

Paternally inherited alleles in male body parts of an ant (*Diacamma* sp.) sex mosaic: implication for androgenetic male production in the Hymenoptera

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Abstract Sex mosaicism, also called gynandromorphism, refers to an accidental phenomenon in dioecious organisms (mainly animals) in which an individual phenotype includes both female and male characteristics. Despite the rarity of this phenomenon, elucidating the mechanisms of naturally occurring sex mosaicism should deepen our understanding of diverse sex determination and differentiation systems in nature. We report the results of a genetic study of a sex mosaic individual of the ant *Diacamma* sp. from Japan's Okinawa Island. Parentage analysis using microsatellite markers revealed that the female and male parts of the sex mosaic showed different inheritance patterns: female parts had alleles consistent with their biparental inheritance, whereas most of the male parts had alleles consistent with their paternal inheritance (i.e., androgenesis). We discuss

plausible cytogenetic mechanisms that gave rise to the male parts of this individual: polyspermy and the subsequent independent cleavage by a surplus sperm pronucleus, and maternal genome elimination after fertilization of an ovule. Moreover, we hypothesize that the androgenetically produced males found in some Hymenoptera might share the same underlying cytogenetic mechanism with hymenopteran sex mosaicism.

Keywords Gynandromorph · Androgenesis ·
Haplodiploid · Male clonality · Polyspermy

Introduction

Sex mosaicism (also called gynandromorphism) has been reported frequently, mostly as single cases, in ants [Yang and Abouheif (2011); Yoshizawa et al. (2009); and references therein]. Due to their haplodiploid sex determination system, the genetic descent of female and male body parts in ant sex mosaics is of particular interest. In haplodiploidy, females arise from fertilized (diploid) eggs that have biparentally inherited genomes, whereas males arise from unfertilized (haploid) eggs that exclusively contain the maternally inherited genome. Previous studies in honeybees and braconid wasps have described various aberrations from the normal haplodiploid inheritance pattern in sex mosaics (e.g., Clark et al., 1968; Rothenbuhler et al., 1952). These aberrations can be summarized from the perspective of heredity as follows: (1) unfertilized eggs develop female parts and (2) fertilized eggs develop male parts. The latter case includes three additional cases: the genome of the male parts is (2a) maternally inherited (gynogenesis), (2b) paternally inherited (androgenesis), or (2c) a combination of both. However, these inheritance patterns were typically

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revealed by artificial crossings of captive strains on the basis of phenotypic markers, and the relevance of these markers to naturally occurring hymenopteran sex mosaics is unclear. In addition, assessment of inheritance patterns using neutral genetic markers remains to be done.

We report a single case of a naturally occurring sex mosaic individual of the ant *Diacamma* sp. from Okinawa Island, the southernmost part of Japan. The strict monogyny (i.e. a single reproductive female, the gamergate, per colony) of this species (Fukumoto et al., 1989) and colony-wide sampling enabled us to infer paternally and maternally inherited alleles of microsatellite markers in the sex mosaic; in other ant species that show frequent production of sex mosaics this would have been difficult because of polygyny, unless high-resolution genetic markers are available (Berndt and Kremer, 1982; Kinomura and Yamauchi, 1994; Yoshizawa et al., 2009). We found that female parts of the sex mosaic had alleles indicating their biparental origin, whereas male parts showed a genotype consistent with paternal inheritance. We discuss plausible cytogenetic mechanisms that gave rise to the male parts of this individual. Moreover, we connect these plausible mechanisms to the androgenetic production of males in some Hymenoptera: on the basis of the co-occurrence of sex mosaics in hymenopteran species with androgenetic males, we hypothesize that this androgenetic male production might share the same underlying cytogenetic mechanism with hymenopteran sex mosaicism.

Materials and methods

During a field survey in Sueyoshi Park, Okinawa, on 23 June 2010, we found a sex mosaic individual inside a colony of the ant *Diacamma* sp. (Fig. 1, see also Movie S1 in the Electronic Supplementary Material). Although biology of this species has been extensively studied during the past two decades (e.g., Fukumoto et al., 1989; Okada et al., 2010), this is the first report of sex mosaicism in this species, as well as in the subfamily Ponerinae. We collected the sex mosaic together with the whole colony and maintained them in an artificial nest (see Text S1 for behavioral observation of the sex mosaic). On the following day, unfortunately, the sex mosaic was found beheaded within the artificial nest, probably by its nestmates. We, therefore, shifted from behavioral to parentage analysis of the sex mosaic.

The paternity of the colony was deduced using the gamergate, worker adults ($n = 16$), and brood ($n = 16$; consisting of 2 eggs, 7 larvae and 7 pupae). DNA was extracted using the Chelex method (Walsh et al., 1991), and genotypes were determined at four polymorphic microsatellite loci: DC-8 (Doums, 1999), Plu-24 (Takahashi et al.,

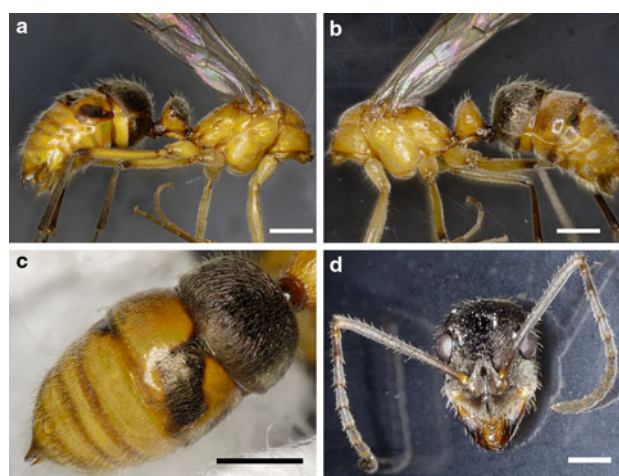


Fig. 1 External morphology of the sex mosaic individual of *Diacamma* sp. Female parts consist of the head (totally female), petiole (partly female), segment III (mostly female), segments IV and V (partly female), whereas male parts prevail in the rest of the body. Scale bars, all 1 mm. **a** Thorax and abdomen (view of right side). **b** Thorax and abdomen (view of left side). **c** Abdomen in dorsal view. **d** Head in front view

2005), DI33 (Viginier et al., 2004), and DspS554; out of the six loci that we tested, these were informative for the colony (see Table S1 for details). The multilocus genotypes we obtained were used to infer the genotype of the male or males that mated with the gamergate, and the inference was confirmed using the software MATE SOFTWARE, ver. 1.0 (Moilanen et al., 2004). Subsequently, the sex mosaic individual, in pure ethanol, was dissected into subparts so as to separate female and male parts phenotypically, and their genotypes were determined with the same protocol.

Results and discussion

Colony-level microsatellite genotyping revealed that the gamergate had mated with two males (M-1 and M-2, Table 1a), and that all the workers could be assigned as daughters of the gamergate sired by one of the two males; i.e., no workers in the colony had genotypes suggesting immigration or gamergate turnover. Previous behavioral studies have found that the male abdomens of *Diacamma* sp. from Okinawa served as mating plugs, suggesting that there is strict monandry (i.e., a single male mate per gamergate) in this species (Nakata et al., 1998). Thus, the prevalence of multiple mating of gamergates should be studied further using microsatellite markers.

Genetic descent of the sex mosaic

The genotypes of all female parts ($n = 8$ parts) of the sex mosaic were consistent with their biparental origin, which

Table 1 Microsatellite genotypes of members of the colony in which the sex mosaic was found. *a* Genotypes of gamergate, her inferred mates (M), workers, and brood, together with their inferred paternity. *b* Genotypes of the female and male parts of the sex mosaic, together with their inferred paternity

	Microsatellite marker				Inferred paternity
	DC-8	Plu-24	DI33	DspS554	
<i>(a) Individual</i>					
Gamergate	159/159	160/172	223/229	98/98	
Males					
M-1	161	160	223	94	
M-2	159	160	229	94	
Workers					
<i>n</i> = 3	159/161	160/160	223/223	94/98	M-1
<i>n</i> = 3	159/161	160/160	223/229	94/98	M-1
<i>n</i> = 1	159/161	160/172	223/223	94/98	M-1
<i>n</i> = 1	159/161	160/172	223/229	94/98	M-1
<i>n</i> = 2	159/159	160/160	223/229	94/98	M-2
<i>n</i> = 1	159/159	160/160	229/229	94/98	M-2
<i>n</i> = 2	159/159	160/172	223/229	94/98	M-2
<i>n</i> = 2	159/159	160/172	229/229	94/98	M-2
Brood					
<i>n</i> = 2	159/161	160/160	223/223	94/98	M-1
<i>n</i> = 4	159/161	160/160	223/229	94/98	M-1
<i>n</i> = 3	159/161	160/172	223/223	94/98	M-1
<i>n</i> = 1	159/161	160/172	223/229	94/98	M-1
<i>n</i> = 3	159/159	160/160	229/229	94/98	M-2
<i>n</i> = 3	159/159	160/172	229/229	94/98	M-2
<i>(b) Body parts</i>					
Female ^a	159/159	160/160	223/229	94/98	M-2
Male ^b	159	160	229	94	^d
Male ^c	159/159	160/160	223/229	94/98	^d

^a The analyzed parts were funiculi (2), scapes (2), mandibles (2), the rest of the head (1), and the epidermis of segment III (1)

^b The analyzed parts were forelegs (8), midlegs (8), hindlegs (8) (for each leg, the following parts were separated: coxa + trochanter, femur, tibia, and tarsus), forewings (2), hindwings (2), mesonotum (1), propodeum (1), epidermis of the tergite of segment VI (1), epidermis of the sternite of segment VI (1), accessory gland (and the surrounding tissues) (1), and genitalia with the epidermis of segment VII (1)

^c The analyzed parts were the pronotum (1) and testis (and the surrounding tissues) (1). Left and right sides of all bilateral external structures were genotyped separately. The number in parentheses indicates the number of body parts genotyped

^d See the “Results and discussion”

was the same as the results for the workers sired by one of the two males (M-2, Table 1b). Most of the male parts analyzed (*n* = 34 out of 36 parts) possessed single alleles at all of the four loci (Table 1b); a comparison with the female parts at the genotypes of the loci DspS554 and DI33 suggested that these parts were haploid or haploid-derived. Furthermore, the genotypes of these parts were exactly the

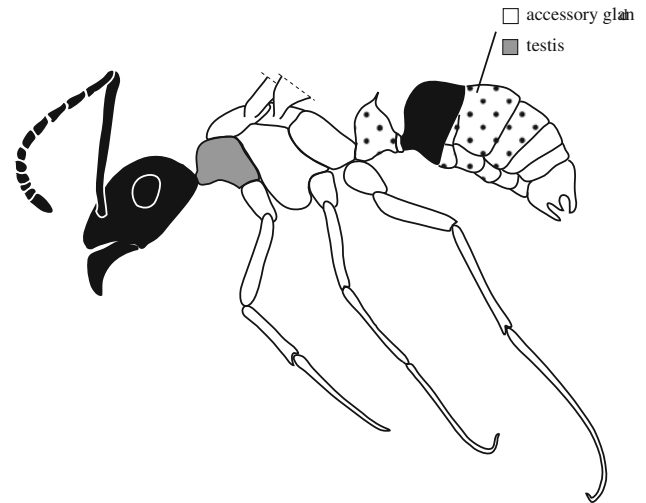


Fig. 2 Schematic lateral diagram showing the phenotype-genotype relationships of the sex mosaic individual of *Diacamma* sp. *Black area* female body parts with the genotype consistent with its biparental origin, *white area* male body parts with the genotype consistent with its paternal origin, *gray area* male body parts with the genotype consistent with its biparental origin, *dotted area* mosaics of female and male body parts that were not genotyped. See Fig. 1 and Table 1 for details

same as that of the male parent of the female parts. The most parsimonious explanation for the observed pattern is that the sex mosaic developed from a fertilized egg through androgenesis in these male parts; i.e., the alleles were paternally inherited (pattern 2b in the “Introduction”). Some of the male parts (*n* = 2, Table 1b), which were identified phenotypically, had the same genotype as the female parts. The simplest interpretation of this result is contamination with female parts during dissection, which is based on the fact that these male parts were adjacent to the female parts (Fig. 2). It is also possible that haploid-derived cells coexisted with diploid cells in some body parts, irrespective of their phenotypes, and this coexistence could be detected in these “male” parts [Yoshizawa et al. (2009) reported such a discordance between ploidy and phenotype in ant sex mosaics].

Because of the small number of alleles available for the analyzed loci, an alternative scenario (random loss of chromosomes from one or more zygote-derived blastomeres in the male parts) could not be ruled out as an explanation of the genotype of the haploid-derived male parts. In this scenario, the alleles at loci Plu-24 and DC-8 could be derived maternally or paternally. However, in both scenarios, allele 94 at locus DspS554 and allele 229 at locus DI33 were unambiguously assigned as paternally derived alleles. An independent population genetic analysis using 34 workers from 17 colonies (2 workers per colony) from Okinawa revealed no indication of linkage disequilibrium between DspS554 and DI33 [*p* = 0.874; analysis performed using GENEPOP 4.0 (Raymond and Rousset, 1995)]. If

Table 2 Co-occurrence of sex mosaicism with androgenetic male production in the Hymenoptera

Family	Species	Androgenetic male production		Female clonality co-occurs?	Report of sex mosaics	
		Study type	Reference		Study type	Reference
Braconidae	<i>Habrobracon juglandis</i> ^a	C	Clark et al. (1968)	No	A	Clark et al. (1968)
Apidae	<i>Apis mellifera</i>	C	Koeniger et al. (1989)	No	A	Rothenbuhler et al. (1952)
Formicidae	<i>Wasmannia auropunctata</i>	G	Fournier et al. (2005)	Yes	F	A. Mikheyev (pers. comm.) ^b
		C	Foucaud et al. (2010)			
	<i>Vollenhovia emeryi</i>	G	Ohkawara et al. (2006)	Yes	F	Kinomura and Yamauchi (1994) ^c
	<i>Paratrechina longicornis</i>	G, C	Pearcy et al. (2011)	Yes	na	

Hymenopteran species for which androgenetic male production has been reported are listed

^a *Ebony* mutant

^b Single observation of an ergatandromorphic individual (worker-male mosaicism)

^c 60% of male-producing colonies contained ergatandromorphic (and some gynandromorphic, i.e., queen-worker mosaic) individuals

C laboratory crossing, G population genetic study, A androgenesis in male parts was confirmed by laboratory crossing, F report of a field-collected sex mosaic individual, na not available

chromosomal loss accounts for the origin of these male parts, the inferred independence of these two loci suggests that the survival of two paternally inherited alleles occurred independently, an event that seems less likely to explain the genetic descent of these male parts.

Androgenesis in male parts of hymenopteran sex mosaics has been confirmed in the honeybee *Apis mellifera* (Rothenbuhler et al., 1952) and the braconid wasp *Habrobracon juglandis* (Clark et al., 1968). If this is the case for haploid-derived male parts of *Diacamma* sp., the present study is the first report of androgenesis in ant sex mosaics.

Potential cytogenetic mechanisms: implications for androgenetic male production in the Hymenoptera

Androgenesis in male parts of hymenopteran sex mosaics has an important implication for androgenetic production of normal males in the Hymenoptera, as Yoshizawa et al. (2009) noted. Androgenetic male production (AMP) in the Hymenoptera has been reported in a honeybee (*Apis mellifera carnica*) and an *ebony* mutant of *Habrobracon juglandis*, with three ant species recently added (*Wasmannia auropunctata*, *Vollenhovia emeryi*, and *Paratrechina longicornis*) (Table 2). AMP has been receiving attention in the context of cooperation and conflict between female and male genomes (Queller, 2005). It should be noted that sex mosaicism has been reported in all of the above species except *P. longicornis*, and some of these species have been confirmed to show androgenesis in male parts (Table 2). This co-occurrence of (androgenetic) sex mosaicism with AMP, together with our present report of tentative androgenesis in male parts of the sex mosaic, leads us to hypothesize that AMP and androgenesis in sex mosaicism in the Hymenoptera might share the same underlying cytogenetic mechanism.

Two potential cytogenetic mechanisms have been proposed that could explain AMP in the Hymenoptera. First, maternal genome elimination may occur after an ovule is fertilized (Fournier et al., 2005). Second, females sometimes lay eggs without nuclei that are then fertilized by males (Foucaud et al., 2007). It is difficult to distinguish between these two mechanisms using only genetic markers, and no cytogenetic study has been performed thus far. Maternal genome elimination might also explain the production of androgenetic male parts in hymenopteran sex mosaics, if it occurs in some of the blastomeres after several rounds of cleavage.

In contrast to AMP, the cytogenetic mechanisms responsible for unusual inheritance patterns in hymenopteran sex mosaics have been studied extensively since the early 20th century [see Nilsson (1987) for a review]. Androgenesis in male parts of these mosaics is believed to occur through polyspermy (Morgan, 1905): a normal ovule is fertilized by two (or more) sperm, followed by dual cleavage by a zygote produced by normal syngamy and by a surplus sperm pronucleus. This process leads to a sex mosaic, in which female parts arise from the zygote (and are, thus, biparentally inherited), and male parts arise from the surplus sperm (and are thus paternally inherited). This mechanism has been empirically supported by the inheritance patterns of phenotypic markers in honeybee sex mosaics (Rothenbuhler et al., 1952). If it occurs widely in the Hymenoptera, as is already known in honeybees (Nachtsheim, 1913) and *H. juglandis* (Speitche, 1936), polyspermy might also be responsible for AMP: polyspermy of an ovule and subsequent dominance of blastomeres that originated from a surplus sperm over those that originated from a zygote would result in (apparently) normal males that have paternally inherited genomes. This potential mechanism requires only a relatively precocious

cleavage by a surplus sperm pronucleus. The fact that male parts covered a much larger area of the body than female parts in most sex mosaics of *C. kagutsuchi* (Yoshizawa et al., 2009) suggests the possibility of such a precocious cleavage. Moreover, if two or more sperm are involved in AMP, the underlying mechanism might be a novel candidate for the study of sperm competition or cooperation (Birkhead et al., 2009). Because the mechanisms of AMP and sex mosaic production might also vary among species, detailed cytogenetic study will be required for each species in future research.

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