, 2006; Oettler et al., 2010). We collected the colonies from cavities in the uppermost 10 cm of the soil after heavy rains, but it is likely that the ants move deeper underground during drier periods. For example, *C. elegans* nests reach to a depth of 40 cm (Lenoir, 2006) and nest chambers of the desert species *C. ulianini* were found as deep as 1.5 m (Marikovskiy & Yakushkin, 1974). Colonies of *C. shuckardi* were small, with fewer than 100 workers, as is typical for *Cardiocondyla* (Heinze, 1999; Oettler et al., 2010). Nevertheless, because of the high density of nests, foragers of *C. shuckardi* appeared to be among the most abundant ants in our collecting sites. The species presumably plays a considerable role in anthropogenically disturbed, open habitat, such as plantations and gardens. Its ecology therefore appears to resemble that of *C. venustula* in South Africa (e.g., Samways, 1983; van Hamburg et al., 2004).

Our study clarifies two important aspects of the reproductive biology of this species. First, its colonies are at least temporarily facultatively polygynous, and second, males are wingless and resemble those of the related species *C. venustula* in size and morphology (Frohschammer and Heinze 2009). In the studied specimens of *C. shuckardi*, pronotal shoulders appeared to be less angular than in the presently available males of *C. venustula*, but because of the limited sample, the large variation of *C. venustula* males (Heinze et al., 2013) and the lack of males from other species of the *C. shuckardi* group, it would be premature to define universal diagnostic features. Injuries in the examined males of *C. shuckardi* and the simultaneous presence of two males in field colonies suggest that males may attack and damage young rivals but do not always engage in lethal fighting with other adult males. Hence, male behavior appears to be similar to that of *C. ve-*

nustula and *C. mauritanica* males (Frohschammer and Heinze, 2009; Heinze et al., 1993). Whether *C. shuckardi* males defend small “territories” within the nests against other males, as observed in *C. venustula* (Frohschammer and Heinze, 2009), remains to be determined.

As in other species of this genus, analyzing the genetic structure of colonies and populations of *C. shuckardi* was difficult because of the extremely low variability of genetic markers. Only three of seven microsatellite loci were variable to some extent. This obviously prevents us from making conclusions about queen mating frequencies or fine-scaled population and colony structure. Nevertheless, the available data clearly suggest that inbreeding is extremely common in *C. shuckardi*. From the inbreeding coefficient we estimate that more than 80% of all matings involve full sibs. This matches the condition in monogynous *C. batesii*, *C. elegans*, and *C. nigra*, where 50–80% of all matings involve brothers and sisters (Schrempf et al., 2005; Lenoir et al., 2007, Schrempf, 2014). Dissection data and the co-occurrence of up to four different worker genotypes in colonies of *C. shuckardi* indicate that several mothers may contribute to the offspring of single colonies. Furthermore, the genotypes indicate that individuals may occasionally be exchanged among nests. One queen and one worker from Mandraka had a multilocus-genotype different from those of all other studied nestmates, suggesting the adoption of alien queens as in *C. elegans* (Lenoir et al., 2007).

The combination of frequent inbreeding, polygyny, and queen or worker adoption results in a high nestmate relatedness value, which presumably would be much lower if corrected for inbreeding (e.g., Pamilo, 1985). However, such a correction is not yet possible in *C. shuckardi*, as the mating frequency of queens is unknown and single males may mate with multiple queens. Regardless of the exact value of relatedness, the genotype patterns suggest that queens mate in their natal nests with brothers and then disperse and seek adoption in other nests, in a manner similar to what has been observed in related species (Schrempf et al., 2005; Lenoir et al., 2007). More detailed studies on ants of the *C. venustula* group will help to better understand their peculiar life history.

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Table 1 - Genotypes of workers and queens of the ant *Cardiocondyla shuckardi* from 18 colonies from four populations on Madagascar (Ilafy, Ambohidrabiby, Tsimbazaza, Mandraka) at three microsatellite loci (1a:Card8, CE2-3A; 1b: CE2-12D). For each locus, the number of workers / queens with a certain genotype is given.

Table 1a

	Card8			CE2-3A													
	128/128	128/130	130/130	82/84	84/84	84/86	84/94	86/86	86/90	86/92	86/94	88/88	90/90	92/92	92/94	94/94	
Ilafy 1	19/6							15/6	1/0					2/0	1/0		
Ilafy 2	10/4							9/4									
Ilafy 3	9/3		1/0					13/3			6/0					1/0	
Ilafy 4	19/5					1/0		5/4		2/0	1/0						
Ilafy 5	10/5					1/0		9/5									
Ilafy 6	10/-							10/									
Ilafy 7	9/1							9/1									
Ilafy 8	10/1							10/1									
Ilafy 9	8/1												8/1				
Ilafy 10	4/-					4/-											
Ilafy 11	10/1					4/1		6/0									
Ilafy 12	10/1							8/1			2/0						
Ilafy 13	8/3							8/3									
Ilafy 14	4/-					4/-											
Ambo	8/5	1/0	1/0	1/0		1/3		3/2		3/0	1/0	1/0					
Tsimb 1	10/5							10/5									
Mand 1			10/5					1/0		1/1					1/1	6/3	
Mand 2	1/1		9/3					1/1		0/1				0/1	6/1	3/0	

Table 1 b

CE2-12D									
	143/143	143/159	159/159	159/167	163/163	163/165	165/165	165/167	167/167
Ilafy 1					2/2	8/3	1/0		1/0
Ilafy 2					1/0	3/0	3/2		
Ilafy 3				2/0				1/1	16/2
Ilafy 4			1/0	4/0					¾
Ilafy 5							10/5		
Ilafy 6								3/-	7/-
Ilafy 7						3/0	2/0	3/1	
Ilafy 8			10/1						
Ilafy 9							1/0	2/1	5/0
Ilafy 10			1/-	1/-				1/-	1/-
Ilafy 11							10/1		
Ilafy 12								4/1	6/0
Ilafy 13							2/0	2/1	3/2
Ilafy 14									4/-
Ambo							4/5		
Tsimb 1									8/2
Mand 1	7/3	1/1	1/1						
Mand 2	4/0	3/2	2/1						1/1

