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Research article

A new type of male dimorphism with ergatoid and short-winged males in *Cardiocondyla* cf. *kagutsuchi*

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Summary. A new type of ant male dimorphism, consisting of wingless (ergatoid) and short-winged (brachypterous) males, was found in a species of the "*Cardiocondyla kagutsuchi*"-complex from Malaysia. The ergatoid males show the typical morphological and behavioral characteristics of those of many other *Cardiocondyla* species. The brachypterous males are morphologically intermediate between ergatoid males and typical winged males of other taxa in this genus. On one hand, they share a number of morphological and behavioral features with ergatoid males that might be adaptations to the loss of flight and intranidal mating: aggressive behavior towards rival males, a prolonged spermatogenesis, which is unique in winged males, paler body coloration, smaller compound eyes, shorter antennal funiculi, more rounded heads – perhaps due to the increased development of mandibular muscles, and an angular pronotum, probably for neck protection. Their short wings appear to protect the petiolar joints during fighting. On the other hand, the brachypterous males have not become as specialized as the ergatoids and to some extent keep the nature of the winged males of other species, i.e., they escape from the nest with a higher probability and with less injuries and do not show a reduction of the ocelli. In the sexual production season, the ergatoid males emerged first in small numbers and then both male morphs emerged in large numbers. The sex ratio was extremely female-biased in the earlier stage of sexual production, probably due to local mate competition.

Key words: Male dimorphism, brachypterous males, ergatoid males, polygyny, alternative reproductive tactics, local mate competition.

Introduction

Species of the myrmicine ant genus *Cardiocondyla* generally have wingless (ergatoid) males and several species have also winged males in addition to ergatoids. Ergatoid males are very similar to workers in external morphology, while the winged males have the typical ant male morphology, i.e., large compound eyes, distinct ocelli, a differentiated thorax with functional wings, etc. (Kugler, 1983; Yamauchi and Kinomura, 1993; Heinze et al., 1998; Cremer et al., 2002a). Ergatoid males engage in lethal fighting with one another and their spermatogenesis continues throughout their lives (Heinze and Hölldobler, 1993). Winged males, in contrast, are not aggressive (Kinomura and Yamauchi, 1987; Stuart et al., 1987; Yamauchi and Kinomura, 1993; Heinze et al., 1998) and their spermatogenesis is completed shortly after the adult eclosion, similar to the condition in other Hymenoptera (Heinze and Hölldobler, 1993).

Here, we report on a new type of dimorphism consisting of ergatoid and short-winged (brachypterous) males in a species belonging to the "*C. kagutsuchi*"-group from Ulu Gombak, Malaysia. "*C. kagutsuchi*" is a complex of sibling species, that are morphologically similar to the cosmopolitan tramp species *C. mauritanica* Forel, 1890, which they seem to replace in Southern and Eastern Asia (Seifert, 2003). Different taxa of this complex have been separated by sequencing of mitochondrial genes, karyology, and cross-breeding studies and also appear to differ consistently in male morphology (J. Heinze, A. Trindl, B. Seifert and K. Yamauchi, in prep.). While colonies from Japan, including the Ryukyu Islands (also referred to as *C. nuda*, Yamauchi and Kinomura, 1993; Japanese Ant Database Group, 2003), produce exclusively ergatoid males, typical *C. kagutsuchi* Terayama, 1999 from Ishigaki Island in the Ryukyu Islands and Southeast Asia have regularly both ergatoid and winged males. The species investigated in the present study appears to be yet another independent taxon with only ergatoid and brachypterous males.

Whereas ergatoid males also occur in a few other ant genera, such as *Anergates, Formicoxenus*, *Hypoponera*, and *Technomyrmex* (Loiselle and Francoeur, 1988; Heinze and Tsuji, 1995), to our knowledge the regular occurrence of brachyptery has as yet been described only from the female sex. Short-winged queens are known from several taxa scattered throughout the phylogenetic tree of ants, for example, from *Nothomyrmecia macrops* and several species of *Myrmecia*, *Cataglyphis*, *Monomorium*, *Tapinoma*, and *Vollenhovia* (Heinze and Tsuji, 1995; Heinze and Keller, 2001). The rather widespread occurrence of brachyptery in *Myrmecia* might suggest that it is a stable phenomenon rather than a transient step in the evolution of winglessness. Here, we describe the morphology and behavior of brachypterous *Cardiocondyla* males and discuss the evolutionary significance of our finding.

Material and methods

Ant collecting and rearing

Cardiocondyla colonies were collected after locating the nest by following foragers back to the nest entrance. One particular colony of a species belonging to the "*C. kagutsuchi*"-complex, collected on June 7, 2000 from its nest 20 cm deep in roadside soil near the field station of the University of Malaya in Ulu Gombak, produced both brachypterous and ergatoid males in the laboratory (M1). In December 2002 and December 2003, five additional colonies of this taxon were found, four (M14, M18, M54, M55) at the same site in Ulu Gombak and one (M13) on the campus of the University of Malaya in Kuala Lumpur. Colony M55 produced only ergatoid males and died after a few months in the laboratory, but the other four colonies regularly produced both ergatoid and brachypterous males. In contrast, eight colonies of other taxa of the "*C. kagutsuchi*"-complex collected in these and other sites in Southeast Asia (M16: University of Malaya, Kuala Lumpur; M6, M15, M51, M52, M53, and m3: Ulu Gombak; I 1 Bogor, Indonesia) never produced brachypterous males, but only ergatoid and long-winged males.

To corroborate that the colonies with brachypterous males are indeed separate from the other "*C. kagutsuchi*", we constructed a neighbor-joining tree (Saitou and Nei, 1987) based on 1427 base pairs of COI/COII (including a 55 base pair non-coding region and 58 base pairs of $\mathrm{tRNA}_{\mathrm{Leu}}$) and 533 base pairs of the 16S rRNA gene (for details see J. Heinze, A. Trindl, B. Seifert and K. Yamauchi, in prep.) using the Kimura 2-parameter distance method (Kimura, 1980) in MEGA 2 (Kumar et al., 2001), with bootstrap values estimated from 5000 replicates.

After growing into a large colony, the colony collected in 2000 was split into several sub-colonies with initially one queen and 10 workers each, which were subsequently used for observations and experiments. Seven such sub-colonies were set up on Feb. 14, 2002 and housed in Petri-dishes (21 cm diameter and 5 cm deep) with a plaster floor (approximately 1 cm thick). A hollow space (1 cm wide, 2 cm long, 2 mm deep), covered by a thin glass plate, served as nest. The sub-colonies were reared in climate chambers at 27 °C and fed fresh pieces of mealworms and sugar solution every 2 or 3 days.

The sub-colonies began producing sexuals from early May on. The pattern of sexual production appeared to change with colony size and to consist of three distinct stages:

1) Ergatoid male production stage with a single queen (stage I). Only ergatoid males and a small number of female sexuals emerged in this earliest stage of sexual production in colonies with single queens. The worker population size at which the first ergatoid males eclosed ranged from 14 to 100 individuals (mean 40.7 ± SD 34.9).
2) Ergatoid male production stage with multiple queens (stage II), i.e. the period from the multiplication of queen number per colony through the adoption of new queens after intranidal mating to the emergence of the first brachypterous males. The worker and queen population size when the first brachypterous males eclosed ranged from 60 to 150 individuals (mean 92.9 ± 29.3) and from 3 to 11 (mean 7.4 ± 3.3), respectively.
3) Mixed male production stage (stage III). Many sexuals, including both ergatoid and brachypterous males were produced in polygynous colonies as the worker population became larger.

The individual numbers of colony queens, sexual pupae and adults were counted almost every day from Feb. 14 to Dec. 14, 2002. The numbers of male and female sexuals were estimated by direct observation in stage I and later by periodic counting and removal of female sexuals and daily counting the number of pupae presumed to eclose by the following day. As removal of female sexuals may have affected colony development, we examined the sex ratio both during the first days of stage III until 20 days after first removal of female sexuals and again during a later period. Furthermore, males found outside the nest were removed and preserved in 100% alcohol solution to examine and count the number of injuries during a certain period within the mixed male production stage. The number of workers was roughly estimated every 1 to 2 weeks.

Morphological measurements

Five ergatoid males produced during stage I and 40 ergatoid males, five intermorphic males, and 40 brachypterous males from stage III were measured for the following body parts using a binocular microscope: maximum head width, maximum head length, maximum eye length, scape length, maximum pronotum width, hind-tibia length, number of ocelli and number of antennal segments. Dry weight was measured just before eclosion using 50 pupae of ergatoid males, 25 pupae of brachypterous males, and 20 pupae of winged female sexuals, all of which were randomly chosen from five sub-colonies at stage III. The samples were dried for 48 hours at 60 °C and weighed to 1 μg with a Mettler M5 scale.

Histology and karyotype analysis

Whole males of different age (one 1, 5, 10, 15, 25, 35, and 50 days old male of each morph and a second 10 days old brachypterous male) were fixed in Bouin solution and embedded in Durcupan/Fluka (2000). The gaster was sagitally sectioned at 0.5 to 2 μm with a Histodiamond (Diatome) and a Reichert-Jung microtome and stained with methylene blue (Cremer et al., 2002b). Chromosomal preparations were made from the testes of 20 white pupae with pink eye coloration from each of five sub-colonies using an improved air-drying technique (Imai et al., 1988).

Behavioral observations

1) Fighting among adult males: In addition to general observations with a video camera through a dissecting microscope, the following treatments were done. One 3 days old ergatoid male and one 3 days old brachypterous male were put in a colony consisting of 1 queen, 10 workers and several small larvae at 29 °C under lighted conditions until one of them was killed or driven out of the nest. This treatment was replicated 5 times.
2) Attack of an adult male on male pupae or eclosing males: One adult of each type of male was kept in an artificial nest with 1 to 3 ergatoid male pupae and 1 to 3 brachypterous male pupae, and 10 workers, and the behavior of the adult male was observed. This observation was replicated 9 times for each male morph.
3) Mating potential of males: One 3 days old virgin male was kept with 20 virgin winged female sexuals (0 to 3 days old) and 10 workers at 27 to 29 °C under dark conditions for 60 hours. Then, all female sexuals were dissected to check for the presence of sperm. This treatment was replicated 9 times for each male morph.

4) Queen dispersal: 20 virgin female sexuals were kept in a colony consisting of 1 queen and 20 workers for 20 days. A second such colony was established with the addition of 5 brachypterous males for 20 days. Female sexuals found outside of the nest were removed every day and dissected to check whether they had been inseminated or not.
5) Estimation of adult life span: a male within one day after eclosion was reared with a queen, 10 workers and several small larvae at 27–29 °C under lighted conditions until the male died. This treatment was replicated 5 times for each male morph.
6) Estimation of the length of the pupal period: a male within one day of pupation was reared with a queen, 10 workers and several small larvae at 27 °C under lighted conditions. The body color was recorded every day until he eclosed. This treatment was replicated 8 times for each male morph.

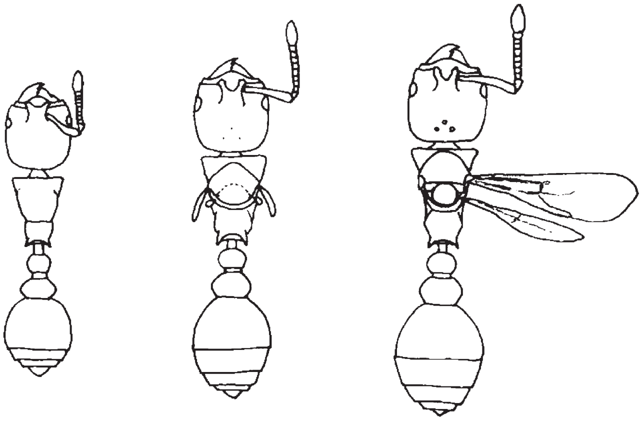

Figure 1. Ergatoid, intermorphic, and brachypterous male of the ant, *Cardiocondyla kagutsuchi* from Ulu Gombak, Malaysia (from left to right)

Results

External morphology of males

The body color of ergatoid males was yellowish. The head was round with small compound eyes, lacking ocelli (Fig. 1). The number of antennal segments was 11, but in an additional investigation, 2 individuals of a total of 112 were found to have only 10 antennal segments. The thorax was simple without a promesonotal suture. The anterior corners of the pronotum were angular in dorsal view. These morphological features are well known also from other ergatoid males in this genus (Kugler, 1983).

Brachypterous males were significantly larger than ergatoid males in all measured body parts (Table 1; two-tailed t-test, $p < 0.05$). Their wings were much shorter than in "normal" winged males of other *Cardiocondyla* species (e.g., Kugler, 1983; Terayama, 1999; Seifert, 2003), the tips reaching the first gastric segment but never exceeding its posteri-

Table 1. Morphological and behavioral measurements in dimorphic males of *C. kagutsuchi*. Data are represented by mean ± standard deviation and range in parentheses

	Ergatoid males		Intermorphic males (n = 5)[1]	Brachypterous males (n = 40)[1]
	Emerged in single queen stage (n = 5)[1]	Emerged in mixed male production stage (n = 40)[1]		
Head length (mm)	0.45 ± 0.02 (0.43–0.46)	0.46 ± 0.02 (0.39–0.50)	0.50 ± 0.01 (0.49–0.51)	0.50 ± 0.02 (0.46–0.56)
Head width (mm)	0.44 ± 0.03 (0.40–0.47)	0.45 ± 0.02 (0.40–0.49)	0.50 ± 0.01 (0.49–0.52)	0.51 ± 0.01 (0.4–0.54)
Eye length (mm)	0.08 ± 0.01 (0.08–0.09)	0.08 ± 0.01 (0.07–0.09)	0.10 ± 0.01 (0.10–0.11)	0.12 ± 0.01 (0.10–0.13)
Scape length (mm)	0.34 ± 0.02 (0.33–0.38)	0.37 ± 0.02 (0.32–0.40)	0.40 ± 0.02 (0.38–0.42)	0.42 ± 0.03 (0.36–0.48)
Pronotum width (mm)	0.31 ± 0.03 (0.27–0.36)	0.34 ± 0.02 (0.29–0.38)	0.39 ± 0.01 (0.38–0.40)	0.41 ± 0.02 (0.38–0.46)
Wing length (mm)	–	–	0.47 ± 0.17 (0.33–0.66)	1.02 ± 0.12 (0.82–1.26)
Hindtibia length (mm)	0.26 ± 0.02 (0.23–0.28)	0.26 ± 0.03 (0.18–0.33)	0.27 ± 0.02 (0.25–0.30)	0.29 ± 0.04 (0.20–0.37)
No. of ocelli	0	0	0 or 3	3
No. of antennal segments	11	11	11	11
Dry weight[3] (mg)		0.069 (50)		0.111 (25)
Pupal period (days)***		5.0 ± 1.3 (n = 8)		9.4 ± 0.7 (n = 8)
Life span (days)*		64.2 ± 30.5 (n = 5)		29.0 ± 7.1 (n = 5)
Mating potential[2]*		6.8 ± 3.2 (n = 9)		10.3 ± 2.5 (n = 9)

[1] Sample size for morphological measurements.
[2] Number of mated females for 60 h.
[3] Average calculated from mass measurements (number of individuals in parenthesis): Female dry weight was also measured: 0.1172 mg (20).
* and *** $p < 0.05$ and $p < 0.0005$, respectively; Mann-Whitney U test.

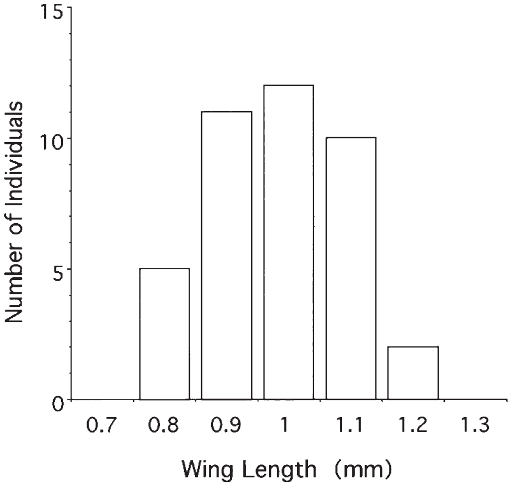

Figure 2. Frequency distribution of wing length in brachypterous *C. kagutsuchi* males

or margin (Fig. 1). Wing length was normally distributed (Shapiro-Wilk's test, W = 0.960, $p < 0.25$; Fig. 2). In contrast to "normal" winged males (e.g., Kugler, 1983; Seifert, 2003), their thorax was broadest at the anterior part of the pronotum with angular corners in dorsal view. The small meso-metanotum was distinctly differentiated into scutum, paraptera, scutellum, and metanotum (Fig. 1). These morphological features suggest that brachypterous males cannot fly, and in fact, we never observed any flight activities. The head was rounded with small compound eyes, three distinct ocelli and 11-segmented antennae. A small number of intermorphic males were found (5 out of 526 examined males). Their wings were very short or rudimentary (Table 1 and Fig. 1). Body color, size, and thoracic structure were intermediate between those of ergatoid and brachypterous males. They either had three or no ocelli and their antennae had 11 segments.

The neighbor-joining tree (Fig. 3) shows that the colonies, which produced ergatoid and brachypterous males, form a distinct cluster separate from other "*C. kagutsuchi*" from Southeast Asia and Japan. One colony (M55) from this group produced only ergatoid males and died after five months in the laboratory without producing any winged males. In contrast to ergatoid males, winged *Cardiocondyla* males are typically not continuously produced (Cremer and Heinze, 2003) and we therefore do not know whether the absence of brachypteorus males in this colony is due to variation in the capability of producing this morph or an artifact of a too short rearing time in the laboratory.

Crossbreeding experiments between sexuals from colonies with brachypterous males and sexuals from colonies producing "normal" males failed consistently (K. Yamauchi, unpublished).

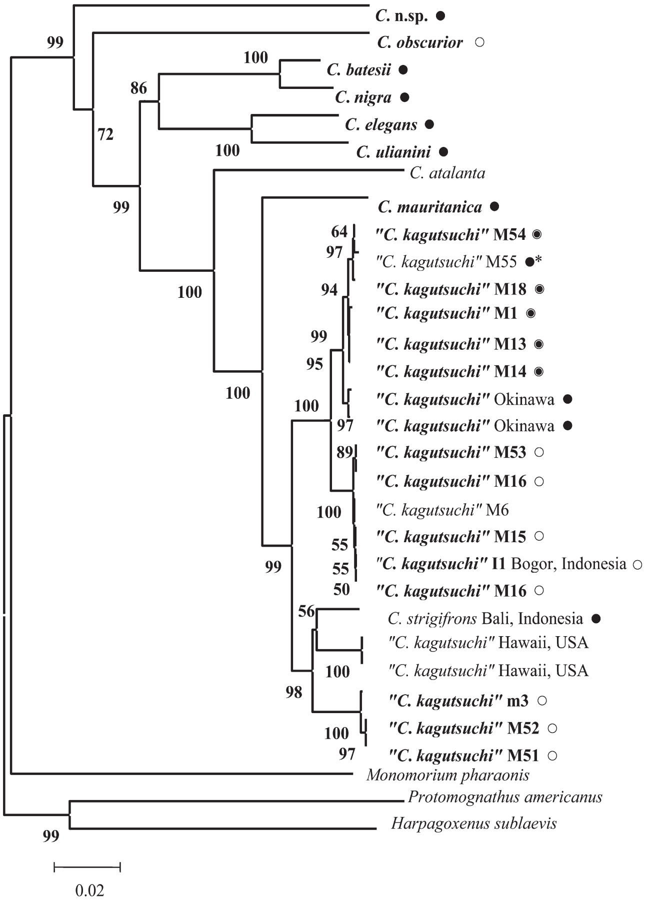

Figure 3. Neighbor-joining tree based on the mitochondrial COI/COII and 16S rRNA genes of several *Cardiocondyla* species. Numbers indicate bootstrap values over 50% in 5000 pseudoreplications. Males are known for all species printed in bold. ○ denotes species with ergatoid and winged males, ● species with only ergatoid males. ◉ indicates the taxon of "*C. kagutsuchi*" from Malaysia with the polymorphism of ergatoid and brachypterous males described here. Combinations of letters and numbers given after the species names are the original collection numbers. *M55 died after 5 months of laboratory culture without producing any winged males

Spermatogenesis and karyotype

All examined males of this taxon, regardless of age and morphology, had well developed testes with all stages of spermatogenesis and mature sperm present. In one of the two examined 10 days old male, testes appeared to show signs of degeneration, whereas spermatogenesis was ongoing in the other. One examined 15 days old brachypterous male had gaps in the tissue; the testes appeared to be dorsally compressed, though spermatogenesis was still ongoing. However, in all older brachypterous males, testes were as well developed as in younger or in ergatoid males, suggesting that both male morphs are characterized by life-long or at least considerably prolonged spermatogenesis (Fig. 4). All of the 100 examined males had haploid chromosome numbers ($n = 14$), suggesting that diploid males are only rarely produced in the studied subcolonies.

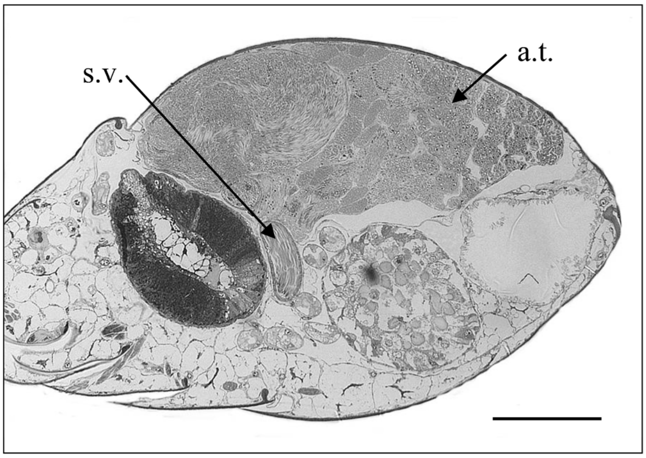

Figure 4. Sagittal section through the gaster of a 50 d old brachypterous *C. kagutsuchi* male, showing fully developed sperm in the posterior part of the testes and the seminal vesicles (s.v.) and spermatogonia in the anterior part of the testes (a. t.). The bar equals 0.1 mm

Aggressive behavior between ergatoid and brachypterous males

Both ergatoid and brachypterous males were observed to kill younger males during the eclosing or callow periods by cutting the body off at the neck or petiole. Generally, males could eliminate almost all younger males by killing or driving them out of the nest. During the most intensive phase of sexual production, however, some young males temporarily co-existed with older males in the nest or managed to escape from the nest when they were attacked, although sometimes they were attacked again and finally killed when carried back into the nest by workers. Ergatoid males won two of five fights with brachypterous males, while brachypterous males won three.

All nine ergatoid males attacked eclosing males of both morphs, while only four of nine brachypterous males attacked eclosing males and in the other cases did not show any special behavior towards eclosing males even when touching them (Fisher's exact probability test, $p < 0.05$).

The ratios of males found outside of the nest to the total number of eclosed males in the mixed male production stage were lower than 50% for ergatoid males and mostly higher than 50% for brachypterous males (Table 2). These differences were significant in six of seven sub-colonies studied. Moreover, morphological examination revealed that 92.1% of brachypterous males and 80.9% of ergatoid males found outside of the nest did not show any visible injury (Table 3). As injuries of the wings may have no influence on subsequent activities, 94.8% of brachypterous males were actually intact. Hence, brachypterous males were significantly less often injured than ergatoid males (χ^2-test, $\chi^2 = 20.03$, $p < 0.001$).

Table 2. Percentage of eclosed males that managed to escape from the nest (total number in parentheses)

Colony code	Ergatoid males	Brachypterous males	χ^2-test
No. 1	12.3 (57)	54.2 (72)	$P < 0.0001$
No. 2	42.8 (215)	69.9 (113)	$P < 0.0001$
No. 3	28.4 (95)	54.1 (109)	$P < 0.0005$
No. 4	30.1 (73)	25.7 (187)	n.s.
No. 5	45.7 (127)	86.2 (145)	$P < 0.0001$
No. 6	19.9 (201)	63.2 (310)	$P < 0.0001$
No. 7	31.3 (243)	74.3 (378)	$P < 0.0001$

Mating potential of males

The number of female sexuals that mated with an ergatoid or brachypterous male within 60 hours varied from three to 12 and from six to 14, respectively. The mating potential of brachypterous males was significantly higher than that of ergatoid males (Table 1). Furthermore, an intermorphic male was confirmed to inseminate at least eight female sexuals during two days.

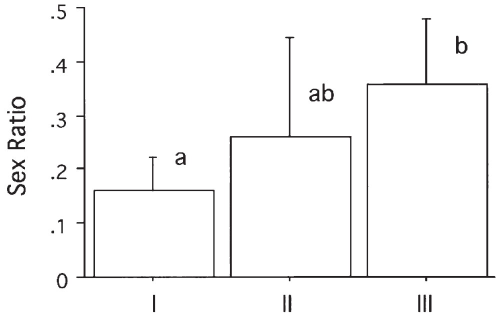

Figure 5. Sex ratio (males/all sexuals; dry weight) in colonies of the ant *C. kagutsuchi* during the three different stages of sexual production (stage I: only ergatoid males, all produced by a single queen; stage II: only ergatoid males, but several queens present; stage III: both short-winged and ergatoid males produced by several queens; (for details see text). Sex ratios differ significantly between samples as indicated by different letters (Mann-Whitney U-tests, $p < 0.005$)

Table 3. Conditions of males after escaping from the nest. Brachypterous males were significantly less frequently injured than ergatoid males ($p < 0.001$, χ^2-test)

	No injury	Injury					Total
		Wing	Leg	Antenna	Leg and antenna	Leg and neck	
Brachypterous males	316	9	5	12	1		343
Ergatoid males	148		17	12	5	1	183

Sex ratio

Sex ratios (males/all sexuals) differed considerably among the three different male production stages. The median sex ratio was significantly lower in the ergatoid male production stage under single queens than in the mixed male production stage (Fig. 5). Per colony, sex ratio varied from 0.09 to 0.33 with a mean of 0.24 ± SD 0.09 in individual number and from 0.06 to 0.23 (mean 0.16 ± 0.06) in dry weight in the ergatoid male production stage under single queens. Sex ratio ranged from 0.14 to 0.68 (mean 0.37 ± 0.20) in number and from 0.09 to 0.56 (mean 0.26 ± 0.18) in dry weight in the ergatoid male production stage under multiple queens, and from 0.24 to 0.62 (mean 0.41 ± 0.12) in number and from 0.20 to 0.57 (mean 0.36 ± 0.12) in dry weight in the mixed male production stage (Table 4). Sex ratios did not differ between the early and late mixed male production stage, i.e., removal of female sexuals did not result in a changed sex allocation pattern. However, in the later phase more brachypterous than ergatoid males were reared.

Table 4. Number of newly eclosed sexuals and the sex ratio during three sexual production stages

I Ergatoid male production stage under a single queen

Colony code	Female	Male (ergatoid)	Sex ratio (number)	Sex ratio (dry weight)
No. 1	3	1	0.25	0.16
No. 2	4	1	0.20	0.15
No. 3	8	4	0.33	0.23
No. 4	10	1	0.09	0.06
No. 5	5	1	0.17	0.11
No. 6	5	2	0.29	0.19
No. 7	2	1	0.33	0.23
Mean ± s.d.	5.3 ± 2.8	1.6 ± 1.1	0.24 ± 0.09	0.16 ± 0.06

II Ergatoid male production stage under multiple queens

Colony code	Female	Male (ergatoid)	Sex ratio (number)	Sex ratio (dry weight)
No. 1	16	3	0.16	0.10
No. 2	35	36	0.51	0.37
No. 3	17	4	0.19	0.12
No. 4	13	28	0.68	0.56
No. 5	8	3	0.27	0.18
No. 6	6	1	0.14	0.09
No. 7	7	8	0.53	0.40
Mean ± s.d.	14.6 ± 10.0	11.9 ± 14.1	0.37 ± 0.20	0.26 ± 0.18

III Mixed male production stage (data from the period from the first production of brachypterous males to twenty days after the first removal of female sexuals are given in parentheses. For details see text)

Colony code	Female	Ergatoid male	Brachypterous male	Sex ratio (number)	Sex ratio (dry weight)
No. 1	413	57	72	0.24	0.20
	(106)	(33)	(19)	(0.33)	(0.26)
No. 2	648	215	113	0.34	0.27
	(196)	(26)	(3)	(0.13)	(0.08)
No. 3	368	95	109	0.36	0.30
	(80)	(50)	(13)	(0.44)	(0.33)
No. 4	426	73	187	0.38	0.34
	(223)	(57)	(110)	(0.43)	(0.38)
No. 5	373	127	145	0.42	0.36
	(86)	(29)	(3)	(0.27)	(0.19)
No. 6	484	201	310	0.51	0.46
	(101)	(105)	(80)	(0.65)	(0.58)
No. 7	378	243	378	0.62	0.57
	(231)	(162)	(97)	(0.53)	(0.45)
Mean ± s.d.	441.4 ± 99.7	144.4 ± 74.6	187.7 ± 114.1	0.41 ± 0.12	0.36 ± 0.12
	(146.1± 67.4)	(66.0 ± 50.2)	(46.4 ± 47.2)	(0.40 ± 0.17)	(0.32 ± 0.17)

Discussion

Some species of *Cardiocondyla* have only ergatoid males, while others have both ergatoid and winged males (Kugler, 1983; Yamauchi and Kinomura, 1993; Heinze et al., 1998; Seifert, 2003). The loss of flight and intranidal mating has led to several morphological changes in ergatoid males, e.g., to small body size, a simplified thorax without wings, small eyes, pale pigmentation, etc. Ergatoid males are highly aggressive towards each other (Kinomura and Yamauchi, 1987; Stuart et al., 1987; Yamauchi and Kinomura, 1993) and their spermatogenesis is prolonged compared to all other Hymenopteran males (Heinze and Hölldobler, 1993; Heinze et al., 1998). Winged males, in contrast, have retained the typical male ant morphology adaptive for mating outside of the nest: large compound eyes, three distinct ocelli, long antennal funiculi, well-differentiated thoracic sutures, functional wings, etc. Their spermatogenesis is completed a few days after eclosion. Winged males, too, mate inside the maternal nest with high mating potential, but within a few days after adult eclosion they disperse from the nest, especially when no virgin female sexuals are available (Yamauchi and Kinomura, 1993; Heinze et al., 1998; Cremer and Heinze, 2003).

The present study revealed a new type of male dimorphism in colonies belonging to one of several species in the "*C. kagutsuchi*"-complex, with brachypterous males as a distinct phenotype in addition to ergatoid males. The ergatoid males have the typical characteristics as mentioned above. The brachypterous males have an intermediate nature between ergatoid males and the normal, long-winged males found in other taxa of the "*C. kagutsuchi*"-complex and related species. Their wings are too small to fly and their pale body coloration and small compound eyes suggest that they mainly live in the nest underground. In addition, intermorphic males, which were morphologically intermediate between ergatoid and brachypterous males, occurred sporadically, probably due to inaccuracies during larval development (see also Cremer et al., 2002b). Similar to ergatoid males, brachypterous males are aggressive and compete for the acquisition of female sexuals. Like in ergatoid males, spermatogenesis appears to be prolonged and even 50 days old males had functional testes. Several morphological changes, such as a round head with strong mandibular muscles and angular pronotum corners, which protect the neck, appear to be associated with male-male aggression. The short wings seem to efficiently protect one of the weakest body parts, the petiolar joints.

However, brachypterous males are not as specialized as ergatoid males and to some extent have retained features of the winged males. This includes the higher probability of escaping from the nest with fewer injuries, the occurrence of ocelli, and a higher sexual activity (Yamauchi and Kinomura, 1993; Heinze et al., 1998). As in species with only ergatoid males (Heinze, 1999), brachypterous males probably have few chances of mating after escaping from the nest because all dispersing female sexuals have already mated in their maternal nests. Although we have no data, it may be possible that brachypterous males can enter alien nests and mate there and that dispersing female sexuals may mate again. Sex allocation ratio differed among sexual production stages. During the ergatoid male production stage with a single queen, it was female-biased. This may be explained by local mate competition (Alexander and Sherman, 1977; Cremer and Heinze, 2002) rather than worker control (Trivers and Hare, 1976), because inbreeding over several generations tends to make all members of a colony almost equally related (Tsuji and Yamauchi, 1994).

In general, polymorphisms as observed in *Cardiocondyla* males may result from genetic polymorphism or phenotypic plasticity. According to previous studies, a genetic determination of male morph appears unlikely. Instead, it has been concluded that male morph is determined by similar social and environmental factors as female caste (Yamauchi and Kinomura, 1993; Heinze and Trenkle, 1997; Cremer and Heinze, 2003). In *C. obscurior,* decreasing temperature, reduction of colony size, and starvation all trigger the production of winged males (Cremer and Heinze, 2003). The fact that in the "*C. kagutsuchi*" investigated in this study only ergatoid males were produced in the earlier stage of sexual production also indicates that social and environmental factors determine male morph.

Male dimorphism is presumably the ancestral condition in *Cardiocondyla* ants and monomorphism with only ergatoid males appears to have evolved convergently in a number of taxa (Yamauchi and Kinomura, 1993; J. Heinze, A. Trindl, B. Seifert and K. Yamauchi, in prep.). This loss of phenotypic plasticity in the male sex might result from mutations in genes that affect the proximate mechanisms of morph differentiation, for example, the density or sensitivity of hormone receptors during a critical period in larval life. Brachypterous males in "*C. kagutsuchi*" may have arisen through a similar mutational shift of developmental thresholds. Given that both male morphs are equally flightless, the question arises whether this type of polymorphism has evolutionary advantages over monomorphism with complete wing loss. Previous studies have only examined the adaptive values of dimorphisms with fully winged and flightless (either brachypterous or completely wingless) morphs (e.g., Roff and Fairbairn, 1991; Denno, 1994; Heinze and Tsuji, 1995; Heinze and Keller, 2000). In any case, it is worth to search for dimorphic ergatoid males in species that were previously thought to be monomorphic, as in *Hypoponera bondroiti* (Yamauchi et al., 1996).

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