RESEARCH ARTICLE

Karyotype instability in the ponerine ant genus *Diacamma*

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Abstract

The queenless ponerine ant *Diacamma ceylonense* and a population of *Diacamma* from the Nilgiri hills which we refer to as '*nilgiri*', exhibit interesting similarities as well as dissimilarities. Molecular phylogenetic study of these morphologically almost similar taxa has shown that *D. ceylonense* is closely related to '*nilgiri*' and indicates that '*nilgiri*' is a recent diversion in the *Diacamma* phylogenetic tree. However, there is a striking behavioural difference in the way reproductive monopoly is maintained by the respective gamergates (mated egg laying workers), and there is evidence that they are genetically differentiated, suggesting a lack of gene flow. To develop a better understanding of the mechanism involved in speciation of *Diacamma*, we have analysed karyotypes of *D. ceylonense* and '*nilgiri*'. In both, we found surprising inter-individual and intra-individual karyotypic mosaicism. The observed numerical variability, both at intra-individual and inter-individual levels, does not appear to have hampered the sustainability of the chromosomal diversity in each population under study. Since the related *D. indicum* displays no such intra-individual or inter-individual variability whatsoever under identical experimental conditions, these results are unlikely to be artifacts. Although no known mechanisms can account for the observed karyotypic variability of this nature, we believe that the present findings on the ants under study would provide opportunities for exciting new discoveries concerning the origin, maintenance and significance of intra-individual and inter-individual karyotypic mosaicism.

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Introduction

Ants are generally classified as highly eusocial species in which the queen and worker castes are morphologically differentiated (Wilson 1971; Hölldobler and Wilson 1990). However, about 100 species belonging to the phylogenetically and morphologically primitive subfamily Ponerinae lack a morphologically distinguishable queen caste (Wheeler 1915; Peeters 1991). In these species, workers have retained the ability to mate and reproduce. In the queenless ponerine ant genus *Diacamma*, reproductive monopoly is achieved by a unique mechanism. Here, all individuals are morphologically identical and eclose with a pair of rudimentary, mesothoracic wing buds called gemmae which apparently release an exocrine signal (Tulloch 1934; Peeters and Billen 1991; Baratte *et al*. 2006a). Gemmae enable individuals to perform sexual calling and are thus necessary for mating to occur. The gamergate (mated egg laying worker (Peeters 1993) however mutilates the gemmae of all the eclosing individuals who eclose after her (Fukumoto *et al*. 1989; Peeters and Higashi 1989). Mutilation of the gemmae leads to irreversible neurological changes in the workers and they loose their ability to perform sexual calling and thus they cannot mate (Gronenberg and Peeters 1993; Baratte *et al*. 2006b). Mutilated workers do not mutilate others, so that after the death of the gamergate, the first worker to eclose retains her gemmae and assumes the role of the gamergate. There is also an interesting evidence that cues for mutilation origi-

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nate in the callows, presumably in the gemmae themselves (Ramaswamy *et al*. 2004). Surprisingly, in some *Diacamma* populations from south India, tentatively called *Diacamma* sp. from Nilgiri (hereafter referred to as '*nilgiri*'), the gamergate does not mutilate her nest mates and yet monopolizes reproduction by using dominance interactions (Peeters *et al*. 1992, in which '*nilgiri*' is mislabelled as *D. vagans*).

Molecular phylogenetic study of the *Diacamma* genus has shown that *D. ceylonense* is closely related to '*nilgiri*' (Baudry *et al*. 2003) and indicates that '*nilgiri*' originates from the most recent divergence in the tree (Veuille *et al*. 1999). These taxa are almost similar in morphology, but for the mutilation of gemmae in *D. ceylonense* and not in '*nilgiri*'. In addition to the behavioural difference related to the mutilation of gemmae, microsatellite and mitochondrial markers have revealed significant genetic divergence between these taxa (Baudry *et al*. 2003).

For all the above-mentioned reasons, we believe that *D. ceylonense* and '*nilgiri*' provide an interesting model system for the study of incipient speciation. To understand the possible mechanism involved in speciation of *Diacamma*, we have undertaken a karyotypic study of *D. ceylonense* and '*nilgiri*'. The karyotype is generally an invariant character of each species and is therefore considered to be of taxonomic value. Nevertheless, chromosomal rearrangements often accompany events of speciation in order to produce species-specific karyotypes. It follows that the study of karyotypic differences among taxonomically closely related species could provide insights into the mechanism of speciation (John 1981; King 1993).

Materials and methods

Colonies of *D. ceylonense* were collected from three different parts of Bangalore: Indian Institute of Science campus, Jakkur, and the Valley School campus (Karnataka 12058′N, 77033′E). *D. indicum* colonies were collected from Malleswaram (Bangalore, Karnataka $12^0$58′N, $77^0$33′E), whereas '*nilgiri*' colonies were collected from different parts of the Nilgiri hill range such as Triambakapura (Karnataka $11^0$47′N, $76^0$45′E) and from Mudumalai (Tamil Nadu, $11^0$37′N, $76^0$34′E) in 2003–2004. All the colonies were kept in artificial plaster of Paris nests in the laboratory at 23°C–27°C and the ants were fed with *Corcyra cephalonica* larvae, termites, cockroaches, honey and water. All colonies used in this study had only one gamergate per colony, which was identified by the presence of gemmae in *D. ceylonense* and *D. indicum* and by egg laying behaviour in '*nilgiri*'.

Chromosomal preparations were made from the cerebral ganglia and hepatic cecae of pre-pupae, pupae, adults and also from ovaries and eggs in the case of gamergate. The modified air-dried procedure of Imai *et al*. (1988) was followed to prepare the slides and well-spread chromosome plates were photographed using a Zeiss microscope (Axioskop 2 plus, Jena, Germany). Only the individuals and the tissues showing good, countable chromosomes were included in the data analysis. The aim of this study was to record a novel type of karyotypic instability. Because of cytological and technical limitations, it was not possible to present all varieties of varying karyotypes.

Results

The major finding of this study is the presence of intra-tissue, intra-individual and inter-individual variability in the karyotype, in males, females as well eggs, in both *D. ceylonense* and '*nilgiri*'. Since this was unexpected, we took the precaution of including *D. indicum* as a control as it is known to have a species-specific, stable karyotype (Imai *et al*. 1984, in which *D. indicum* is incorrectly labelled as *D. vagans*). The observed karyotype ranges, sampling locations, number of colonies and the number of individuals that were subjected to karyotype analysis are given in table 1. Data for each individual are shown in table 2. This report exclusively deals with the numerical variation in chromosomes. Because of the very small size of the chromosomes and the nature of the material, the analysis of the C-bands and morphology of the chromosomes could not be ascertained in spite of repeated attempts to fine-tune the technique.

Diacamma ceylonense

Altogether 55 individuals, including adults, pupae and eggs from nine colonies of three populations were analysed. Thirty chromosome spreads were obtained from cerebral ganglia and hepatic cecae of 13 males. Out of 13 males, 11 showed a consistent haploid number of $n = 5$ or 6 chromosomes. On the other hand, one male from IISc population

Table 1. Collection data and range of karyotype variation in different populations of *D. ceylonense, 'nilgiri' and D. indicum.* Number in the paranthesis represents total number of spreads obtained.

Species	Population studied	Number of colonies	Number of males	Karyotype range of males	Number of females	Karyotype range of females	Number of eggs	Karyotype range of eggs
D. ceylonense	IISc	3	7	n = 4–7 (17)	16	$2n$ = 6–31 (28)	2	9–12 (4)
	Jakkur	3	4	n = 5–6 (11)	5	$2n$ = 10–30 (7)	*	*
	Valley School	3	2	n = 5 (2)	13	$2n$ = 5–35 (28)	6	5–15 (8)
'*nilgiri*'	Triambakapura	3	6	n = 5–14 (19)	12	$2n$ = 5–54 (169)	*	*
	Mudumalai	3	2	n = 7–9 (3)	7	$2n$ = 8–33 (12)	*	*
D. indicum	Malleswaram	3	5	n = 7 (12)	4	$2n$ = 14 (13)	*	*

*Not studied.

Table 2. Karyotype of all the individuals and the range at the colony level, population level and at the species level.

Species	Population	Colony no.	Individual no.	Tissue	Female	Male	Sex unknown
D. ceylonense	IISc	Dc 89	1	CG		5 (2)	
D. ceylonense	IISc	Dc 89	2	CG		5 (3)	
D. ceylonense	IISc	Dc 89	3	HC		5 (1)	
D. ceylonense	IISc	Dc 89	4	HC	10 (1)		
D. ceylonense	IISc	Dc 89	5	Egg			12 (1)
D. ceylonense	IISc	Dc 89	6	Egg			9 (2)
							10 (1)
D. ceylonense	IISc	**Dc 89**	**All individuals**		**10 (1)**	**5 (6)**	**9–12 (4)**
D. ceylonense	IISc	Dc 94	7	CG		6–7 (3)	
				HC		4 (2)	
D. ceylonense	IISc	Dc 94	8	HC		5 (1)	
D. ceylonense	IISc	Dc 94	9	HC	25 (1)		
D. ceylonense	IISc	Dc 94	10	CG	19 (1)		
D. ceylonense	IISc	Dc 94	11	CG	31 (1)		
D. ceylonense	IISc	Dc 94	12	CG	12 (1)		
D. ceylonense	IISc	**Dc 94**	**All individuals**		**12–31 (4)**	**4–7 (6)**	
D. ceylonense	IISc	Dc 97	13	HC	18 (1)		
D. ceylonense	IISc	Dc 97	14	HC	6–18 (4)		
D. ceylonense	IISc	Dc 97	15	CG	10 (1)		
D. ceylonense	IISc	Dc 97	16	CG	8–16 (4)		
D. ceylonense	IISc	Dc 97	17	HC	18 (1)		
D. ceylonense	IISc	Dc 97	18	HC	14–16 (2)		
D. ceylonense	IISc	Dc 97	19	CG	12 (1)		
D. ceylonense	IISc	Dc 97	20	CG	22–28 (3)		
				HC	10 (1)		
D. ceylonense	IISc	Dc 97	21	CG	8–14 (2)		
D. ceylonense	IISc	Dc 97	22	CG	12 (1)		
D. ceylonense	IISc	Dc 97	23	CG	11–20 (3)		
				HC	16–28 (2)		
D. ceylonense	IISc	Dc 97	24	CG		5 (3)	
D. ceylonense	IISc	Dc 97	25	CG		6 (2)	
D. ceylonense	IISc	**Dc 97**	**All individuals**		**6–28 (26)**	**5–6 (5)**	
D. ceylonense	**IISc**	**All colonies**			**6–31 (31)**	**4–7 (17)**	**9–12 (4)**
D. ceylonense	Jakkur	Dc 87	26	CG		6 (3)	
D. ceylonense	Jakkur	Dc 87	27	CG		5 - 6 (3)	
D. ceylonense	Jakkur	Dc 87	28	CG		5 (2)	
D. ceylonense	Jakkur	Dc 87	29	CG		6 (3)	
D. ceylonense	Jakkur	Dc 87	30	HC	10 (1)		
D. ceylonense	Jakkur	Dc 87	31	CG	30 (1)		
D. ceylonense	Jakkur	**Dc 87**	**All individuals**		**10–30 (2)**	**5–6 (11)**	
D. ceylonense	Jakkur	Dc 95	32	HC	30 (1)		
D. ceylonense	Jakkur	Dc 95	33	HC	22 (1)		
D. ceylonense	Jakkur	**Dc 95**	**All individuals**		**22–30 (2)**		
D. ceylonense	Jakkur	Dc 96	34	CG	14–22 (3)		
D. ceylonense	**Jakkur**	**All colonies**			**10–30 (7)**	**5–6 (11)**	
D. ceylonense	Valley School	Dc 90	35	Egg			5 (1)
D. ceylonense	Valley School	Dc 90	36	CG		5 (1)	
D. ceylonense	Valley School	**Dc 90**	**All individuals**			**5 (1)**	**5 (1)**
D. ceylonense	Valley School	Dc 91	37	CG		5 (1)	

Table 2 *(contd)*

Species	Population	Colony no.	Individual no.	Tissue	Female	Male	Sex unknown
D. ceylonense	Valley School	Dc 91	38	Ovary	12 (1)		
D. ceylonense	Valley School	Dc 91	39	Ovary	27–28 (2)		
D. ceylonense	Valley School	Dc 91	40	Egg			9(1)
D. ceylonense	Valley School	Dc 91	41	Egg			8(1)
D. ceylonense	Valley School	Dc 91	42	Egg			15(1)
D. ceylonense	Valley School	Dc 91	43	Egg			9(1)
D. ceylonense	Valley School	Dc 91	44	Egg			7(3)
D. ceylonense	Valley School	Dc 91	45	Ovary	7 - 30 (4)		
D. ceylonense	Valley School	**Dc 91**	**All individuals**		**7–30 (7)**	**5 (1)**	**7–15 (7)**
D. ceylonense	Valley School	Dc 93	46	CG	35 (1)		
D. ceylonense	Valley School	Dc 93	47	CG	25 (1)		
				HC	7 (1)		
D. ceylonense	Valley School	Dc 93	48	HC	12 (2)		
D. ceylonense	Valley School	Dc 93	49	CG	12 - 28 (4)		
D. ceylonense	Valley School	Dc 93	50	CG	7 - 10 (3)		
D. ceylonense	Valley School	Dc 93	51	HC	30 (1)		
D. ceylonense	Valley School	Dc 93	52	CG	5 - 16 (4)		
D. ceylonense	Valley School	Dc 93	53	HC	32 (1)		
D. ceylonense	Valley School	Dc 93	54	CG	16 (2)		
D. ceylonense	Valley School	Dc 93	55	CG	10 (1)		
D. ceylonense	Valley School	**Dc 93**	**All individuals**		**5–35 (21)**		
D. ceylonense	**Valley School**	**All colonies**			**5–35 (28)**	**5 (2)**	**5–15 (8)**
D. ceylonense	**All populations**				**5–35 (66)**	**4–7 (30)**	**5–15 (12)**
'nilgiri'	Triambakapura	Dn 32	56	CG		7(1)	
'nilgiri'	Triambakapura	Dn 33	57	CG	12 (3)		
'nilgiri'	Triambakapura	Dn 33	58	CG	5 - 34 (10)		
'nilgiri'	Triambakapura	Dn 33	59	whole	19 - 40 (15)		
'nilgiri'	Triambakapura	Dn 33	60	whole	11 - 30 (17)		
'nilgiri'	Triambakapura	Dn 33	61	whole	11 - 54 (63)		
'nilgiri'	Triambakapura	Dn 33	62	whole	6 - 50 (39)		
'nilgiri'	Triambakapura	Dn 33	63	CG	22 - 36 (3)		
'nilgiri'	Triambakapura	Dn 33	64	whole	7 - 47 (11)		
'nilgiri'	Triambakapura	Dn 33	65	CG	34 (1)		
'nilgiri'	Triambakapura	Dn 33	66	HC	27 - 32 (2)		
'nilgiri'	Triambakapura	Dn 33	67	HC	22 (1)		
				Ovary	12 - 20 (3)		
'nilgiri'	Triambakapura	**Dn 33**	**All individuals**		**5–54 (168)**		
'nilgiri'	Triambakapura	Dn 40	68	HC		5 - 14 (5)	
				CG		5 - 10 (5)	
'nilgiri'	Triambakapura	Dn 40	69	CG		5 (1)	
'nilgiri'	Triambakapura	Dn 40	70	CG		5 - 6 (4)	
'nilgiri'	Triambakapura	Dn 40	71	HC		5 - 6 (2)	
'nilgiri'	Triambakapura	Dn 40	72	HC		8 (1)	
'nilgiri'	Triambakapura	Dn 40	73	CG	9 (1)		
'nilgiri'	Triambakapura	**Dn 40**	**All individuals**		**9 (1)**	**5–14 (18)**	
'nilgiri'	**Triambakapura**	**All colonies**			**5–54 (169)**	**5–14 (19)**	
'nilgiri'	Mudumalai	Dn 34	74	CG	26 (1)		
'nilgiri'	Mudumalai	Dn 34	75	CG	19 - 33 (3)		
'nilgiri'	Mudumalai	**Dn 34**	**All individuals**		**19–33 (4)**		
'nilgiri'	Mudumalai	Dn 35	76	CG	8–13 (2)		
'nilgiri'	Mudumalai	Dn 35	77	HC	22 (1)		

Table 2 *(contd)*

Species	Population	Colony no.	Individual no.	Tissue	Female	Male	Sex unknown
'nilgiri'	Mudumalai	Dn 35	78	CG	10–24 (2)		
'nilgiri'	Mudumalai	**Dn 35**	**All individuals**		**8–24 (5)**		
'nilgiri'	Mudumalai	Dn 36	79	whole	16 (1)		
'nilgiri'	Mudumalai	Dn 36	80	whole	26 (2)		
'nilgiri'	Mudumalai	Dn 36	81	CG		7 (2)	
'nilgiri'	Mudumalai	Dn 36	82	CG		9 (1)	
'nilgiri'	Mudumalai	**Dn 36**	**All individuals**		**16–26(3)**	**7–9 (3)**	
'nilgiri'	**Mudumalai**	**All colonies**			**8–33 (12)**	**7–9 (3)**	
'nilgiri'	**All populations**				**5–54 (181)**	**5–14 (22)**	
D. indicum	Malleswaram	Di 1	83	Whole	14 (7)		
D. indicum	Malleswaram	Di 1	84	CG		7 (2)	
D. indicum	Malleswaram	Di 1	85	HC		7 (1)	
D. indicum	Malleswaram			CG		7 (4)	
D. indicum	Malleswaram	Di 1	86	HC		7 (1)	
D. indicum	Malleswaram	Di 1	87	CG		7 (3)	
D. indicum	Malleswaram	Di 1	88	TE		7 (1)	
D. indicum	Malleswaram	Di 1	89	Ovary	14 (4)		
D. indicum	Malleswaram	**Di 1**	**All individuals**		**14 (11)**	**7 (12)**	
D. indicum	Malleswaram	Di 2	90	HC	14 (1)		
D. indicum	Malleswaram	Di 4	91	HC	14 (1)		
D. indicum	**Malleswaram**	**All colonies**			**14 (13)**	**7 (12)**	

CG, cerebral ganglia; HC, hepatic cecae; TE, Testis; whole, prepupa where the tissue could not be identified. Numbers in the parenthesis represents total number of spreads obtained. Bold characters indicate the karyotype range at the colony, population and species level.

showed 6 and 7 chromosomes in different cells of the cerebral ganglia indicating intra-tissue variation and only four chromosomes in the cells of hepatic cecae revealing inter-tissue difference (table 2; individual 7). Another male from Jakkur population showed 5 and 6 chromosomes in the cerebral ganglia tissue (table 2; individual 27). Thus, in *D. ceylonense*, the haploid complement varied from 4 to 7 chromosomes with $n = 5$ occurring most frequently (figures 1a and 2). Females exhibited greater karyotypic diversity. Sixty-three spreads from cerebral ganglia and hepatic cecae of 34 females were obtained. The diploid number ranged from 5 to 35 with numbers 10 and 12 occurring frequently (figures 1b and 3). Like males, chromosomal numerical variation was found within a tissue, and also among tissues (table 2, individuals 20, 23 and 47). We also analysed seven spreads from the ovarian tissue of three gamergates from colony Dc 91, Valley School population. The first gamergate from colony Dc 91 showed $2n = 12$ in her ovarian tissue. The subsequent gamergate showed $2n = 27$ and 28. The third gamergate which emerged after the death of the second gamergate, showed a variable karyotype of $2n = 7, 9, 24$ and 30, in different cells of the ovarian tissue (table 2, individual 45; figure 1c)

We screened eggs of one colony from IISc population and two colonies from Valley School population. The eggs from colony Dc 89-IISc population showed 9, 10 and 12 chromosomes. Eggs dissected from colony Dc 91, Valley School population had 7, 8, 9 and 15 chromosomes whereas an egg from another colony from the same population showed only five chromosomes. The ploidy of the preparations from the eggs could not be ascertained because we did not know whether the eggs were fertilized or not.

'nilgiri'

A total of 27 individuals including adults and pupae from six colonies of two populations were analysed. Cerebral ganglia and hepatic cecae of eight males were dissected and 22 good spreads were obtained. The haploid complement ranged from $n = 5$ to 14, with $n = 5$ as the most common number (figures 4a and 5). A male from Triambakapura population showed variable karyotype having $n = 5, 6$ and 10 in different cells of cerebral ganglia and $n = 5, 6, 9$ and 14 in different cells of the hepatic cecae thus showing intra-tissue as well as inter-tissue variation (table 2, individual 68). Intra-tissue variation was seen in two more males where one of them showed $n = 5$ and 6 chromosomes in cerebral ganglia and the other showed $n = 5$ and 6 chromosomes in different cells of hepatic cecae (table 2, individual 70 and 71). Two males from Mudumalai population showed $n = 7$ and $n = 9$ in the cerebral ganglia tissue. In '*nilgiri*' too, females exhibited greater karyotypic diversity than males. Hundred and eighty-one good spreads were obtained from cerebral ganglia and hepatic

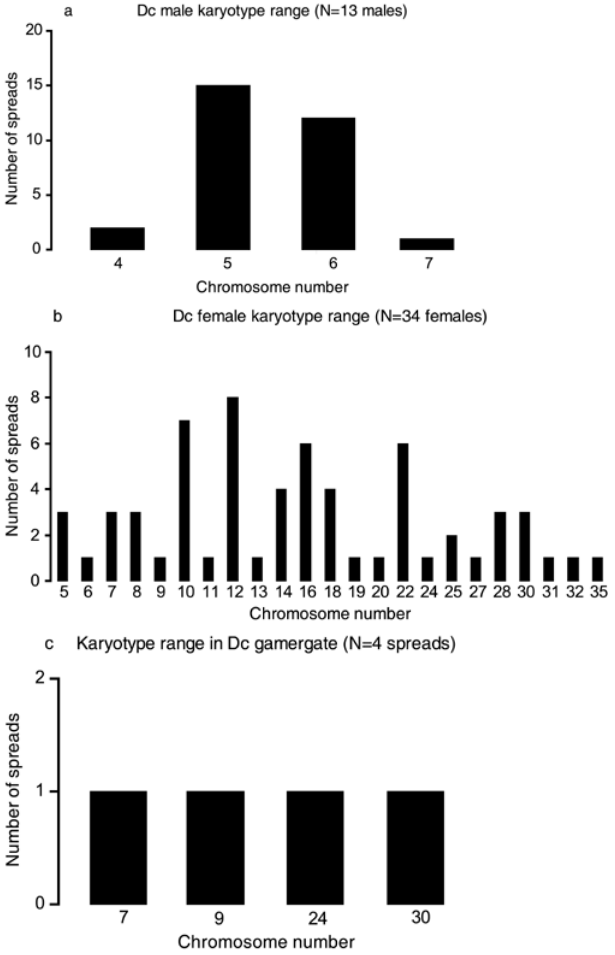

Figure 1. *D. ceylonense*, a profile of range of karyotype variations in (a) males (b) females of all the populations analysed (c) intra-individual variations of karyotype in the ovarian tissue of the gamergate.

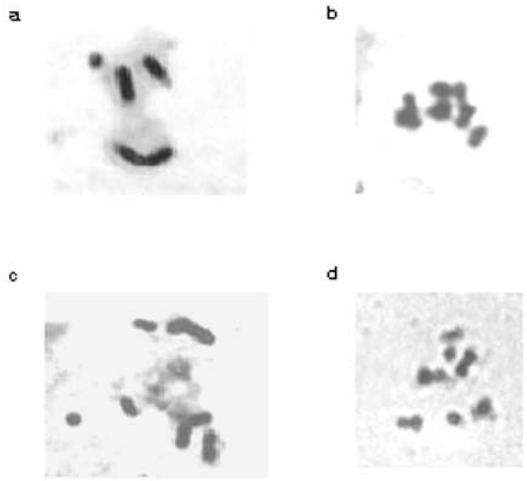

Figure 2. Karyotype variation in *D. ceylonense* males. (a) $n = 4$; (b) $n = 5$; (c) $n = 6$; (d) $n = 7$.

cecae of 19 females. Out of 19, seven were early stage pre-pupae. We assumed that these pre-pupae were females as the

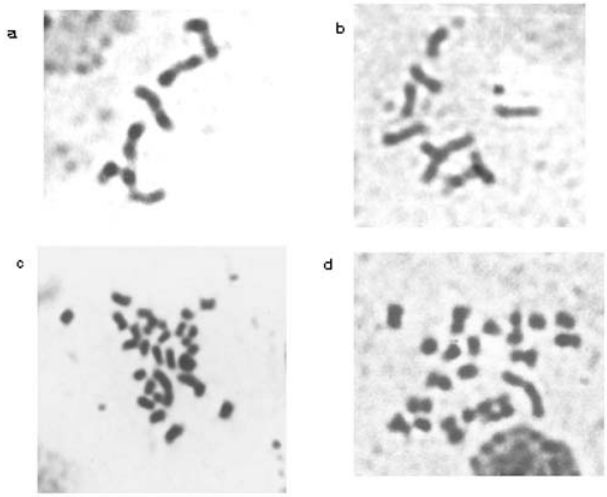

Figure 3. Karyotype variation in *D. ceylonense* females. (a) $2n = 6$; (b) $2n = 10$; c) $2n = 24$; (d) $2n = 22$.

colony was producing mostly females at the time of dissection. The diploid number ranged from $2n = 5$ to 54 with frequent numbers of $2n = 17$ and 30. (figures 4b and 6). We observed four spreads from the ovarian tissue and hepatic cecae of the gamergate from Triambakapura population. Hepatic cecae showed $2n = 22$, whereas ovaries showed 12, 14 and 20 chromosomes (table 2, individual 67). Considering the intra-tissue variation, cerebral ganglia showed more intra-tissue variation within female pupae. For example, one female pupa from Triambakapura population showed $2n = 5, 9, 15, 16, 31, 33$ and 34 in different cells of cerebral ganglia (table 2, individual 58; figure 4c). The nature of variation was similar for the Mudumalai population females which showed a range of $2n = 8$–33. We could not analyse the eggs for any '*nilgiri*' population.

Diacamma indicum

Nine adults and pupae from three colonies of a single population were examined. In contrast to *D. ceylonense* and '*nilgiri*', all the individuals in this species had a constant karyotype with $n = 7$ in males and $2n = 14$ in females, in all the tissues examined. The complement consists of five metacentric, one submetacentric and one dot chromosome(s) (table 2, individuals 83–91; figure 7).

Discussion

The chromosome number is considered to be an important and invariant feature of every species and therefore plays an important role in taxonomic and phylogenetic studies. Significant variations in chromosome number involving standard members of the karyotype are rare. However, there are some notable exceptions. For example, studies on grasshoppers and locusts have revealed polysomy in the male germ line due to one or more members of the karyotype (Lewis and John 1959; Sharma *et al*. 1965; Hewitt and John 1968; Gosalvez and Lopez-Fernandez 1981; Peters 1981; Viseras and Camacho 1982; Talavera *et al*. 1990; Channaveerappa 1996). In *Gastrimargus africanus orientalis*, male germ-line

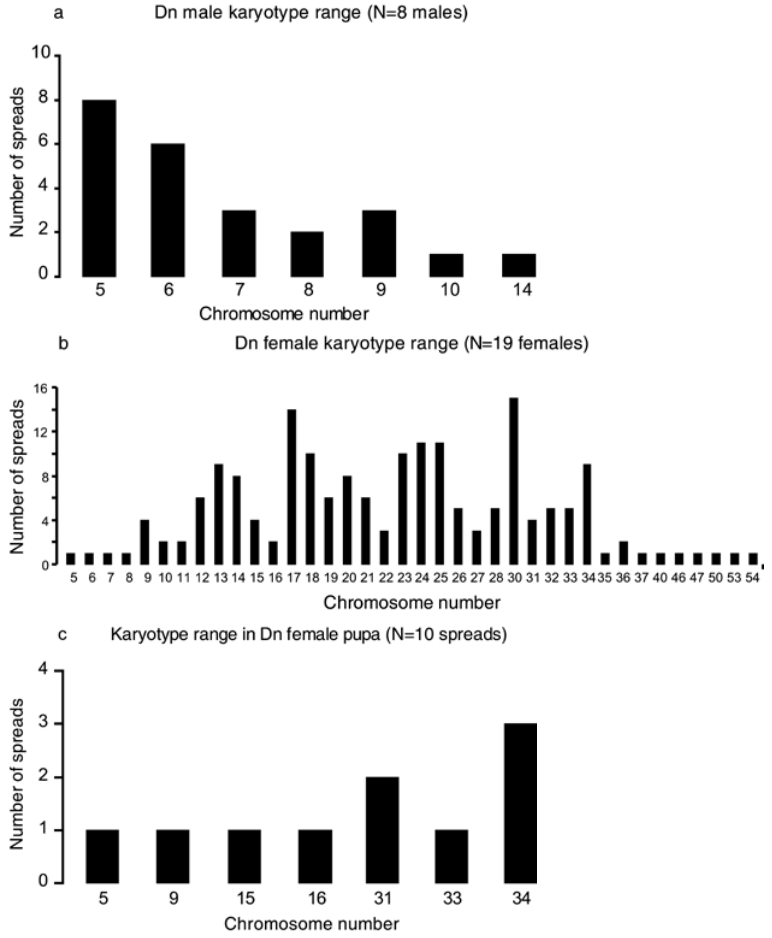

Figure 4. *'nilgiri'* - a profile of range of karyotype variations in (a) males, and (b) females, of all the populations analysed; (c) intra-individual variations of karyotype in the cerebral ganglia tissue of a female pupa.

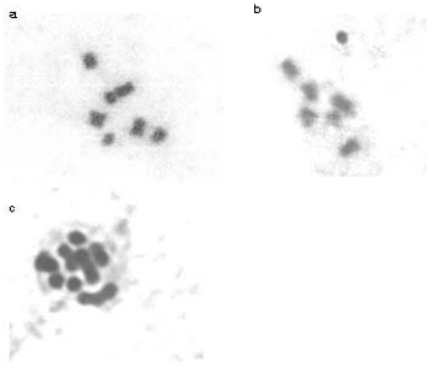

Figure 5. Karyotype variation in *'nilgiri* males (a) $n = 6$; (b) $n = 7$; (c) $n = 8$.

karyotypic mosaicism was not only due to extra representation but also due to loss of some of the chromosomes (Channaveerappa and Ranganath 1997). Imai *et al*. (1977, 1994) and Crosland and Crozier (1986) have found karyotypic variability within and among *Myrmecia pilosula* sibling species complex with chromosome numbers ranging from $2n = 2$ to 32.

In this study we have uncovered significant karyotypic variability in *D. ceylonense* and '*nilgiri*', representing a fairly extreme level of karyotypic mosaicism, with variation within a tissue and among tissues of an individual. Imai *et al*. (1988) has suggested a number of mechanisms that can bring about spontaneous changes in chromosome numbers. However, it is difficult to see how the mechanisms suggested by Imai *et al*. (1988); Imai (1986) can give rise to the observed variability unless one imagines these mechanisms to operate repeatedly in every individual and every cell division.

Another mechanism that could generate karyotypic variability is inter-species hybridization although this can only account for inter-individual variation and not intra-individual variation. For example, extensive inter-individual karyotypic diversity is observed in laboratory hybrid populations of *Drosophila nasuta* and *Drosophila albomicans*. In some populations, over a period of time, the karyotypic polymorphism disappeared and was replaced by a stable karyotype, thus forming cytoraces (Tanuja *et al*. 1999; Ranganath 2002; Ranganath and Aruna 2003). Similarly, McAllister (2002) reported chromosomal variation in the form of a cline in the

Table 3. Expected haploid number of chromosomes in sperms and eggs as well as possible diploid number of chromosomes in adults.

D. ceylonense

Eggs→	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	24	26
Sperms↓	Diploid number in adults																		
4	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	28	30
5	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	29	31
6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	30	32
7	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	31	33

'nilgiri'

Eggs→	5	6	7	8	9	10	11	12	13	14	15	16	17	23
Sperms↓	Diploid number in adults													
5	10	11	12	13	14	15	16	17	18	19	20	21	22	28
6	11	12	13	14	15	16	17	18	19	20	21	22	23	29
7	12	13	14	15	16	17	18	19	20	21	22	23	24	30
8	13	14	15	16	17	18	19	20	21	22	23	24	25	31
9	14	15	16	17	18	19	20	21	22	23	24	25	26	32
10	15	16	17	18	19	20	21	22	23	24	25	26	27	33
14	19	20	21	22	23	24	25	26	27	28	29	30	31	37

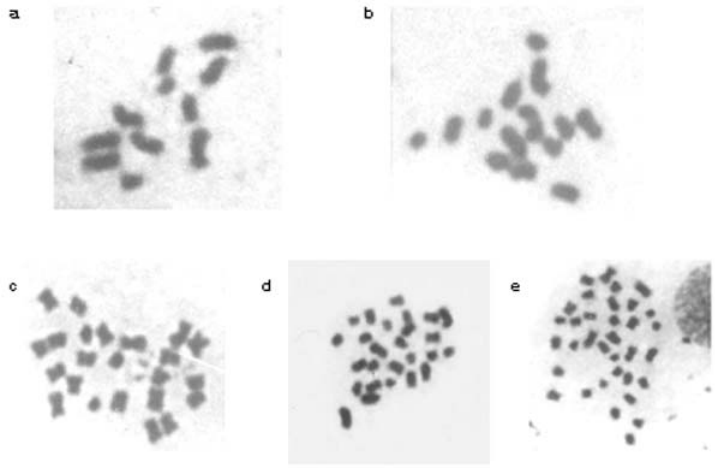

Figure 6. Karyotype variation in *'nilgiri'* females. (a) $2n = 11$; (b) $2n = 14$; (c) $2n = 22$; (d) $2n = 26$; (e) $2n = 34$.

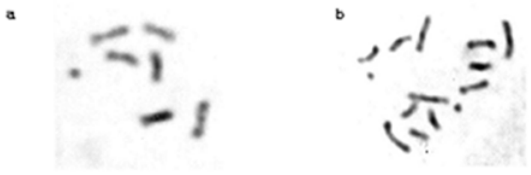

Figure 7. *Diacamma indicum* standard male and female karyotypes (a) $n = 7$; (b) $2n = 14$.

naturally occurring hybrid zone of *Drosophila americana americana* and *Drosophila americana texana*. Such karyotypic instability is also seen in the hybrid zones of grasshoppers, mammals and birds (Hewitt and Barton 1980). Therefore, one of the ways of accounting for the observed inter-individual chromosomal diversity in *Diacamma* populations under study is by considering each of the population as an assemblage of hybrid individuals. Though we have not found a single colony where both *D. ceylonense* and '*nilgiri*' co-existed, long-range hybridization studies between these two ants are yet to be made. Otherwise, one may look for a 'hybrid zone' in nature to explore the possibilities of introgression.

The observed numerical variability, both at intra-individual and inter-individual levels, does not appear to have hampered the sustainability of the chromosomal diversity in each population under study. This may be because the chromosomes may have 'minimum interactions' during prophase of meiosis (Imai *et al*. 1986; Imai 1986; Imai *et al*. 1999, 2001). In spite of the karyotype mosaicism, the fertility of the individuals is not affected. Colonies breed in field as well as in laboratory condition which suggests some kind of buffering mechanism to take care of the karyotype noise.

Given the different observed haploid and diploid numbers and inferring the karyotypes of the eggs and sperm, we can theoretically examine the possibilities for the origin of different karyotypes in different individuals. Since there is variation within ovary and testis, we may get more than 10 types of eggs and 5 types of sperms in each species. Table 3 predicts the expected diploid number of chromosomes in different individuals of a population with a possibility of 26 and 25 types for *D. ceylonense* and '*nilgiri*', respectively. But in the present investigation, we have not recovered karyotypes of all these expected theoretical numbers. It could be due to the small sample size, or all the karyotypes in eggs may not be viable. In this study, we have seen that for *D. ceylonense*, $n = 5, 6$ and $2n = 10$ and 12 are the more frequent karyotypes and for '*nilgiri*', $n = 5$ and 6 and $2n = 17$ and 30 are the frequent karyotypes. However, we cannot treat them as the standard karyotypes. Most of the individuals did not show a consistent karyotype. This would suggest extensive inter-individual variability but cannot account for intra-individual variability, which could be due to mitotic instability, chromosomes rearrangements and minimum interactions among chromosomes. As we did not get the so-called 'standard karyotype' and standard variation in any individual, it is difficult to count the chromosome arm number in this case. We did not find the presence of B-chromosome in any individual. Also, thinking of the possibility of intracellular symbionts, they may be present in few individuals but it is difficult to imagine their presence in all the individuals of the population.

It is also unlikely that the observed variability is an artifact of our experimental procedures, because we have taken the precaution of including *D. indicum* in our study and this species displays a consistent karyotype of $n = 7$ and $2n = 14$ with no intra-individual or inter-individual variability whatsoever.

As of now, it is premature to decide about the exact relation between *D. ceylonense* and '*nilgiri*' either as two different species or as subspecies. As discussed earlier, long range hybridization studies will be necessary to determine the species status of '*nilgiri*'. It could also clarify if the behavioural difference raises enough barriers for reproductive isolation.

This preliminary data is so exciting that further study with molecular probes is required to analyse the fate of each and every chromosome particularly during mitosis and meiosis. Although no known mechanisms can account for the observed intra-individual and inter-individual karyotypic variability in *D. ceylonense* and '*nilgiri*', we believe that this pair of closely related ant populations would provide opportunities for exciting new discoveries concerning the origin, maintenance and significance of intra-individual and inter-individual karyotypic mosaicism.

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