For Phil Ward, with our thanks and best wishes
Ross

Chromosoma (Berl.) 59, 341 – 393 (1977)

CHROMOSOMA

Karyotype Evolution in Australian Ants

Hirotami T. Imai[1], Ross H. Crozier[2], and Robert W. Taylor[3]

[1] Department of Cytogenetics, National Institute of Genetics, Misima, Shizuoka-ken, 411 Japan
[2] School of Zoology, University of New South Wales, Kensington, N.S.W., 2033 Australia
[3] Division of Entomology, C.S.I.R.O., Canberra, A.C.T., 2601 Australia

Abstract. 105 Australian ant species, including members of the important primitive genera *Amblyopone* and *Myrmecia,* were karyotyped using a C-banding air-drying technique. The observed haploid numbers in this survey ranged from 2n=84 (the highest known in the Hymenoptera) to 2n=9. Seven types of chromosome rearrangement were detected, namely: Robertsonian rearrangements, pericentric inversions, saltatory changes in constitutive heterochromatin, simple reciprocal translocations, complex translocations accompanied by significant loss of euchromatin, supernumerary (B-) chromosome variation, and chromosome deletion. Most ant karyotype evolution is explicable in terms of the first three of these. No evidence was found for polyploidy or centric dissociation being of evolutionary significance in ants. The C-band analysis supports a model in which pericentric inversions converting acrocentrics to other types greatly predominate over those with reverse effects. There appears to be little, if any, correlation between whether a species is morphologically primitive or advanced and its karyotype organization. The data provide little support for the ancestral chromosome number in ants having been high with subsequent reduction ("fusion hypothesis"), but rather suggest that the ancestral number was either very low with subsequent increase ("fission hypothesis") or coincident with the present mode ("modal hypothesis"). Moreover, for these ant data, the modal hypothesis is interpretable as a subset of the fission hypothesis.

Introduction

With 185 ant species karyotyped during the last 15 years, the family Formicidae is now one of the cytologically best-known in the Hymenoptera (for a general review, see Crozier, 1975). Most previous work was done on Northern Hemisphere species, and only 31 Southern Hemisphere species have been studied – 24 of them Australian. Australia has a particularly large and diverse ant fauna, which Brown and Taylor (1970) estimated to include some 1,100 species, an

estimate now known to be low (R.W. Taylor, unpublished). Although this fauna was derived from the Old World tropics, some of the most primitive living genera, such as *Myrmecia* and *Amblyopone*, remain prominent elements of the Australian ant biota, which differs substantially from that of the Northern Hemisphere due to the long-term isolation of the southern continent. Chromosomal observations of Australian ants are thus likely to be of great importance in advancing our knowledge of the family, and especially of its karyotype evolution.

This paper describes the results of a survey of 105 Australian ant karyotypes using an improved air-drying technique allowing C-banding. In the light of these observations we also discuss karyotype evolution in ants and comment on how our findings relate to karyotype evolution in general.

Materials and Methods

Biological Materials. Ants were collected from the 32 localities listed in Table 1. With a single exception in Papua New Guinea, all these localities are in the Australian states of New South Wales, Queensland, and South Australia, and all collections were made during 1975. Where it is necessary to refer to localities, we do so using the code numbers given in Table 1.

We cultured winter-collected colonies for one to three months in plastic containers in the laboratory until stages suitable for cytological observation appeared among the brood. The cultivation time was one or two weeks in spring. In summer, preparations from most colonies were made either on the day they were collected or the day after. Preparations were made in the field immediately after collection for some series (AAGW, AAGX) using short-term colchicine treatment. No chromosomal aberrations or other evidence of cytologically-deleterious effect were seen in preparations from the longer-term cultures.

Chromosome Preparation Technique. Most preparations were made from the cerebral ganglia of worker prepupae; others involved the gonads of early male and queen pupae. We used an improved air-drying technique incorporating elements of a previously-used air-drying technique (Crozier, 1968a, and in press) and the improved squash technique developed by Imai and Kubota (1972; Imai, 1974). The new technique, although simple enough to allow making chromosome preparations in the field, yields high-quality metaphases and results in C-banding without subsequent treatment.

The steps in our technique are: (1) Dissect out required organs in colchicine-hypotonic solution (0.005% w/v colchicine in 1% sodium citrate solution) on a cavity slide and remove as much as possible of the fat body, tracheae and epithelial membranes. For species with a low mitotic index, from four to fifteen hours colchicine-pretreatment (Crozier, 1970a, and in press) increases the number of metaphases suitable for standard analysis of chromosome morphology but this long treatment period also leads to the chromosomes being generally too condensed for C-band analysis. (2) Transfer the organs to fresh colchicine-hypotonic solution on another depression slide using a Pasteur pipet and leave for 20 min at room temperature. (3) Transfer the material onto a freshly wiped pre-cleaned slide (washed in detergent solution, rinsed in distilled water, and stored in absolute ethanol) using the Pasteur pipet. With one end of the slide on a damp absorbent plastic sponge, tilt it so as to drain off most of the hypotonic solution, and draw off as much as possible of the drop around the organ(s) using a dissecting needle. (4) Incline the slide at 10–13° onto the sponge and apply several drops of freshly-prepared Fixative I (60% acetic-ethanol, i.e., 3:3:4, glacial acetic acid: absolute ethanol: distilled water) so that the fixative flows over the organ(s) and drains off the end of the slide. (5) Place the slide under a dissecting microscope and add two further drops of Fixative I directly onto the material. After a few seconds, macerate the organs as completely and quickly as possible using dissecting needles. This maceration dissociates the tissues into single cells and clumps of cells. (6) Immediately (i.e., before the cell suspension dries) add two drops of freshly-prepared Fixative II (absolute acetic-ethanol, i.e., 1:1, glacial acetic

Table 1. Localities and codes of Australian ants used in this study. Collection data: each code (e.g., AAFU) refers to colonies collected at the same locality at the same time. All collections were made in 1975. Unless otherwise indicated, localities are Australian

Code(s)	Locality
AAFU	Kanangaroo State For., nr Jenolan Caves, N.S.W.
AAFZ, AAGB	Smith's Lake Field Sta. environs, nr. Bulahdelah, N.S.W.
AAGA	Ferny Ck., Bachelor State For., nr. Bulahdelah, N.S.W.
AAGC, AAGR, AAGT	Peter Meadows Rd. at ck. nr. Hanson's Rd., Leumeah, N.S.W.
AAGD	Blaxland, nr. Penrith, N.S.W.
AAGE, AAGF, AAGG	Lady Carrington Dr., Royal Nat. Pk., N.S.W.
AAGH, AAHM	Nr. "Picadilly Circus", A.C.T.
AAGI	Nr. Cotter Dam, A.C.T.
AAGJ	Black Mountain Reserve, A.C.T.
AAGK	1.5 km SW of Sanderston, nr. Adelaide, S.A.
AAGL	1.5 km NW of Kapana, nr. Adelaide, S.A.
AAGM	7 km SE of Sedan, nr. Adelaide, S.A.
AAGN	Innes Nat. Pk., SW York Penn., S.A.
AAGO	Daly Head, SW York Penn, S.A.
AAGP	Bulldog Rd., 7 km S Edie Ck., Papua New Guinea
AAGS	Wakehurst Parkway, French's Forest, N.S.W.
AAGU	Silver City H'Way at Darling R. Ana Branch, N.S.W.
AAGW	15.4 km N Coombah Roadhouse, S. of Broken Hill, N.S.W.
AAGX	11.8 km S Coombah Roadhouse, S. of Broken Hill, N.S.W.
AAGZ, AAHA, AAHD, AAHE, AAHF, AAHK	Fowler's Gap. Res. Stat., N. of Broken Hill, N.S.W.
AAHB	8 km E of Florida, E. of Cobar, N.S.W.
AAHC	Vittoria, W. of Bathurst, N.S.W.
AAHI	Stephen's Ck., N. of Broken Hill, N.S.W.
AAHJ	Mt. Keira, N.S.W.
RWT75-143, -146	Curtain Figtee rainfor., nr. Yungaburra, N.Q.
RWT75-162, -165, -166, -167	Lake Eacham Nat. Pk., N.Q.
RWT75-185	Dalrymple Heights, nr. Mackay, N.Q.
RWT75-147, -148	Mt. Lewis, nr. Julatten, N.Q.
RWT75-183, -184	Eungella Nat. Pk., nr. Mackay, N.Q.

acid: absolute ethanol). After 15–30 sec, drain most of the Fixative II and remaining Fixative I by inclining the slide *laterally* (with a long edge bottom-most) onto the sponge. (7) Immediately (i.e., before the preparation dries) add two drops of Fixative III (glacial acetic acid) and after about 10 sec again drain off most fixative by inclining the slide *laterally*. Then place the slide horizontally and allow it to dry completely.

The preparations were stained, after having dried for at least one day, using freshly-prepared Giemsa solution (Merck solution diluted 1:24 in M/15 Sørensen's pH 6.8 buffer: KH_2PO_4 4.54 g/l, Na_2HPO_4 4.75 g/l) for ten min at room temperature. After staining, each slide was rinsed for about one second (two washing strokes) in running tap water and then drained. No coverslip need be mounted.

Chromosome Nomenclature. For brevity, we use the following abbreviations: metacentrics (M), submetacentrics (SM), subtelocentrics (ST), acrocentrics (A), telocentrics (T), and minute dots (m – either acrocentric or telocentric). An objective classification of chromosomes into three groups, "T", "A" and "M, SM or ST", is now possible based on the non-random localization of the centromere (Imai, 1976). For mammals, Imai (1976) finds that when the centromere position is indicated by the weight of the short arm (S_w) given as a percentage of the weight of the total

haploid set, the three above groups are definable as having, respectively, $S_w < 0.1$, $0.1 \leqq S_w \leqq 0.6$ and $S_w > 0.6$. However this new system cannot yet be applied to ants because insufficient measurements are available to identify the S_w values distinguishing the three chromosome types in this group. In the absence of this information, we rely on intuitive criteria and note that over 90% of "acrocentric" and "meta-, submeta- and subtelocentrics" so classified by mammalian cytogeneticists fall into two categories, one "M, SM or ST" as above, and the other combining Imai's (1976) "A" and "T" groupings (Imai, 1973), giving us some confidence in intuitive classifications. While differentiation of Imai's (1976) third category into M, SM and ST types does not reflect any basic difference in chromosome organization with respect to centromere position, it is useful for classifying chromosomes, and we have been guided by the criteria of Levan et al. (1964) in making such distinctions. We have relied mostly on judgement in distinguishing A from T chromosomes, according to short arm length. Our classification in this paper is thus hopefully a transitional one, to be superseded in future when the critical values of S_w have been worked out.

Species Studied; Identification and Deposition of Specimens. The colonies examined of each species in this study are listed in Table 2, along with the locality code, cytological details, and figure references for each. Unless otherwise stated, all were identified by R.W.T.

Many of the species involved cannot presently be determined using conventional scientific names. In such cases, they have been identified to genus, and the species within each genus assigned numbers followed by the letters ANIC [e.g., *Prolasius sp. 1* (ANIC)]. The resulting codes each refer to a putative biological species, as represented in the Australian National Insect Collection (ANIC). This procedure follows a formal numbering system, developed by R.W.T., which provides ant species in the ANIC with provisional but constant "handles" in lieu of formal names, until such time as names can be assigned to them. The system operates to allow any currently unnameable species, which might be encountered by different authors in separate studies, to receive the same code designation in all publications referring to it. Ultimately these publications will include a generic monograph or other paper in which each such species will either be described as new or identified as one already named. This system is necessary because (1) many, if not most, Australian species do not yet possess scientific names, and (2) those species which have been named often cannot be recognized in available collections due to their poor original descriptions, the lack of monographic studies, and inaccessibility of type material. Species encountered by us that can be confidently named have of course been identified by name. Those that seem to be certainly undescribed (and thus unnamed) have been assigned numbers. Piecemeal naming of such species in other than a revisionary context is not worthy of present attention. Voucher specimens from all colonies studied are deposited under the appropriate names or numbers in the ANIC.

Observations

A. Chromosome Polymorphisms

1. Robertsonian Polymorphism

We found Robertsonian polymorphisms in six species: two each in the genera *Rhytidoponera* and *Myrmecia* and one in *Aphaenogaster* and *Sphinctomyrmex* (Fig. 1, Table 2).

In seven colonies of *Rhytidoponera metallica* ("Eastern form", see below) from New South Wales the following chromosome numbers were found in each: 2n=36 (10 M) and 37 (9 M) in AAHI-1 (Fig. 1a), 2n=41 (5 M) and 42 (4 M) in AAGR-12, 2n=42 (4 M) and 43 (3 M) in AAGR-11 (Fig. 1b), 2n=43 (3 M) and 44 (2 M) in AAHB-2, and 2n=46 (0 M) in AAGB-1, AAGB-4 and AAGB-6. The diploid chromosome number and the number of bi-armed chromosomes ranges between 2n=36 (10 M) and 46 (0 M), but the number

Table 2. Chromosome numbers of Australian ants: ♀ denotes a worker ant, ♂ a male, and Q a queen (=♀). ANIC is the Australian National Insect Collection

Taxon	Colony	Chrom. number (n) 2n	Ind. no. obs.	Modal cell no. obs.	Figs.
Myrmecioid complex					
Subfamily Myrmeciinae					
Tribe Myrmeciini					
Myrmecia					
brevinoda	AAGC-3	84	7♀, 2Q	60, 18	4e and 12h
cephalotes	AAGX-5	66	5♀	27	12f
forficata	AAFU-10	50, 51	6♀, 1♀	54; 10	1f
fulvipes	AAHM-1	60	1♀	12	12e
sp. cf. fulvipes	AAGR-14	12	1♀	20	2b and 12b
	AAGT-12	12	1♀	10	
gulosa	AAGR-2	38	8♀	52	
	AAGT-1	38	4♀	36	12d
nigrocincta	AAGC-2	22	1♀	19	
	AAGR-1	22	10♀	86	12c
	AAGT-2	22	6♀	60	2c–k
pilosula	AAHM-2	31, 32	2♀, 1♀	18, 13	5a–c
(=*ruginoda*)	AAGR-13	10	3♀	30	2a and 12a
	AAGT-11	9, 10	2♀	36, 29	9
pyriformis	AAGC-1	(41), 81	2♂, 2Q, 1♀	14, 7, 3	12g
Subfamily Dolichoderinae					
Tribe Dolichoderini					
Dolichoderus					
scabridus	AAGR-3	28	10♀	91	4f
Tribe Leptomyrmecini					
Leptomyrmex					
erythrocephalus	AAHC-1	(12)	6♂	60	13a
Tribe Tapinomini					
Technomyrmex					
albipes	AAGR-8	16	5♀	50	13b
	AAGC-7	16	7♀	84	
Iridomyrmex					
nitidus	AAGR-23	16	7♀	57	13d
purpureus group	AAGK-1	18	1♀	9	
(blue form)	AAGM-8	18	3♀	22	
	AAGW-15	18	4♀	33	13g
(black form)	AAHF-1	18	3♀	16	
? sp. 7 (ANIC)	AAGT-14	22	5♀	31	13k
(?=*Bothriomyrmex ? pusillus*)					
darwinianus group					
sp. 8 (ANIC)	AAGI-2	(7), 14	2♂, 5♀	11, 45	13l
? sp. 9 (ANIC)	AAHF-4	12	5♀	45	13m
itinerans group					
sp. 10 (ANIC)	AAGC-10	16	3♀	20	
	AAGH-6	16	3♀	24	
	AAGH-11	16	3♀	28	13c
gracilis group					
sp. 13 (ANIC)	AAGN-2	18	6♀	31	4b and 13f
sp. 14 (ANIC)	AAGK-2	18	3♀	25	13i

Table 2 (continued)

Taxon	Colony	Chrom. number (n) 2n	Ind. no. obs.	Modal cell no. obs.	Figs.
sp. 15 (ANIC)	AAGJ-9	18	5♀	46	6f
	AAGB-3	18	5♀	50	13j
sp. 16 (ANIC)	AAGW-16	18	6♀	54	13e
sp. 17 (ANIC)	AAGU-3	18	6♀	30	13h
Subfamily Formicinae					
Tribe Melophorini					
Notoncus					
? ectatomoides	AAGH-3	(22), 44	10♂, 3♀	77, 23	14a
Prolasius					
sp. 1 (ANIC)	AAGJ-4	(9), 18	3♂, 4♀	19, 40	14b
sp. 2 (ANIC)	AAGH-4	18	1♀	10	
	AAGI-1	18	5♀	50	14c
Tribe Plagiolepidini					
Stigmacros					
sp. 1 (ANIC)	AAGG-4	38	3♀	22	
	AAGT-10	38	4♀	30	14d
sp. 3 (ANIC)	AAHD-2	(10), 20	3♂, 4Q	30, 20	14e
	AAGG-7	(10)	2♂	24	
Tribe Formicini					
Paratrechina					
sp. 1 (ANIC)	AAGR-9	30	7Q	50	4c and 14f
Tribe Camponotini					
Calomyrmex					
sp. 1 (ANIC)	AAGW-1	28	4♀	18	14g
Camponotus					
consobrinus	AAGS-1	(23), 46	7♂, 1Q	36, 2	16a
sp. 1 (ANIC)	AAGR-7	(23)	5♂	21	
	AAGR-24	(23)	5♂	26	
	AAGT-5	46	4♀	18	16c
sp. 2 (ANIC)	AAGM-5	(23)	2♂	13	16b
sp. 3 (ANIC)	AAFZ-3	48	2♀	28	
	AAGJ-3	48	7♀	39	16e
	AAGR-4	48	6♀	42	
sp. 5 (ANIC)	AAGU-1	32, 64	6♀, 1♀	56, 4	15b
sp. 8 (ANIC)	AAGX-8	32	4♀	26	15c
sp. 9 (ANIC)	AAFZ-5	38	6♀	55	
	AAGJ-2	38	2♀	18	15e
sp. 10 (ANIC)	AAGW-5	46	1♀	7	
	AAGW-17	46	3♀	13	16d
sp. 11 (ANIC)	AAGW-14	32	3♀	20	
	AAHA-3	32	6♀	37	15d
sp. 12 (ANIC)	AAGM-3	38	2♀	10	15f
sp. 13 (ANIC)	AAFZ-4	(10)	1♂	17	
	AAFZ-6	20	2♂	19	
	AAGD-1	20	10♀	80	15a
	AAGR-19	20	6♀	46	
	AAGT-15	20	3♀	28	
sp. 14 (ANIC)	AAGC-8	(19), 38	1♂, 2♀	16, 27	15g
	AAGW-9	38	5♀	25	15h

Table 2 (continued)

Taxon	Colony	Chrom. number (n) 2n	Ind. no. obs.	Modal cell no. obs.	Figs.
Opisthopsis					
rufithorax	AAGW-13	50	5♀	27	14h
Polyrhachis					
sp. 1 (ANIC)	AAGW-10	42	2♀	13	14i
ammon	AAGT-3	(21), 42	2♂, 1♀	20, 3	14j
	AAGT-4	(21)	7♂	43	
Poneroid complex					
Subfamily Ponerinae					
Tribe Amblyoponini					
Amblyopone					
cf. fortis	RWT75-162	44	8♀	41	17a
australis	RWT75-185	(24)	6♂	33	4a
	AAGH-1	48	11♀	41	17b
	AAGH-2	48	8♀	35	
	AAFU-13	48	1♀	10	
Tribe Ectatommini					
Heteroponera					
relicta	RWT75-166	(11), 22	2♂, 4♀	20, 38	17c
Rhytidoponera					
chalybaea	AAHJ-2	42	4♀	31	17d
impressa	AAGA-2	42	4♀	52	17e
metallica					
(Western form I)	AAGW-6	22, 23, 24	1♀, 1♀, 1♀	9, 10, 10	8b–d
(Western form II)	AAGL-1	24	9♀	72	
	AAGM-1	24	6♀	60	8a
(Eastern form)	AAHI-1	36, 37	4♀, 1♀	18, 4	1a
	AAGR-11	42, 43	4♀, 2♀	18, 19	1b
	AAGR-12	41, 42	3♀, 3♀	21, 14	
	AAHB-2	43, 44	2♀, 2♀	5, 11	
	AAGB-1	46	1♀	46	
	AAGB-4	46	5♀	37	17f
	AAGB-6	46	5♀	42	
purpurea	RWT75-167	38	7♀	38	17g
victoriae	AAGH-5	42	3♀	20	
maniae	AAHK-1	39, 44	1♀, 2♀	4, 14	1c, d
	AAGX-2	44, 45, 46	1♀, 2♀, 1♀	8, 6, 1	
	AAGW-12	45, 47, 48	1♀, 5♀, 2♀	6, 28, 18	
mayri	AAGM-11	50	2♀	16	17h
	AAGX-6	50	9♀	52	
	AAHF-2	50	4♀	37	
aciculata	AAHB-3	52	5♀	29	17i
Tribe Ponerini					
Hypoponera					
sp. 1 (ANIC)	AAGH-9	(19), 38	1♂, 2♀	8, 11	18a
sp. 2 (ANIC)	RWT75-148	38	5♀	23	18b
Bothroponera					
sp. 2 (ANIC)	AAHA-5	60	2♀	16	5f
Brachyponera					
lutea	AAGW-4	16	2♀	20	18c

Table 2 (continued)

Taxon	Colony	Chrom. number (n) 2n	Ind. no. obs.	Modal cell no. obs.	Figs.
Cryptopone					
? rotundiceps	RWT75-147	12	8♀	78	18d
Tribe Odontomachini					
Odontomachus					
sp. 1 (ANIC)	AAGX-7	44	3♀	9	
	AAGX-1	44	2♀	12	18e
Tribe Sphinctomyrmecini					
Sphinctomyrmex					
steinheili	AAGT-13	45, 46	1♀, 4♀	2, 15	
	AAGT-16	46	8♀	31	18f
Tribe Cerapachyini					
Cerapachys					
brevis	AAGZ-1	(23), 46	2♂, 3♀	15, 14	18g
Subfamily Myrmicinae					
Tribe Myrmicini (broad sense)					
Aphaenogaster					
longiceps	AAGC-5	46	4♀	21	
	AAGF-5	45,46	4♀, 4♀	29,30	1e
Monomorium					
sp. 1 (ANIC)	AAHD-3	22	5♀	29	
	AAGM-4	22	6♀	58	20a
sp. 2 (ANIC)	AAGI-3	42	4♀	32	4d and 20b
Pheidole					
sp. 20 (ANIC)	AAGE-3	20	2♀	14	19a
sp. 21 (ANIC)	AAGH-7	20	7♀	54	19b
sp. 22 (ANIC)	AAGJ-7	20	2♀	20	19c
sp. 23 (ANIC)	AAGL-2	20	7♀	70	19d
	AAGL-3	20	7♀	67	
sp. 24 (ANIC)	AAGL-4	18	5♀	49	19e
sp. 25 (ANIC)	AAGL-5	20	7♀	49	19f
sp. 26 (ANIC)	AAGO-1	20	4♀	37	19g
sp. 27 (ANIC)	AAGT-9	20	5♀	44	19h
sp. 28 (ANIC)	AAGT-17	20	5♀	45	19i
sp. 29 (ANIC)	AAHD-1	20	6♀	46	6g
sp. 30 (ANIC)	AAGW-18	20	6♀	30	6h
sp. 31 (ANIC)	RWT75-146	20	4♀	24	19j
sp. 32 (ANIC)	RWT75-183	20	7♀	60	19k
Oligomyrmex					
sp. 6 (ANIC)	RWT75-184	38	3♀	17	20c
Podomyrma					
adelaidae	AAGW-3	49, 50, 51	3♀, 2♀, 1♀	13, 13, 1	10
Vollenhovia					
sp. 3 (ANIC)	AAGP-1	40	9♀	52	5d, e
Chelaner					
rothsteini	AAGU-2	22	4♀	40	
	AAGW-2	22	10♀	85	6a, b
	AAGW-8	22	5♀	46	
	AAHE-2	22	3♀	28	6c

Table 2 (continued)

Taxon	Colony	Chrom. number (n) 2n	Ind. no. obs.	Modal cell no. obs.	Figs.
whitei	AAHA-2	24	2♀	20	20d
sp. 1 (ANIC)	AAGH-10	32	7♀	50	6d
sp. 2 (ANIC)	AAGJ-6	22	5♀	46	20e
Xiphomyrmex					
sp. 2 (ANIC)	AAHE-1	18	6♀	58	20f
sp. 3 (ANIC)	AAGR-5	20	5♀	45	
	AAGR-6	20	5♀	48	20g
sp. 4 (ANIC)	AAGT-6	18	4♀	34	20h
	AAGT-7	18	5♀	39	
Tribe Meranoplini					
Meranoplus					
minor	AAGA-3	22	7♀	62	20i
sp. 4 (ANIC)	AAGR-22	22	5♀	47	20j
sp. 5 (ANIC)	AAGW-7	22	8♀	67	21a
Mayriella					
abstinens	AAGH-8	18	8♀	80	21b
Tribe Crematogastrini					
Crematogaster					
sp. 1 (ANIC)	AAGT-8	(12), 24	4♂, 2Q	35, 18	
	AAGR-10	24	6♀	44	6e and 21c
sp. 2 (ANIC)	AAGM-6	26, 39	3♀, 1♀	22, 4	21d
Tribe Dacetini					
Strumigenys					
friedae	RWT75-165	24	5♀	32	21e
Colobostruma					
sp. 1 (ANIC)	AAGF-2	22	8♀	62	21f
Orectognathus					
versicolor	AAGA-1	(11)	4♂	42	
	AAGF-1	22	3♀	34	21g
darlingtoni Taylor (n. sp.)					
	RWT75-143	(11), 22	1♂, 10♀	10, 87	21h

of major chromosome arms in diploid karyotypes is always 46 (i.e., the *nombre fondamentale* is 46). Similar observations (n=17–22, 2n=41–43) have been previously reported (Crozier, 1969) for colonies from Victoria. We therefore conclude that at least the six largest chromosome pairs are involved in the *metallica* Robertsonian polymorphism.

Another Robertsonian series was found in the congeneric *Rhytidoponera maniae*. The following karyotypes were seen in the three colonies studied: 2n=39 (11 M) and 44 (6 M) in AAHK-1 (Figs. 1c and 1d), 2n=44 (6 M), 45 (5 M) and 46 (4 M) in AAGX-2, and 2n=45 (5 M), 47 (3 M) and 48 (2 M) in AAGW-12. Although both *metallica* and *maniae* have the six largest pairs of chromosomes involved in Robertsonian polymorphism, the polymorphisms in the two species have clearly arisen independently because the two *nombres fondamentales*

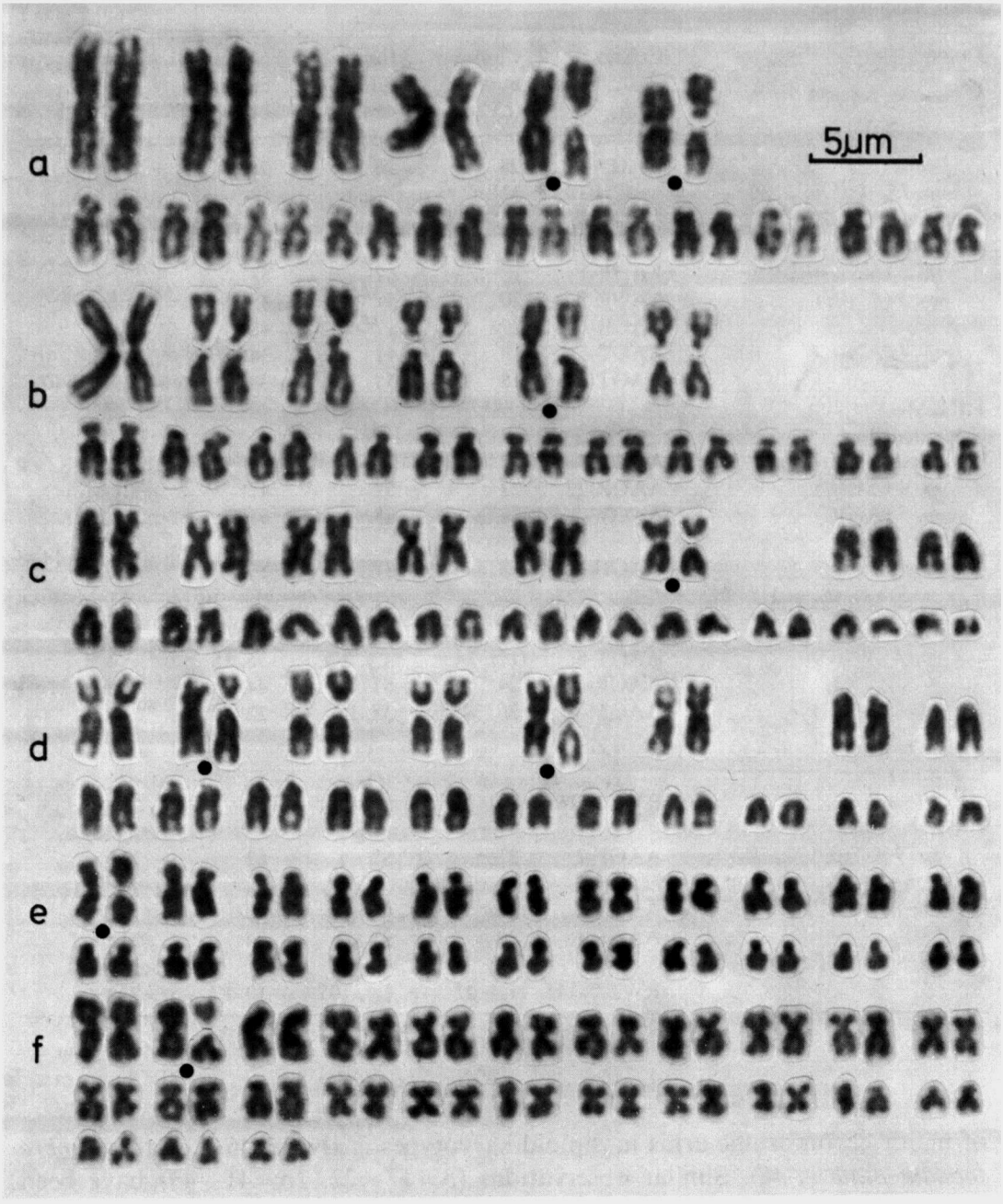

Fig. 1a–f. Karyotypes with Robertsonian polymorphisms. Heterozygous pairs for this rearrangement are indicated by solid circles. *Rhytidoponera metallica* **a** 2n = 36 (10M) heterozygous for pairs 5 and 6, and **b** 43 (3M) heterozygous for pair 5. *Rhytidoponera maniae* **c** 2n = 39 (11M) heterozygous for pair 6, and **d** 44 (6M) heterozygous for two pairs. *Aphaenogaster longiceps* **e** 2n = 45 heterozygous for one pair, and *Myrmecia forficata* **f** 2n = 51 heterozygous for one pair

differ (N.F. = 50 for *maniae* but 46 for *metallica*). Moreover, on morphological criteria, the two species are clearly not closely related within the genus.

While all four workers in one *Aphaenogaster longiceps* colony (AAGC-5) had 2n = 46, those from another (AAGF-5) had either 2n = 45 or 46. In 45-

chromosome karyotypes, the largest metacentric is single and there are two telocentrics corresponding to its two arms; this polymorphism is therefore Robertsonian (Fig. 1e). Robertsonian polymorphism involving a single pair was also found in a colony (AAFU-10) of *Myrmecia forficata.* Here the metacentric involved is the second-largest in size. Six individuals were found with 2n=51 and one with 2n=50 (Fig. 1f).

Myrmecia pyriformis (AAGC-1) and *Sphinctomyrmex steinheili* (AAGT-13, AAGT-16) may also have Robertsonian polymorphisms. In *M. pyriformis,* two males had n=41 (Fig. 12g) and three workers 2n=81, while in one colony of *S. steinheili* diploids with 2n=45 and 46 were found. While our photographic record for these two species is not as complete as we would wish, that the odd-numbered karyotypes had one M or SM chromosome in each case, suggests that the numerical polymorphism is Robertsonian.

Robertsonian polymorphism is also known in the Japanese ant *Pheidole nodus* (2n=37, 38, 39; n=17, 18, 19, 20; Imai and Kubota, 1972, 1975). Thus, seven ant species (2.5%) out of the 280 karyotyped are now known to have Robertsonian polymorphisms. Note that species with such polymorphisms have relatively high chromosome numbers for ants, with n=17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 40 and 41 (ant haploid numbers range from 3 to 42, with the modal number at n=11 and the median at n=15).

2. Pericentric Inversion Polymorphism

A typical example of pericentric inversion polymorphism was observed in chromosome 2 of *Myrmecia pilosula.* This chromosome pair was subtelocentric in colony AAGR-13 (Fig. 12a), but in both of the specimens from AAGT-11 one chromosome was subtelocentric but the other metacentric (Fig. 9). In one colony (AAGM-1) of *Rhytidoponera metallica* (Western form-II), the largest SM chromosome seems to be heterozygous for a pericentric inversion, because the arm ratio is r=1.5 in one homolog but r=2.0 in the other (Fig. 8a). A third example is the smallest chromosome pair of *Monomorium sp. 2,* where one homolog is acrocentric but the other obviously metacentric (Fig. 20b).

The smallest chromosome of *Amblyopone australis* is also polymorphic for a pericentric inversion, as seen in colony AAGH-1 (Fig. 17b). In addition to these examples, inversion polymorphisms are known in the Australian *Iridomyrmex gracilis* (Crozier, 1968b) and the North American *Tapinoma sessile* (Crozier, 1970b). Although rather few pericentric inversion polymorphisms have thus been reported in ants, perhaps partly because of technique limitations, comparative karyotype analysis between related species, as discussed below, reveals that this rearrangement-type has been fixed frequently.

3. Pericentric Inversion Detection via C-banding Analysis

Pericentric inversions are usually detected as polymorphisms, because chromosomes similar in size differ in centromere position. It is therefore difficult to

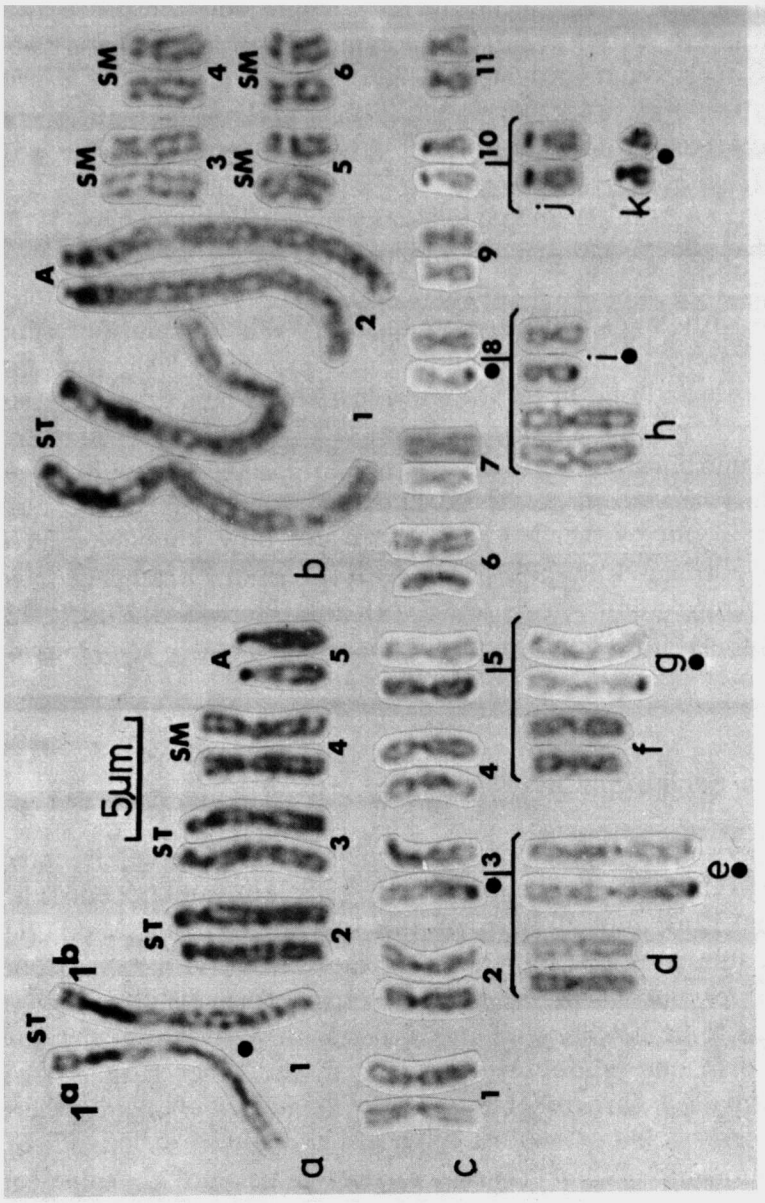

Fig. 2a–k. C-band karyotypes of three *Myrmecia* species. Heterozygous pairs for C-band polymorphism are indicated by solid circles. **a** *M. pilosula*. **b** *M. sp. cf. fulvipes*. **c** *M. nigrocincta*. **d–k** Partial karyotypes of *M. nigrocincta*. **e, g, i** Chromosomes with heterozygous terminal heterochromatin. **j, k** Acrocentrics homozygous (**j**) or heterozygous (**k**) for totally heterochromatic short arms. **d, f, h** Chromosomes negative for terminal heterochromatin

identify this type of rearrangement using only homozygous individuals. This technical difficulty is, however, considerably reduced when C-banding analysis is possible.

Figure 2a–c shows the C-banding patterns observed in three colonies of "jumper" bull-dog ants, *Myrmecia pilosula* (AAGR-13), *M. sp. cf. fulvipes* (AAGR-14) and *M. nigrocincta* (AAGT-2), respectively. Apart from chromosome 1, the AAGR-13 and AAGR-14 karyotypes are very different, with the former having $2n = 6ST + 2M + 2A = 10$ and the latter having $2n = 4ST + 8M = 12$. Chromosome 1, however, is the largest in both species, is of similar size and arm ratio in the two species, and also has a long C-banding positive region in the proximal position of the long arm in both cases. Despite the differences in the other chromosomes, it is tempting to suggest that these marked similarities

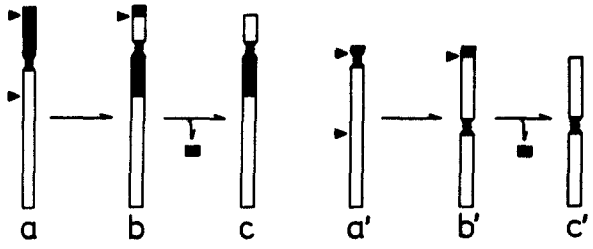

Fig. 3. Schematic representation of pericentric inversion breaking down acrocentrics, p.i. (A or T→M, SM or ST). Black part; C-band positive heterochromatin. White part; euchromatin. Arrow heads; break points assumed. Solid circle; centromere. For details see text

indicate that pair 1 in *pilosula* and *sp. cf. fulvipes* are homologous and derived from a common ancestor. The argument which follows concerning the evolution of banding patterns does not, however, hinge upon this interpretation. There is one small but important difference between chromosome 1 of the two species: in AAGR-13 (Fig. 2a) the short arm is wholly euchromatic (C-banding negative) whereas that of AAGR-14 (Fig. 2b) has a block of terminal heterochromatin. A plausible model for the phylogeny of these two chromosomes is: (1) chromosome 1 of the common ancestor had a long heterochromatic short arm (Fig. 3a), (2) a pericentric inversion (break-points shown in Fig. 3a) converted this hypothetical chromosome into the form shown in Figure 3b, which is that seen in AAGR-14, (3) loss of the terminal heterochromatin resulted in the form seen in AAGR-13 (Fig. 3c). We include an initial, hypothetical chromosome with a wholly heterochromatic short arm in the above scheme in order to illustrate what we suggest is a major pathway of chromosomal change in ants. Our reasons for this suggestion will shortly become apparent.

The polymorphism for terminal heterochromatic segments observable in *M. nigrocincta* represents, we believe, the stage where terminal heterochromatin is being lost to the population. Four chromosome pairs (3, 5, 8 and 10) are C-banding positive in the AAGT-2 karyotype (Fig. 2c). Chromosome 10 is acrocentric, with a wholly-heterochromatic short arm, as is usual for ant acrocentrics. The other three pairs are bi-armed with characteristic C-bands at the tips of the long arms, in some homologs at least. We can denote the presence of C-bands in both homologs as C^{++}, heteromorphism as C^{+-}, and homozygosity for lack of these C-banding positive segments as C^{--}. The frequencies observed for each possible genotype in each chromosome for six nestmates are: Chromosome 3 (0 C^{++}, 3 C^{+-}, 3 C^{--}), chromosome 5 (0 C^{++}, 1 C^{+-}, 5 C^{--}), chromosome 8 (0 C^{++}, 2 C^{+-}, 4 C^{--}) and chromosome 10 (1 C^{++}, 5 C^{+-}, 0 C^{--}). Note that in some chromosomes there has been total loss of terminal heterochromatin from both homologs (Fig. 2d, f and h). A plausible evolutionary pathway linking these chromosomes is illustrated in Figures 3a′, b′ and c′. The initial chromosome is an acrocentric with a small heterochromatic short arm (Fig. 3a′), resembling chromosome 10 (Fig. 2j). Of the two breaks involved in the pericentric inversion (Fig. 3a′), one must be very close to the centromere, as shown, because pericentromeric heterochromatin could not be seen in the resulting chromosomes. The two subsequent steps, shown in Fig. 3b′ and c′, resemble those shown in Figure 3b and c. Chromosomes 2 of

M. pilosula also show terminal heterochromatin at the 3b′ stage (Fig. 2a). We propose that the pathway shown in Figures 3a′, b′ is a major one in ant chromosomal evolution, because bi-armed chromosomes usually have apparently negligible pericentromeric heterochromatin blocks. We note at this stage that our analysis suggests that five *Myrmecia* chromosomes were converted "orthogenetically" from acrocentrics to bi-armed types by pericentric inversions. We denote such pericentric rearrangements as p.i. (A or T→M, SM or ST).

4. C-banding (Constitutive Heterochromatin) Patterns

Figure 4 shows some examples of C-banded ant karyotypes. *Iridomyrmex sp. 13* (Fig. 4b), with a formula of 2n=10 (M or SM)+8A=18, has a typical animal C-banding pattern with all M or SM chromosomes having distinct pericentromeric heterochromatin blocks and all the acrocentrics having totally heterochromatic short arms. In the *Amblyopone australis* karyotype (RWT75-185), however, only 5 (M or SM) chromosomes out of the 24 in the haploid set have well-developed pericentromeric heterochromatin, the remainder being C-band negative (Fig. 4a). Such C-band negative chromosomes were observed in many species in this study. While failure to observe C-bands doubtless arises sometimes from technical limitations, some bi-armed chromosomes are clearly C-band negative, as in *Myrmecia nigrocincta* (AAGT-2, Fig. 2c), *M. sp. cf. fulvipes* (AAGR-14, Fig. 2b) and *Monomorium sp. 2* (Fig. 4d). The short arms of acrocentrics are usually C-banding positive, as in Figures 4c, d, and 2a, but, as mentioned above, chromosome 10 in *Myrmecia nigrocincta* (AAGT-2) is often C-band negative (Fig. 2k). We found remarkable and extreme C-banding patterns in the karyotypes of *Myrmecia brevinoda, Bothroponera sp. 2* and *Dolichoderus scabridus,* with either the long or the short arm of most chromosomes in the first two species being totally heterochromatic (Figs. 4e and 5f). Extraordinarily marked pericentromeric heterochromatin blocks characterize all the chromosomes of *D. scabridus* (Fig. 4f). Those chromosomes with very long C-bands seem best interpreted as having arisen through "growth" of constitutive heterochromatin, such as by tandem gene duplication or else saltatory replication (Britten and Kohne, 1969).

There is ample evidence in our data of rapid change in constitutive heterochromatin in ant chromosomes, and some of it suggests that there can be both rapid duplication and "growth", and deletion. Chromosome 1 of *Myrmecia pilosula* (AAGR-13), as discussed above, has a very long C-band in the proximal region of the long arm (Fig. 2a); this segment in one homolog (denoted chromosome 1^a) is about two-thirds the length it is in the other (denoted chromosome 1^b). Chromosome 1^a is more similar to chromosome 1 in *M. sp. cf. fulvipes* (AAGR-14, Fig. 2b). If we are correct in our suggestion (see above) that these chromosomes in the two species are homologous, then the greater length of this heterochromatic segment in chromosome 1^b indicates duplication rather than deletion as the cause of the polymorphism in *pilosula*. The C-banding patterns of *M. brevinoda* and *M. pyriformis* reinforce our belief that heterochromatin growth can be rapid in ants. These species are morphologically fairly similar, and the euchromatic sections of their karyotypes are also quite similar. *M. brevinoda* has 2n=84, with the long arms of all chromosomes totally het-

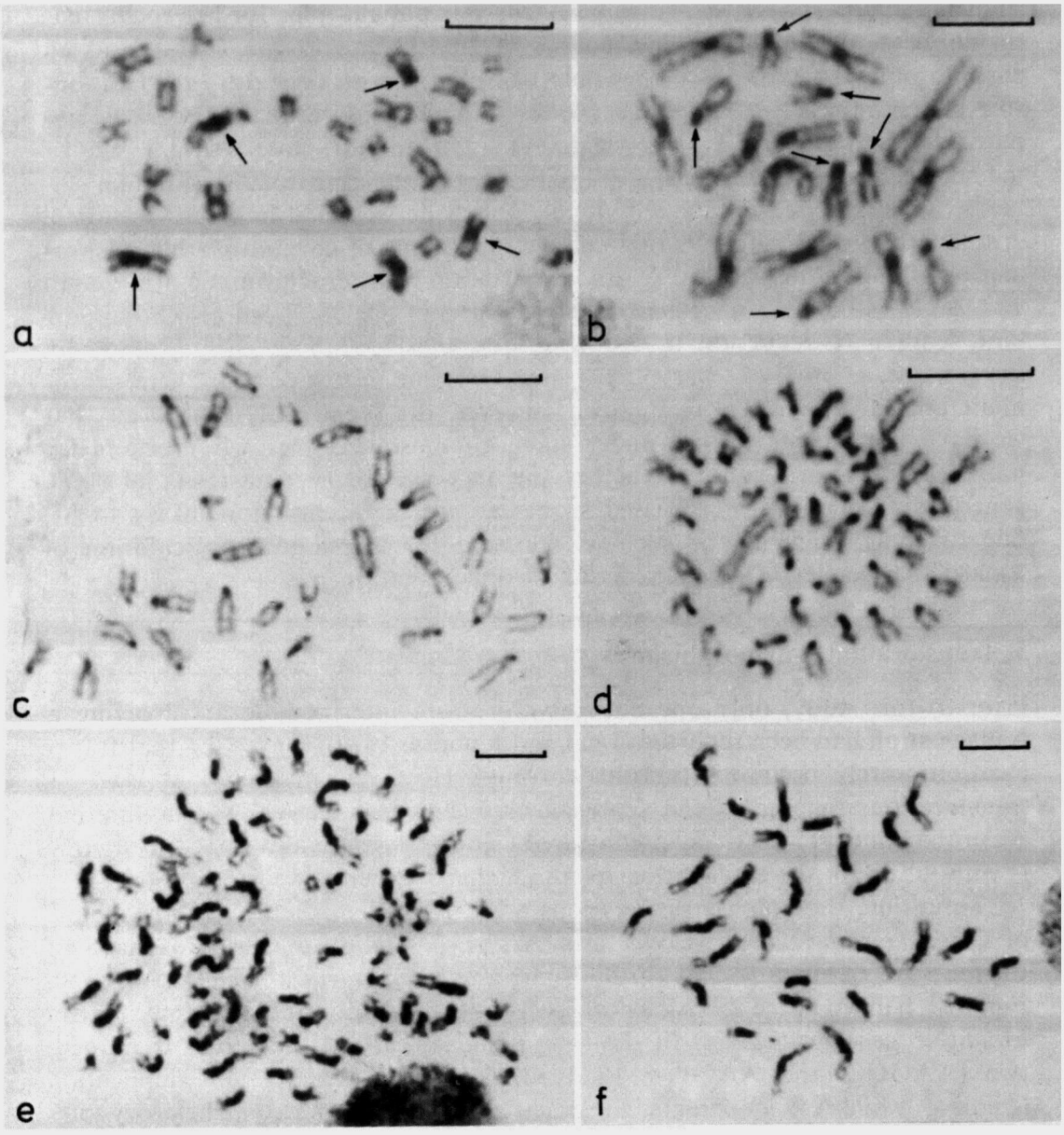

Fig. 4a–f. C-banded metaphases in various ants. Arrows (in **a** and **b**) indicate pericentromeric heterochromatin. **a** *Amblyopone australis* (n=24). **b** *Iridomyrmex sp. 3* (2n=18). **c** *Paratrechina sp. 1* (2n=30). **d** *Monomorium sp. 2* (2n=42). **e** *Myrmecia brevinoda* (2n=84). **f** *Dolichoderus scabridus* (2n=28). Scales are 5 μm

erochromatic (Fig. 12h), whereas in *pyriformis* (n=41) most of the chromosomes are normal ant acrocentrics (Fig. 12g). Because the *brevinoda* karyotype is quite unusual and markedly different in heterochromatic patterns from that of the closest relative to *brevinoda* examined (*pyriformis*), we suggest that the heterochromatic long arms in *brevinoda* developed by "growth" after the separation of the *brevinoda* and *pyriformis* lineages. Deletion of the heterochromatic segments in *brevinoda* to yield the *pyriformis* karyotype seems unlikely, because it is *brevinoda* which has the unusual karyotype for the genus. The A.C.T.

sibling of *pilosula* (AAHM-2) may be extensively polymorphic for heterochromatin duplications. In this sibling, counts of 2n=32 and 31 are found, and all chromosomes have variously-sized heterochromatic arms (Fig. 5c) – the relationship between these two "*pilosula*" species will be discussed further below. Compare the karyotypes of two nestmates (Fig. 5a and b); note that the size of the heterochromatic arms varies dramatically between individuals, although stable between cells of the same individual. This wide variation in heterochromatic arm length strongly suggests that change in amount of constitutive heterochromatin is rapid in this species. A similar variation in heterochromatic arm length was found within *Vollenhovia sp. 3* (Fig. 5d and e). Deletion can also be important, leading to reduction in arm size, and must occur at various points in the genome, as otherwise karyotypes would become more and more heterochromatic and larger and larger in size. However, the remarkably long arms that we have found suggest that under some circumstances "growth" occurs far more readily than deletion. Whether this increase occurs as a result of some generalized selection for repeated sequences, or, as seems more likely to us, due to some single factor such as selection for increased representation of a particular sequence family, remains an open question.

5. Translocation Polymorphism

Prior to this study, only one ant polymorphism interpretable as involving a translocation had been reported (Imai and Kubota, 1972), suggesting that translocations rarely become established in ants. However, we found at least 11 translocations in nine of the species included in this survey. We doubt that Australian ants are relatively predisposed to translocations; improved technique is without doubt the explanation for the higher frequency we find.

Three inter-homolog translocations were found, involving chromosome 2 of *Chelaner rothsteini* (AAGW-2 and AAHE-2, Fig. 6a–c), chromosome 5 of *Chelaner sp. 1* (Fig. 6d), and chromosome 6 of *Crematogaster sp. 1* (AAGR-10, Fig. 6e). This type of translocation seems to be important as a mechanism *leading to gene duplication.* In the four *Chelaner rothsteini* colonies examined, two (AAGU-2 and AAGW-8) had the karyotype lacking the duplication (Fig. 6a), while AAGW-2 included similar homozygotes as well as heterozygotes for the translocation, and AAHE-2 included homozygotes for the translocation (Fig. 6c). The affected chromosome has a duplicated segment on the long arm (Fig. 7a). Naturally, one could imagine the process running in reverse, leading to deletion, but this is more likely to be deleterious and hence seems the less probable mechanism. Such a duplication of chromosome segments is also interpretable by the so-called unequal crossing-over found in *Drosophila* by Bridges (1936).

Figure 6f–h shows three reciprocal translocations, which were found respectively in *Iridomyrmex sp. 15* (AAGJ-9), *Pheidole sp. 29* (AAHD-1), and *Pheidole sp. 30* (AAGW-18).

Highly complex translocation polymorphisms involving chromosome deletion occur in species in the genera *Rhytidoponera* and *Myrmecia.* Two Western *Rhytidoponera metallica* colonies were analysed. In AAGM-1, taken in South Australia, the workers had uniformly 2n=24, but in the New South Wales colony

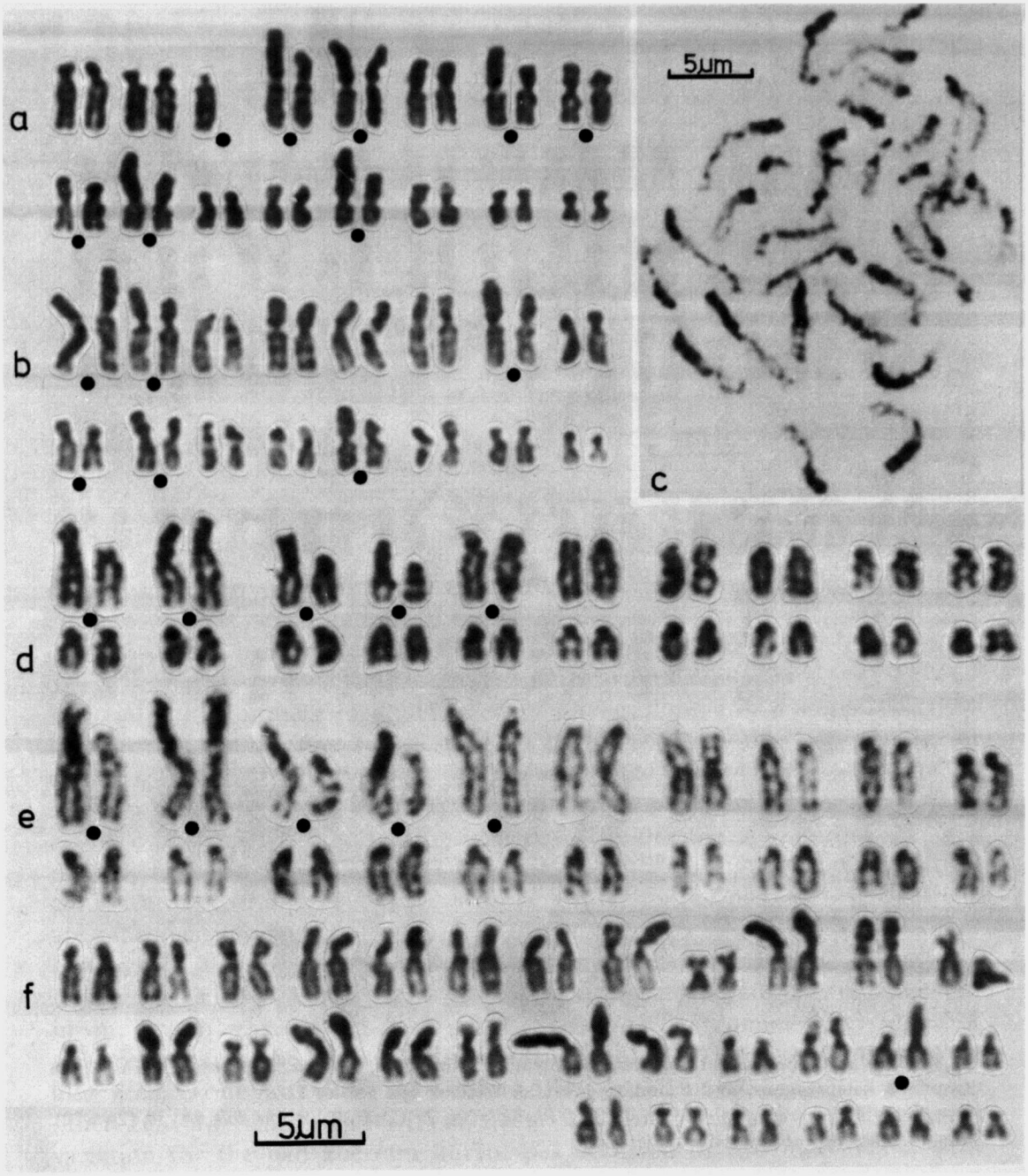

Fig. 5a–f. C-banded karyotypes with extremely long heterochromatic arms. Chromosome pairs heterozygous for polymorphisms are indicated by solid circles. **a** *Myrmecia pilosula* (2n = 31) with monosomy for chromosome 3. **b** *Myrmecia pilosula* (2n = 32), note the marked differences in short arm sizes within pairs and between this karyotype and that shown in **a**. **c** *M. pilosula* (C-banded metaphase). **d** *Vollenhovia sp. 3* (2n = 40), of which a C-banded karyotype is shown in **e**. **f** *Bothroponera sp. 2* (2n = 60)

Fig. 6a–h. Karyotypes with translocation polymorphisms. **a**, **b**, **c** *Chelaner rothsteini* (2n=22, **a** normal, **b** heterozygous, and **c** homozygous). **d** *Chelaner sp. 1* (2n=32). **e** *Crematogaster sp. 1* (2n=24). **f** *Iridomyrmex sp. 15* (2n=18). **g** *Pheidole sp. 29* (2n=20). **h** *Pheidole sp. 30* (2n=20). Polymorphic chromosomes are underlined

AAGW-6 the diploid numbers ranged from 22 to 24. The AAGM-1 karyotype has two independent translocations (Fig. 8a), denoted Tr. 1 and Tr. 2, and a pericentric inversion as mentioned above. As well as the Tr. 1 and Tr. 2 rearrangements, two further translocations (Tr. 3 and Tr. 4) were found in the AAGW-6 karyotypes (Fig. 8b–d). The chromosome number variation between the AAGW-6 karyotypes results from the intriguing segregation of the Tr. 2 rearrangement. This translocation shifts one arm of a small SM chromosome onto the long arm of a ST chromosome (Figs. 7b and 8b). The 23-

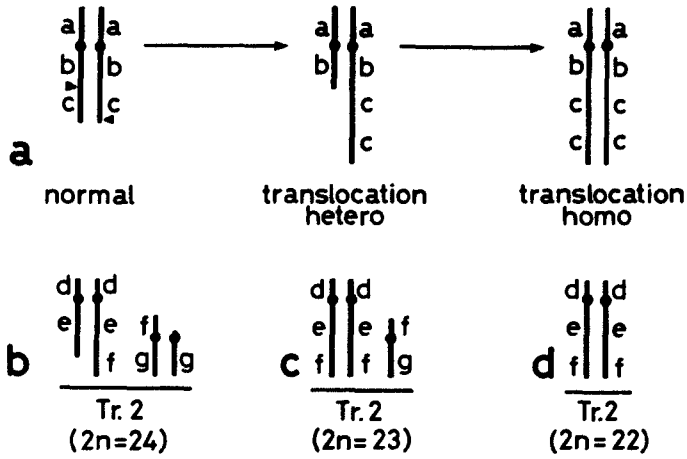

Fig. 7a–d. Diagrammatic representation of chromosome rearrangements in *Chelaner* (**a**) and *Rhytidoponera* (**b, c, d**). For details see text. Arrow heads are breakage points assumed. Letters represent marker regions indicating chromosome homology

chromosome karyotype includes two altered ST chromosomes and one SM chromosome (Fig. 8c), and clearly most of the short arm of the original SM chromosome is here trisomic and most of the long arm monosomic (Fig. 7c). The 22-chromosome karyotype is a derivative type: While the SM short-arm material is now present in double-dose again, the long-arm material has now been lost altogether (Fig. 7d), although it is not clear how this loss of material can be tolerated. The karyotypes found in AAGM-1 and AAGW-6 are similar in many ways, with both having the Tr. 1 and Tr. 2 rearrangements, as well as five other similar chromosome pairs. However, the large SM chromosomes 1 of AAGM-1 are absent in AAGW-6 (Fig. 8a and b), suggesting that further complicated rearrangements are involved. So far as the present investigations are concerned, we could not get evidence that those unusually large chromosomes are C-band positive.

The other complex pattern of translocation heterozygosity was observed in one colony (AAGT-11) of *Myrmecia pilosula*. The common karyotype of this species is $2n=2SM+6ST+2A=10$ (Fig. 12a). Two other karyotypes were also found, one with $2n=1M+2SM+5ST+1A+1m=10$ (Fig. 9a) and the other $2n=1M+2SM+5ST+1A=9$ (Fig. 9b). Inter-cell and intra-individual variation for the two aberrant karyotypes occurred in two individuals, with cell frequencies as follows: individual one, 16 cells with ten chromosomes and eight with nine; individual two, 13 cells with ten chromosomes and 28 with nine). The reduction in chromosome number clearly results from loss of the minute chromosome, and this chromosome is mitotically unstable. C-band analysis revealed that these unusual karyotypes resulted from two rearrangements involving at least six breaks in all. One chromosome 2 sustained a pericentric inversion, and chromosomes 1, 4 and 5 were involved in a four-break rearrangement. Our interpretation (Fig. 9c) of the latter changes is that two breaks occurred in the long arm of chromosome 5 (one very near the centromere and the other about one third of the way down the arm from the centromere),

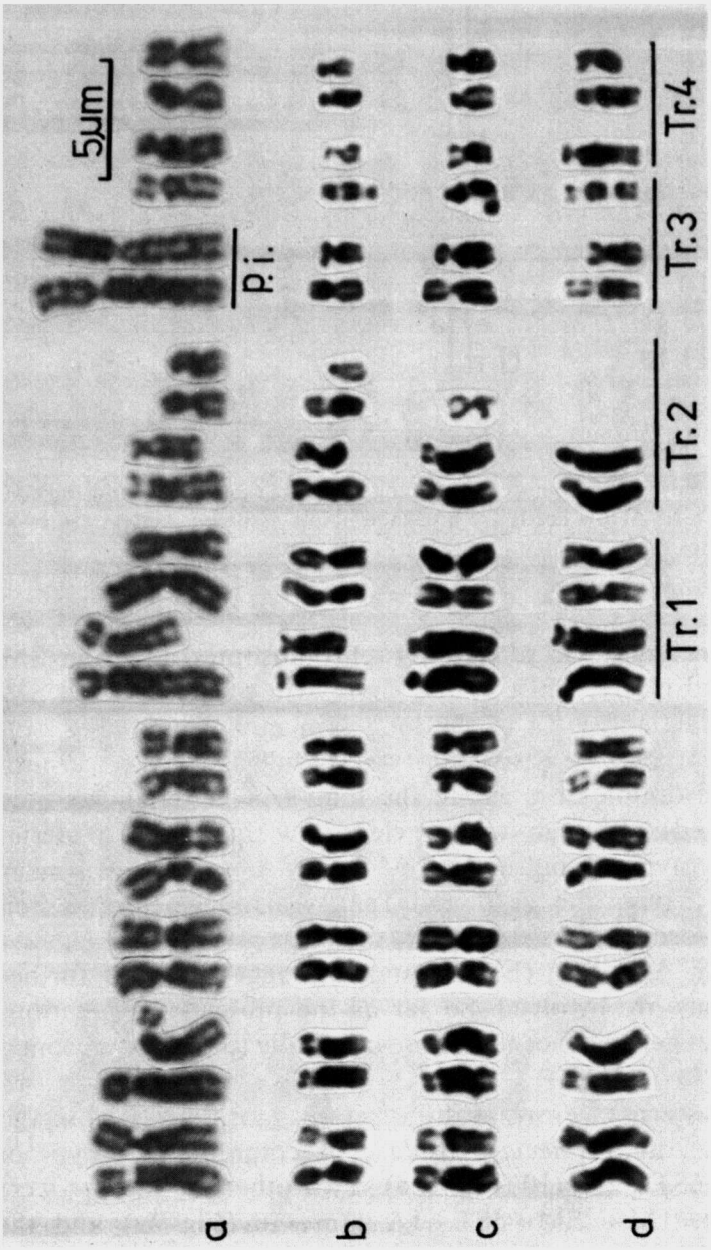

Fig. 8a–d. Karyotypes of *Rhytidoponera metallica* (Western forms). The unchanged members of complement shown to left of figure. Tr.: translocation. p.i.: pericentric inversion. **a** Western form II (AAGM-1) with 2n=24. **b**, **c**, **d** Western form I (AAGW-6, **b** 2n=24, **c** 2n=23, **d** 2n=22)

breaking this element into three fragments [denoted as the minute short arm (m), 1/3rd, and 2/3rd fragments]. The other two breaks occurred close to the tips of chromosomes 4 and 1. The chromosome 5 2/3rd fragment became attached to the tip of the long arm of chromosome 4. The 1/3rd fragment became attached to the end of chromosome 1, and the broken end on this new element was sealed by one of the two detached tips. The mitotically-unstable minute element could then be sealed similarly by the remaining detached tip fragment. The short arm of the acrocentric chromosome 5 is heterochromatic (Fig. 9c), which may be the reason for the observed instability, although it is also worth noting that it may lack a centromere of adequate size.

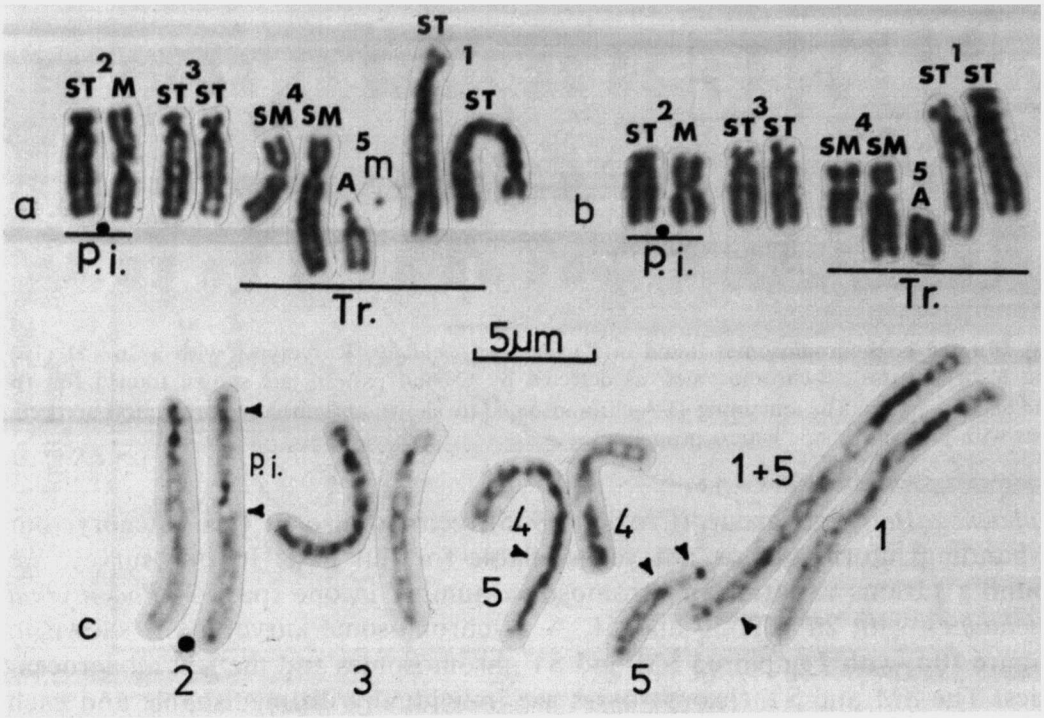

Fig. 9 a–c. Karyotypes of *Myrmecia pilosula* (AAGT-11) with a complex translocation accompanying chromosome loss. **a** A karyotype with one minute (2n=10). **b** A karyotype without the minute (2n=9). **c** A C-banded karyotype. Arrow heads indicate breakage points assumed. The No. 2 chromosomes are heterozygous for pericentric inversion and 4 break rearrangement occurred among the Nos. 1, 4 and 5 chromosomes. *p.i.*: pericentric inversion. *Tr.*: translocation. *m*: minute chromosome (C-band positive). Chromosome pairs with solid circle indicate heterozygosity for pericentric inversion

With the complete elimination of the minute chromosome, as seems likely, this *pilosula* rearrangement can be regarded as a kind of tandem fusion (a "double tandem fusion"?) which has occurred between ST and A chromosomes. A tandem fusion has been suggested during *Iridomyrmex* karyotype evolution (Crozier, 1975), but the change involved is also explicable as involving a pericentric inversion followed by a centric fission or fusion. No tandem fusion between two A chromosomes has been previously reported for ants. Although tandem fusions may have occurred in some grasshoppers, this rearrangement is rare in animal karyotype evolution generally (White, 1973).

Although we cannot be certain yet about the ultimate fate of translocations such as those mentioned above, it seems highly significant that they occurred in species with numbers below or at the median for ants (i.e., in species with n=3, 4, 5, 9, 10, 11, 12 and 16). We have already pointed out the opposite distributional bias in the occurrence of Robertsonian polymorphisms and will discuss the significance of these asymmetries further below.

6. Supernumerary (B-) Chromosomes

Typical B-chromosomes have been found in *Leptothorax spinosior* (Imai, 1974); the chromosome number variation seen in some siblings of the North American

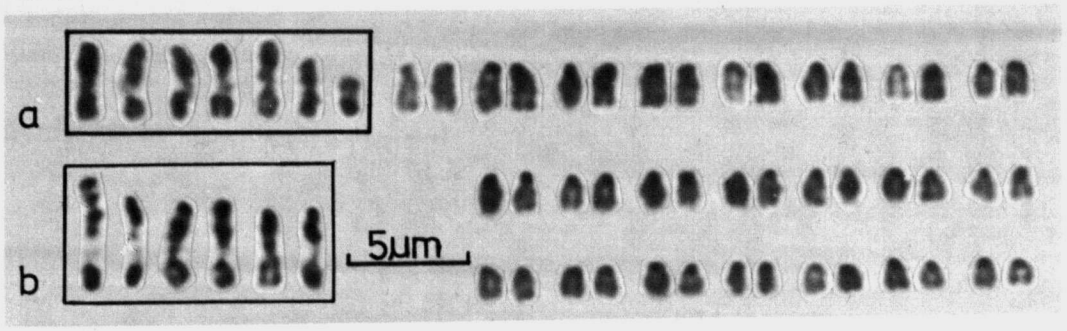

Fig. 10a and b. B-chromosomes found in *Podomyrma adelaidae*. Karyotypes with **a** 2n=51 (7B) and **b** 2n=50 (6B). B-chromosomes, as detected by C-band pattern, are shown framed for 7B and 6B karyotypes. The remaining 44 A-chromosomes are shown unframed and are typical acrocentrics with pericentromeric heterochromatin

Aphaenogaster rudis group (Crozier, 1975) seem to also fit this category, but C-banding information is not yet available for this case. In our survey, we found a curious variation in chromosome number in one species—*Podomyrma adelaidae*—with 2n=49, 50 and 51. A 51-chromosome karyotype is shown in Figure 10a, with 7 unpaired SM and ST chromosomes and the rest all acrocentrics. The SM and ST chromosomes are individually distinguishable and each is mostly heterochromatic when C-banded, whereas the others show no C-band positive material excepting pericentromeric regions (Fig. 10b). The number of these heterochromatic marker chromosomes varied between individuals, with 49-, 50- and 51-chromosome individuals having, respectively 5, 6 and 7 such chromosomes. Intra-individual variation was also observed, with 51-chromosome (Fig. 10a) and 50-chromosome (Fig. 10b) cells observed in the same preparation. Our sample of ants did not produce suitable stages in the laboratory, so that all our preparations were made in the field and hence were not of the highest quality; however, we feel that this case can be tentatively classified as one involving B-chromosomes. If this is correct, then B-chromosomes are known from at least three ant species out of 280 examined cytologically. Their frequency is thus low. Moreover not one of the three cases occurs in a species with a Robertsonian polymorphism.

7. Deletion

We found three presumed cases involving deletion of chromosomal material, on the assumption that heterochromatin growth occurs only at the centromere region. However, if this assumption is invalid it is possible that in one or more of these cases an actual addition of material may have occurred. We found one case each in the two forms of *Myrmecia pilosula* and one in *Rhytidoponera metallica* (Western form—I). As discussed previously, a minute heterochromatic chromosome is in the process of being lost in the low-number *M. pilosula* form following a presumed complex tandem fusion (Fig. 9). In the case of *Rhytidoponera metallica,* euchromatic material has been deleted as a result of an unusual segregation pattern following a translocation (Fig. 8c and d), but in the high-numbered *pilosula* case chromosome 3 has been lost in some colonies but not others (Fig. 5a). The latter two cases are quite unexpected;

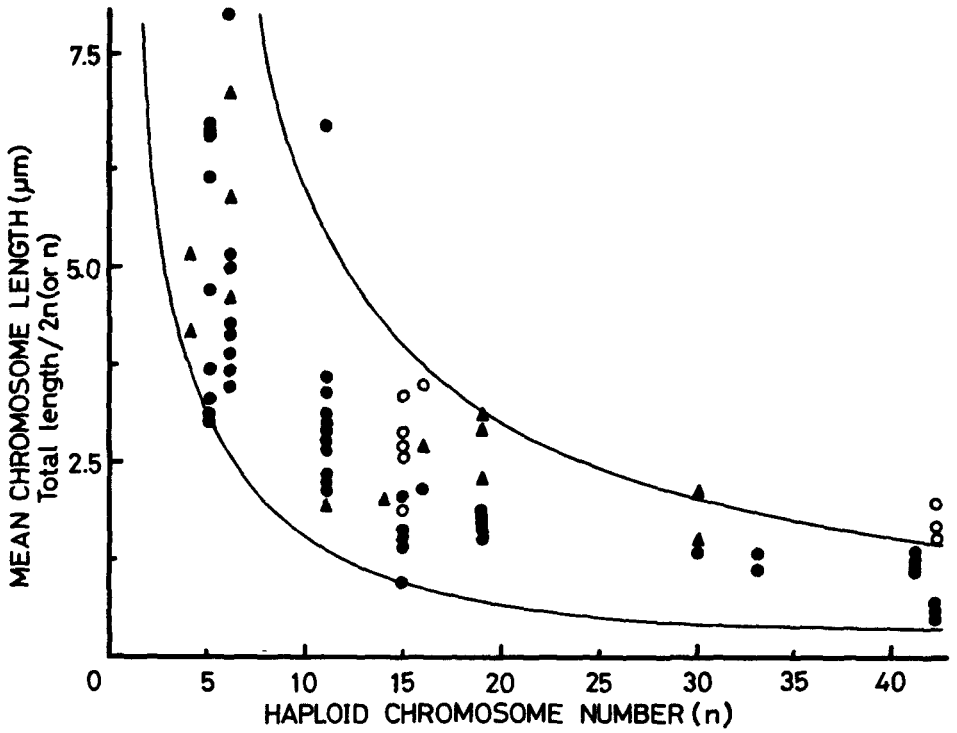

Fig. 11. Relationship between Mean Chromosome Length (MCL) and haploid number in myrmeciine (●, ○) and ponerine (▲) ant karyotypes. The materials used cover all species of the Tribe Myrmeciini and the Tribe Ponerini investigated in this paper, and three ponerine ants, *Ponera scabra* (n=4), *Brachyponera sinensis* (n=11), and *Cryptopone sauteri* (n=14) studied by Imai and Kubota (1972). Assuming that no changes involve polyploidy, the shape of the distribution is indicated by two curves constructed using the relationship MCL $\propto$ 1/n, with the starting point for one curve a karyotype with elongated and for the other one with contracted chromosomes. Solid symbols refer to measurements of euchromatic length only; adjacent open symbols involve heterochromatin as well

inasmuch as each probably involves the loss of a significant number of loci, we doubt that such rearrangements have played an important role in ant karyotype evolution.

8. Polyploidy

Occasional polyploid individuals have been reported previously in ants (see Crozier, 1975), and we found two such individuals, one triploid (*Crematogaster sp. 2*, 3n=39) and one tetraploid (*Camponotus sp. 5.*, 4n=64). Despite this occasional production of polyploid individuals, we can reject polyploidy as a significant factor in ant karyotype evolution. Indeed, there is a well-known general tendency in ants for low-numbered karyotypes to have large chromosomes and high-numbered karyotypes to have small ones (Crozier, 1975), suggesting that the total genome size is fairly constant. We may examine this conclusion more precisely by using the relationship between mean chromosome size and chromosome number. If chromosome number change in ant evolution is primarily a result of polyploidy, then there should be little or no correlation between mean chromosome length per karyotype and chromosome number. However,

if Robertsonian rearrangements rather than polyploidy constitute the principal factor leading to numerical change, then mean chromosome length will vary inversely with haploid number. We measured a series of species with a wide range of chromosome number from two divergent and groups, namely seven ponerine ants with n=4, 6, 11, 14, 16, 19 and 30, and nine *Myrmecia* species with n=5, 6, 11, 15, 16, 19, 25, 26, 30, 33, 41 and 42. The total length of each haploid set was divided by n and that of diploid sets by 2n to calculate mean chromosome size. The shape of the resulting distribution (Fig. 11, solid circle) fits the model involving Robertsonian change as the main mode of chromosome number change and rejects a significant role for polyploidy. Thus, although there is an occasional increase of genome size through growth of constitutive heterochromatin (Fig. 11, open circle), the euchromatic genome length is relatively constant in ants.

B. Comparative Karyotype Analysis with Respect to Rearrangement Frequency and Cytotaxonomy

This study covers 99 "nominal" ant species placed in five subfamilies: Myrmeciinae (9 species), Dolichoderinae (14), Formicinae (22), Ponerinae (18), and Myrmicinae (36), though there are 105 species when siblings are taken into account. The chromosomal polymorphisms found are described above and seem to be a biased sample of evolutionarily-important rearrangements. Robertsonian and pericentric inversion polymorphisms, which are both considered to be principal modes of chromosomal change in animals, occurred less frequently than translocations, generally considered a type of rearrangement seldom contributing to karyotype evolution. It is possible to at least partly correct for this bias by comparing the karyotypes of closely-related species and thus identify those rearrangements that actually become established and fixed. This is the chief objective of the sections below.

The species examined were identified using conventional taxonomic techniques. Members of some genera (e.g., *Amblyopone, Camponotus, Myrmecia* and *Rhytidoponera*) are often difficult to sort confidently to species on such criteria. In ants, as in other animals, it is a general rule, with some notable exceptions, that the karyotype is reasonably uniform within species but often differentiated between them. Applying this rule, we find that some morphologically extremely similar (and hitherto unrecognized) biological species can be distinguished, and these examples of chromosomally-characterized sibling species are discussed below.

In making the identifications (R.W.Taylor) it is emphasised that, in most cases, conventional taxonomic characters will likely enable adequate diagnosis and separation of these sibling species *once their status as good biological species is established.* Cytological studies provide important evidence contributing to the confident sorting of such species, as does intensive field work (involving sympatric associations, detailed distributional analyses, etc.) and expanded taxonomic research (involving scanning electron microscopy, isozyme studies, examination of male genitalia, etc.). Without such evidence, decision making by taxonomists is often severely hampered. Australian ant taxonomy has been impaired greatly in the past by deficiencies in these areas.

We have not effected any formal name changes as a result of our findings here, and feel that nomenclatorial re-arrangements in such taxonomically "difficult" taxa as the clearly portmanteau "species" *Amblyopone australis, Myrmecia pilosula, M. fulvipes,* and *Rhytidoponera metallica* should be held in abeyance until better biological evidence enables us to more properly evaluate the *real* situation of these taxa, *in nature.* At that time a formal nomenclature, with real biological meaning, can be proposed. In the meantime, these names should be recognized as defining areas of taxonomic doubt, and the provisional ANIC numbers discussed above can be used to discriminate putative component species where necessary.

1. Subfamily Myrmeciinae

The genus *Myrmecia* (comprising the so-called "bull-dog" ants) is generally accepted as being the most primitive in the world today, excepting the enigmatic once-caught *Nothomyrmecia* of the same subfamily. The known range of chromosome numbers, with 2n=9, 10, 12, 22, 30, 31, 38, 50, 51, 60, 66, 81 and 84 (Table 2) is large by the standards of any animal group, and 2n=84 is by far the highest number known in the Hymenoptera (outside *Myrmecia*, various other ants with 2n=52, 54 and 56 have the next-highest hymenopteran numbers). Karyotype analysis suggests that the following four species are closely related: *brevinoda* (2n=84, N.F.=84 not counting heterochromatic arms, Fig. 12 h), *pyriformis* (2n=81, N.F.=84, Fig. 12 g), *forficata* (2n=50 and 51, N.F.=88, Fig. 1 f) and *gulosa* (2n=38, N.F.=76, Fig. 12 d). Although chromosome number varies widely within this group (2n=38−84), the range in fundamental number (N.F.=76−88) is much less, suggesting that these karyotypes form a broadly related group between the terminal members of which there have been at least 23 Robertsonian changes and at least six pericentric inversions. One pericentric inversion and three Robertsonian changes are plausible as relating the karyotypes of *cephalotes* (2n=66, N.F.=66, Fig. 12 f) and *fulvipes* (2n=60, N.F.=68, Fig. 12 e). With fundamental numbers lower than those in the previous group, *cephalotes* and *fulvipes* may represent a different lineage. However, the information on sibling species we present below serves as a warning to would-be speculators about *Myrmecia* karyotype evolution. Such sibling species were found for the *pilosula* and *fulvipes* groups. In the case of *fulvipes,* one colony (AAHM-1) has almost identical worker morphology to two colonies (AAGR-14, AAGT-12) collected elsewhere, but the karyotypes from the two localities are quite different with the former having 2n=60 (N.F.=68, Fig. 12 e) and the latter 2n=12 (N.F.=22, Fig. 12 b). This marked karyotypic difference indicates that there is a very low probability indeed that these colonies are from the same species and, because Fr. B.B. Lowery noted small differences in the morphology, the low-numbered form has been denoted *sp. cf. fulvipes.* It should be noted that the nominal species *M. fulvipes* as defined by Brown (1953) includes several variant forms, some of which were accorded nomenclatorial recognition by authors preceding Brown. Our *sp. cf. fulvipes* might eventually be identified as one of these named forms. Certainly, the notion that *M. fulvipes,* and its proposed synonyms as suggested by Brown, constitute a single variable biological species gains little support from our findings.

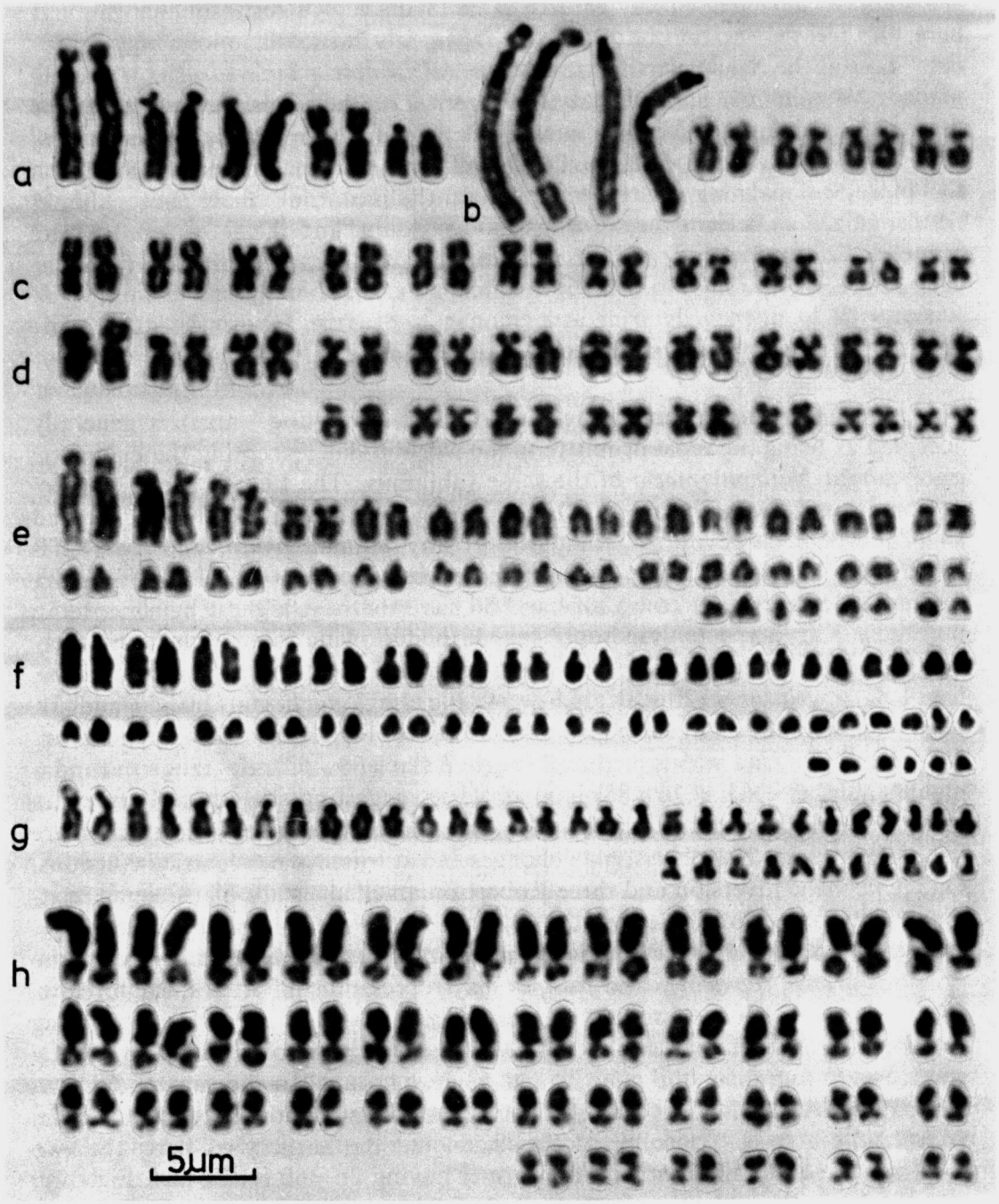

Fig. 12a–h. Karyotypes of Bull-dog ants (*Myrmecia*). **a** *M. pilosula* (2n = 10). **b** *M. sp. cf. fulvipes* (2n = 12). **c** *M. nigrocincta* (2n = 22). **d** *M. gulosa* (2n = 38). **e** *M. fulvipes* (2n = 60). **f** *M. cephalotes* (2n = 66). **g** *M. pyriformis* (n = 41). **h** *M. brevinoda* (2n = 84)

No apparently significant morphological differences were detected between workers of a *pilosula* cytotype from Canberra with 2n=31 and 32 (Fig. 5a and b) and one taken at a lower elevation, near Sydney, with 2n=9 and 10 (Fig. 9 and 12a). One Victorian *pilosula* (=*ruginoda*) collection had n=15 (Crozier, 1975). Clearly, *pilosula* is also a composite entity, comprising at least two chromosomally-characterized sibling species. Note that, as discussed above, *pilosula* (low-n form) and *fulvipes* apparently have homologous chromosomes 1 in terms of size and C-band pattern. The similarity of the karyotypes of these two forms is most surprising, because they are generally not considered closely related and were assigned to different species groups by Clarke (1951).

2. Subfamily Dolichoderinae

We examined 14 dolichoderine ant species (Fig. 13, Table 2), with ten of these being members of the genus *Iridomyrmex*, which is well-developed in Australia. *Iridomyrmex*, however, is a taxonomically-difficult genus at species level, due to the morphological reduction of the workers. We tentatively recognise four karyotypic groups among the species of this survey: (1) *darwinianus* group I (2n=12, *sp. 9*), (2) *darwinianus* group II (2n=14, *sp. 8*), (3) the *nitidus* and *itinerans* group (2n=16), and (4) a group including *purpureus* and *gracilis* (2n=18). The numerical difference between groups (1) and (2) is minor, but it seems to involve complex rearrangements because all chromosomes in both species are metacentric, although their relative size relationships differ (Fig. 13 l and m). Change has similarly been complex between groups (2) and (3) (Figs. 13c and e). However, as pointed out by Crozier (1968b), groups (3) and (4) are simply related by a single Robertsonian change; we can now see that chromosome 2 (SM) of the 16-chromosome karyotype of group (3) is equivalent to chromosomes 7 and 8 in the group (4) karyotype, although the latter have obvious short arms, suggesting a tandem growth of constitutive heterochromatin (e.g., see Fig. 13d and e). The karyotype is basically the same within the 2n=16 (Fig. 13a and d) and 2n=18 (Fig. 13e, h–j) groups, except for the changes due to two pericentric inversions involving 18-chromosome species. One pericentric inversion is indicated by chromosome 6 in sp. 13 (Fig. 13f) which is ST as against SM in other 2n=18 species. A related species is polymorphic for the original SM chromosome 6 and either the same or a similar inversion (Crozier, 1968b). Another pericentric inversion is discernible in chromosome 7 of *purpureus*-group colony (AAGW-15), in that this chromosome is acrocentric in other 2n=18 species, but SM in AAGW-15 (Fig. 13g).

Colony AAGT-14 has been tentatively identified as *Iridomyrmex sp. 7*, but there is a possibility that it might actually be the species previously named *Bothriomyrmex pusillus*. Identification in this case is hampered by the great morphological reduction and resulting paucity of characters in this species and the inaccessibility of *pusillus* type material. In any case, however, this species is karyotypically distinct from the other *Iridomyrmex* species (Fig. 13k).

3. Subfamily Formicinae

There is a wide range in chromosome number in the 22 species of formicines we examined, with 2n=18, 20, 28, 38, 42, 44, 46, 48 and 50 (Figs. 14–16,

Fig. 13a–m. Karyotypes of Dolichoderine ants. **a** *Leptomyrmex erythrocephalus* (n = 12). **b** *Technomyrmex albipes* (2n = 16). **c** *Iridomyrmex sp. 10* (2n = 16). **d** *I. nitidus* (2n = 16). **e** *I. sp. 16* (2n = 18). **f** *I. sp. 13* (2n = 18), with a small pericentric inversion in chromosome-6. **g** *I. purpureus* group (2n = 18), with a pericentric inversion in one chromosome. **h** *I. sp. 17* (2n = 18). **i** *I. sp. 14* (2n = 18). **j** *I. sp. 15* (2n = 18). **k** *I. ? sp. 7* (2n = 22). **l** *I. sp. 8* (2n = 14). **m** *I. ? sp. 9* (2n = 12)

Table 2). Our analysis suggests that this karyotype diversity arose from a combination of Robertsonian rearrangements and pericentric inversions.

A series of Robertsonian rearrangements is indicated in *Stigmacros* and *Camponotus*. *Stigmacros sp. 1* has 19 pairs of acrocentric chromosomes (Fig. 14d) whereas in *S. sp. 3*, with 2n=20, all the chromosomes are metacentric (Fig. 14e). Because the fundamental number is similar in the two species despite the wide numerical divergence (N.F.=38 for *sp. 1* and 40 for *sp. 3*), it is possible to relate these karyotypes by 9 Robertsonian changes and one pericentric inversion.

Camponotus is one of the largest genera of ants, and, although the taxonomy of the Australian species is confused, there are clearly about 200 of them, of which we have examined 12 (Figs. 15 and 16). These 12 species fall into five karyotypic groups: (1) species with 2n=20 and N.F.=40 (*sp. 13*), (2) 2n=32 and N.F.=50 (*spp. 5, 8* and *11*), (3) 2n=38 and N.F.=52 (*spp. 9, 12* and *14*), (4) 2n=46 and N.F.=52 (*consobrinus, spp. 1, 2* and *10*). and (5) 2n=48 and N.F.=52 (*sp. 3*). Inasmuch as its fundamental number is much lower than those of the others, *sp. 13* seems karyotypically distant from the other species surveyed. The other groups have nearly the same fundamental numbers (N.F.=50–52), despite the wide variation in chromosome numbers (2n=32–48), suggesting a comparatively close relationship, with the differences being plausibly accounted for by a minimum of eight Robertsonian changes and at least one pericentric inversion.

Evidence for an important role in karyotype change for pericentric inversions was found among species of *Prolasius* and *Camponotus*. Two species of *Prolasius, sp. 1* (Fig. 14b) and *sp. 2* (Fig. 14c), have the same chromosome number (2n=18) but differ in the arm ratios of chromosomes 2, 3 and 8, suggesting fixation of pericentric inversions in these chromosomes between species. In *Camponotus*, we found four fixed pericentric inversions in three species, namely, chromosome 12 and chromosome 13 in *sp. 11* (Fig. 15d), chromosome 8 in *sp. 14*, (Fig. 15h), and chromosome 4 in *sp. 10* (Fig. 16d). In each of the four cases the derived (inverted) chromosomes have the centromere nearer the centre of the chromosome (A→M, SM or ST).

Note that the karyotypes of some species cannot be distinguished, such as *Camponotus sp. 5* and *C. sp. 8* (Fig. 15b and c), *C. sp. 9, sp. 12* and *sp. 14* (Fig. 15e–g), and *C. consobrinus, sp. 1* and *sp. 2* (Fig. 16a–c). This great, possibly total, similarity of karyotype between related species indicates that speciation can occur without any obvious karyotypic divergence. The same situation holds in the myrmicine genus *Pheidole*, as discussed below.

4. Subfamily Ponerinae

The 22 ponerine ant species karyotyped (Figs. 17 and 18, Table 2) include two clusters of sibling species. The chromosome numbers observed are 2n=12, 16, 22, 23, 24, 36, 37, 38, 39, 41, 42, 43, 44, 45, 46, 47, 48, 50, 52 and 60.

The very primitive cosmopolitan genus *Amblyopone* is well-developed in Australia, whence is found the type-species, *A. australis*. *A. australis*, according to Brown (1958), is a widespread species, rather variable morphologically. Brown

Fig. 14a–j. Karyotypes of Formicine ants (I). **a** *Notoncus ?ectatomoides* (2n = 44). **b** *Prolasius sp. 1* (2n = 18). **c** *P. sp. 2* (2n = 18). **d** *Stigmacros sp. 1* (2n = 38). **e** *S. sp. 3* (2n = 20). **f** *Paratrechina sp. 1* (2n = 30). **g** *Calomyrmex sp. 1* (2n = 28). **h** *Opisthopsis rufithorax* (2n = 50). **i** *Polyrhachis sp. 1* (2n = 42). **j** *P. ammon* (n = 21)

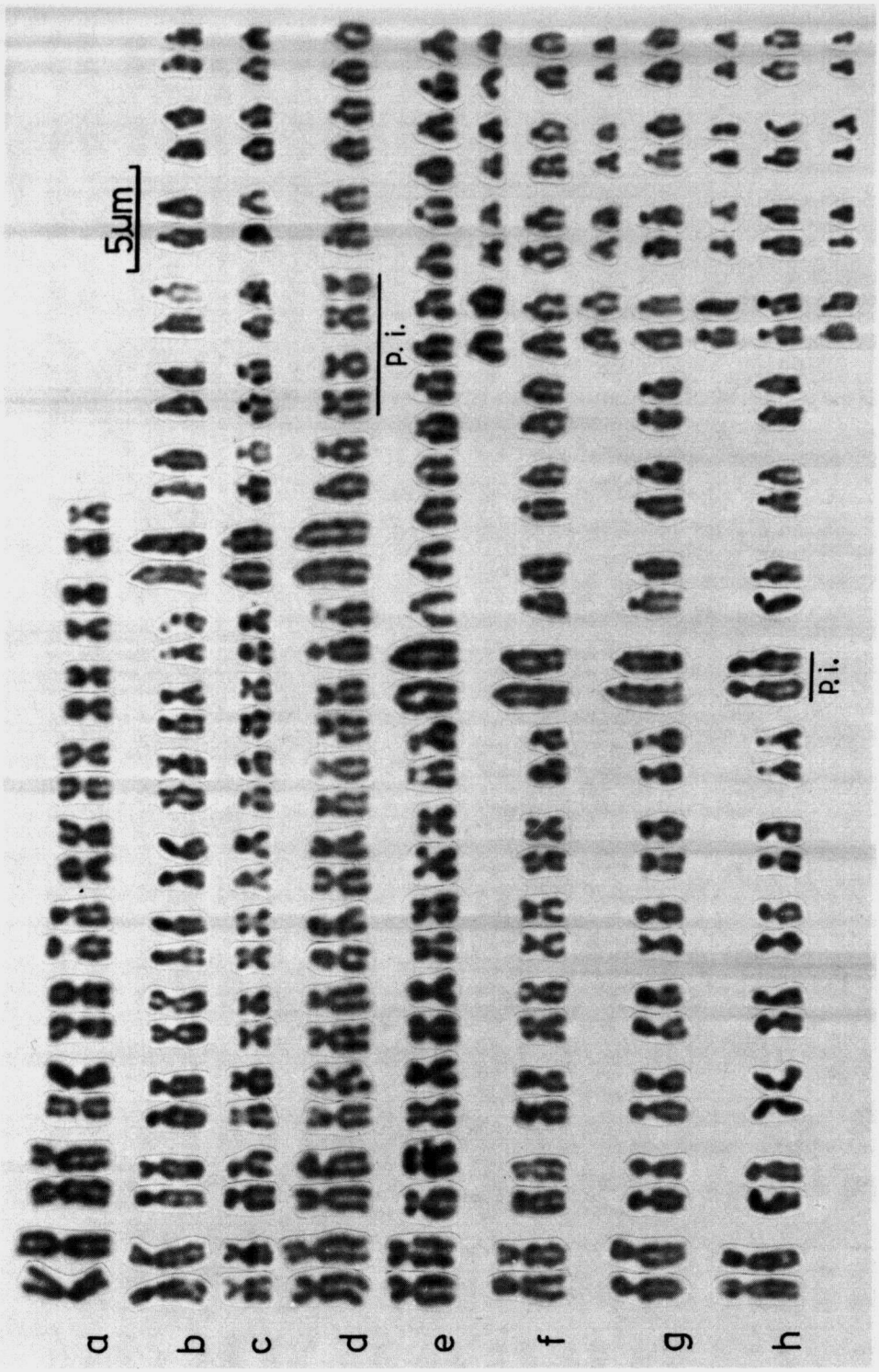

Fig. 15a–h. Karyotypes of Formicine ants (II), with *fixed* pericentric inversions indicated. **a** *Camponotus sp. 13* (2n = 20). **b** *C. sp. 5* (2n = 32). **c** *C. sp. 8* (2n = 32). **d** *C. sp. 11* (2n = 32). **e** *C. sp. 9* (2n = 38). **f** *C. sp. 12* (2n = 38). **g** *C. sp. 14* (2n = 38). **h** *C. sp. 14* (2n = 38)

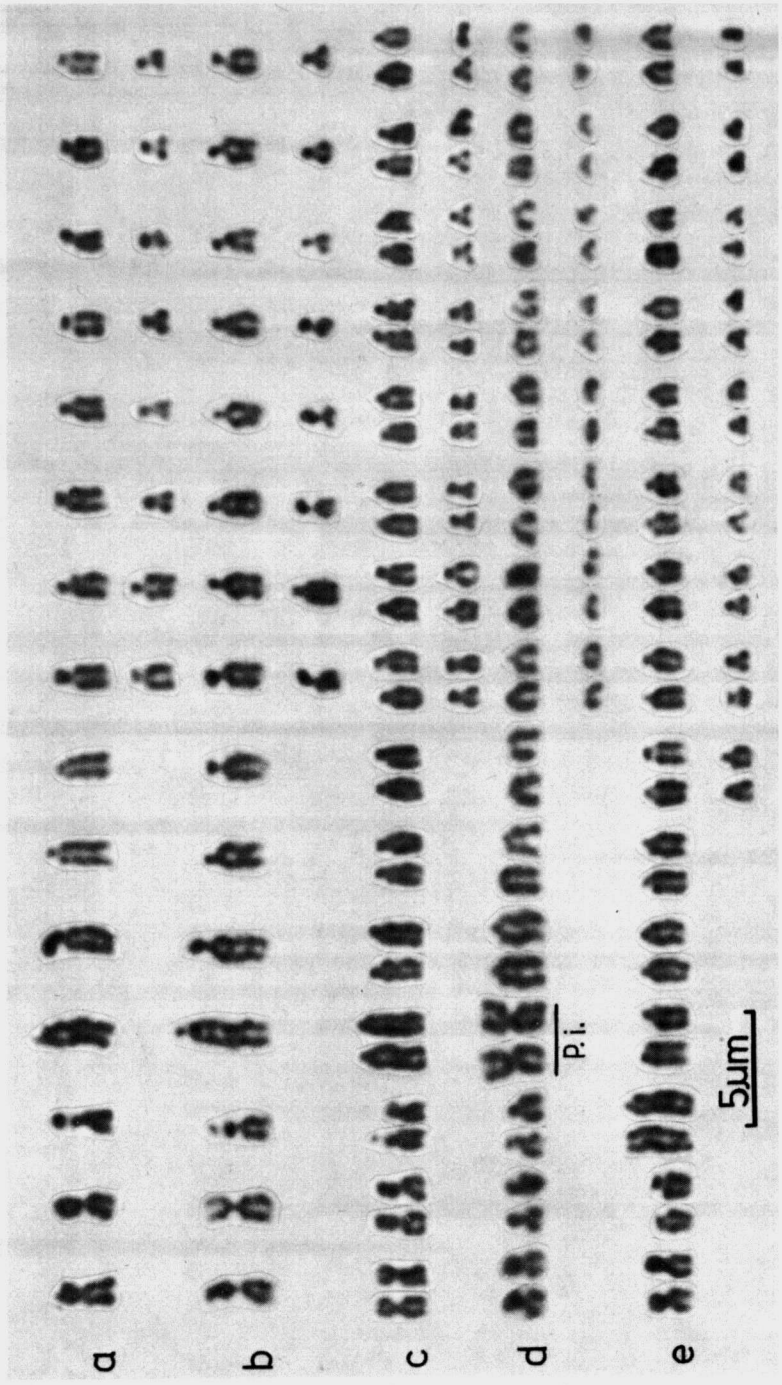

Fig. 16a–e. Karyotypes of Formicine ants (III), with a *fixed* pericentric inversion indicated. **a** *Camponotus consobrinus* (n=23). **b** *C. sp. 2* (n=23). **c** *C. sp. 1* (2n=46). **d** *C. sp. 10* (2n=46). **e** *C. sp. 3* (2n=48)

included as synonyms of *australis* a number of nominal species and subspecies named by earlier authors. It now appears likely that this portmanteau "*australis*" includes at least two karyotypically-distinguishable sibling species. One of these has 2n=44 (N.F.=84, Fig. 17a) and the other has 2n=48 (N.F.=94, Fig. 17b), with at least two Robertsonian changes and three pericentric inversions separat-

Fig. 17a–i. Karyotypes of Ponerine ants (I). **a** *Amblyopone australis* (*cf. fortis*) (2n = 44). **b** *A. australis* (2n = 48) the smallest pair is polymorphic for a pericentric inversion, as indicated; two examples of this pair from other individuals are shown framed. **c** *Heteroponera relicta* (2n = 22). **d** *Rhytidoponera chalybaea* (2n = 42). **e** *R. impressa* (2n = 42). **f** *R. metallica* (Eastern form, 2n = 46). **g** *R. purpurea* (2n = 38). **h** *R. mayri* (2n = 50). **i** *R. aciculata* (2n = 52)

ing the two karyotypes. The pericentric inversion polymorphism in the smallest member of the 2n=48 complement confirms the occurrence of pericentric inversions in the "*australis*" lineage. The higher-numbered form occurs widely in eastern Australia south of Dalrymple Heights, Queensland (Table 2), while the sole colony (RWT75-162) of the other was collected in North Queensland. These forms may therefore be largely allopatric in distribution. The 2n=44 form may correspond with the species described by Forel (1910) as *fortis*, synonymized by Brown (1958) with *australis*, and is listed as *sp. cf. fortis* in our table.

The *Rhytidoponera metallica* case seems particularly complex. All the colonies studied (Table 2) had workers of very similar morphology, but this "species" can be divided into at least two karyotypically distinct forms, which we denote as the Eastern and Western forms. As discussed above, the Eastern form has Robertsonian polymorphism in a number of chromosomes, and the Western form, while lacking Robertsonian polymorphism, has a complex translocation polymorphism (Figs. 1a, b and 8). One of us (R.W.T.) feels that the Western form can be further subdivided morphologically into a Western form I (AAGW-6) and a Western form II (AAGL-1, AAGM-1). This suggestion is plausible karyotypically, because translocations 3 and 4 of form I are not detectable in form II, so that overall at least three chromosome pairs are non-homologous between the two forms (Fig. 8). However, some caution seems advisable before finally accepting the two Western forms as different sibling species because of the evidence for possible gene flow given by both being polymorphic for translocations 1 and 2.

There is much variation in fundamental number between *Rhytidoponera* species, with N.F.=46 (*metallica*), 48 (*chalybaea* and *impressa*), 50 (*maniae*), 52 (*purpurea*), about 70 (*mayri*), and about 88 (*aciculata*). This wide range suggests that pericentric inversions and probably also tandem increases of constitutive heterochromatin have been very important during the chromosomal evolution of this group, with some 20 such events involved. Two pericentric inversions distinguish the two *Hypoponera* species studied, *H. sp. 1* (2n=38, N.F.=48, Fig. 18a) and *H. sp. 2* (2n=38, N.F.=52, Fig. 18b).

5. Subfamily Myrmicinae

The 36 myrmicine species observed have chromosome numbers of 2n=18, 20, 22, 24, 26, 32, 38, 40, 42, 45, 46, 49, 50 and 51 (Figs. 19–21, Table 2).

Pheidole is a large cosmopolitan genus with probably around 100 Australian species, of which we karyotyped 13. Twelve of these have 2n=20 and their karyotypes are almost identical (Fig. 19). These results, together with previous data (Crozier, 1975), suggest 2n=20 for most *Pheidole* species. The single differing *Pheidole* species, *sp. 24*, has 2n=18 (Fig. 19e) and a fundamental number of N.F.=36 as against N.F.=40 for the 2n=20 species. While the numerical difference between the two *Pheidole* karyotypes is minor, the differences between them involve highly complex chromosomal rearrangements. Chromosomes 2 through 9 are very similar in appearance, but chromosome 1 differs markedly

Fig. 18a–g. Karyotypes of Ponerine ants (II). **a** *Hypoponera sp. 1* (2n=38). **b** *H. sp. 2* (2n=38), differing from *H. sp. 1* by two pericentric inversions. **c** *Brachyponera lutea* (2n=16). **d** *Cryptopone? rotundiceps* (2n=12). **e** *Odontomachus sp. 1* (2n=44). **f** *Sphinctomyrmex steinheili* (2n=46). **g** *Cerapachys brevis* (2n=46)

in both size and morphology between these groups. Thus, if we measure the percentage of a chromosome against the total haploid karyotype length (C) and the arm ratio (r), we find that these values for chromosome 1 are C=21.4 and r=1.1 in the 18-chromosome species and C=16.0 and r=2.0 in the 20-chromosome species. We also note that the combined length of chromosomes 1

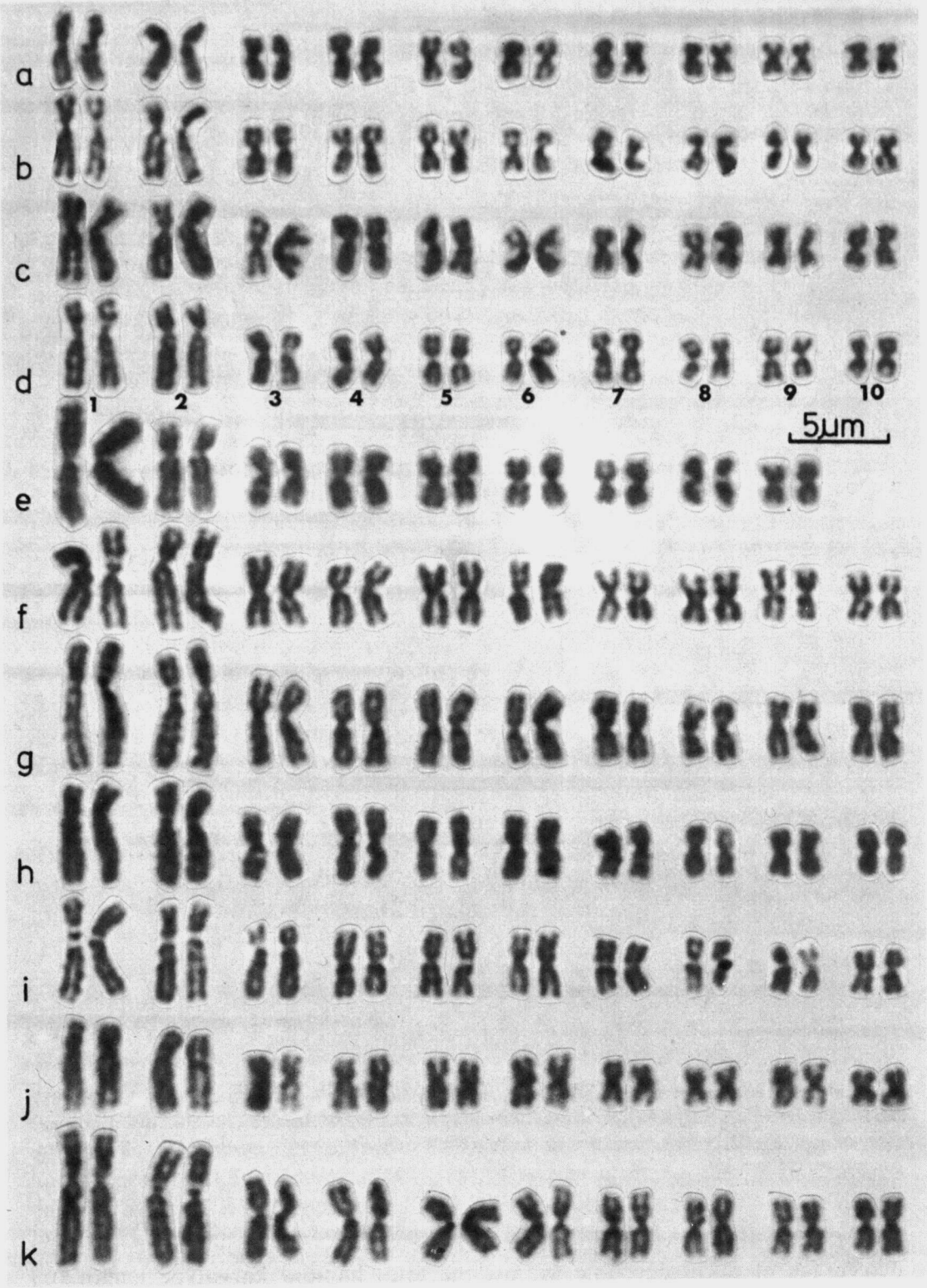

Fig. 19a–k. Karyotypes of Myrmicine ants (I). *Pheidole sp. 20* (2n=20). **b** *P. sp. 21* (2n=20). **c** *P. sp. 22* (2n=20). **d** *P. sp. 23* (2n=20). **e** *P. sp. 24* (2n=18). **f** *P. sp. 25* (2n=20). **g** *P. sp. 26* (2n=20). **h** *P. sp. 27* (2n=20). **i** *P. sp. 28* (2n=20). **j** *P. sp. 31* (2n=20). **k** *P. sp. 32* (2n=20)

and 10 in the 20-chromosome species is, at $C_{(1+10)}$, similar to that of chromosome 1 in the other karyotype. Under four assumptions, we can construct a plausible scheme relating the *Pheidole* karyotypes. The assumption that, in this genus, there has been an overall conservation of the euchromatic material is reasonable in view of much evidence from other organisms for such stability, and also in view of our own data (see p.363). Our observations also suggest conservation of heterochromatin in *Pheidole*. The assumption that there are no further karyotypes to be found which will be incompatible with the scheme below is a strong one, but we note that *Pheidole* is a karyotypically conservative genus. In our scheme, the sequence of events leading to the present-day karyotypes is: (1) the ancestral *Pheidole* karyotype included a submetacentric chromosome 1 with arm lengths of 7.0 and 16.0 (r=2.3), (2) this hypothetical chromosome yielded two telocentrics via centric fission, (3) these telocentrics underwent pericentric inversions to yield the present chromosomes 1 and 10 in modern 20-chromosome species, and (4) the initial hypothetical chromosome 1 underwent pericentric inversion to yield the metacentric (r=1.1) chromosome 1 of *Pheidole sp. 24*. The derivation of the 18-chromosome karyotype from the 20-chromosome one seems considerably less likely than the reverse, because in that case chromosomes 1 and 10 would become converted into acrocentrics by pericentric inversions, which, as we discuss further below, seems to be a very rare rearrangement in ants, although pericentric inversions as such are common.

A stability in chromosome number similar to that of *Pheidole* may also hold for *Meranoplus* where all three species surveyed in this study and one of two studied previously (Crozier, 1975), have 2n=22 (Figs. 20i, j and 21a).

Multiple rearrangements seem to have occurred in the karyotype evolution of *Monomorium*, with *M. sp. 1* having 2n=22 and N.F.=44 (Fig. 20a) and *sp.2* having 2n=42 and N.F.=51 (Fig. 20b). At least ten Robertsonian changes and three pericentric inversions are required for any scheme relating these two karyotypes. Further complexity in this case is revealed by the fact that whereas there is little differentiation between the chromosomes of *sp. 1*, three previously-karyotyped 22-chromosome *Monomorium* species all have one chromosome small and either acrocentric or subacrocentric (Crozier, 1975). *Crematogaster* seems to be another variable genus, with *sp. 1* having 2n=24 and N.F.=48 but *sp. 2* having 2n=26 and N.F.=50 (Fig. 21c and 21d), suggesting that these karyotypes might be related via one Robertsonian change and one pericentric inversion. *Xiphomyrmex* has evidently also undergone more karyotypic change than would be suspected from the numbers found (2n=18 in *sp. 2* and *sp. 4*, and 2n=20 in *sp. 3*). Chromosomes 2, 4, 5, 6, 7 and 8 seem morphologically identical in all three species (Fig. 20f–h). However, chromosomes 1 and 9 are similar only in *sp. 2* and *sp. 3*, while *sp. 3* and *sp. 4*, but not *sp. 2*, share chromosome 3. The short arm (C=3.6) of chromosome 3 of *sp. 2* resembles the acrocentric chromosome 10 (C=3.4) of *sp. 3* in length, and the length of the long arm of chromosome 3 in *sp. 2* (C=12.1) is similar to the total length of chromosome 3 in both *sp. 3* and *sp. 4* (C=12.8). Chromosome 1 is SM (C=20.0) in both *sp. 2* and *sp. 3*, but ST (C=19.0) in *sp. 4*. We indicate in Figure 22 what we believe to be the most plausible model relating these

Fig. 20a–j. Karyotypes of Myrmicine ants (II). **a** *Monomorium sp. 1* (2n = 22). **b** *M. sp. 2* (2n = 42), the smallest pair is polymorphic for a pericentric inversion. **c** *Olygomyrmex sp. 6* (2n = 38). **d** *Chelaner whitei* (2n = 24). **e** *C. sp. 2* (2n = 22). **f** *Xiphomyrmex sp. 2* (2n = 18). **g** *X. sp. 3* (2n = 20). **h** *X. sp. 4* (2n = 18). **i** *Meranoplus minor* (2n = 22). **j** *M. sp. 4* (2n = 22)

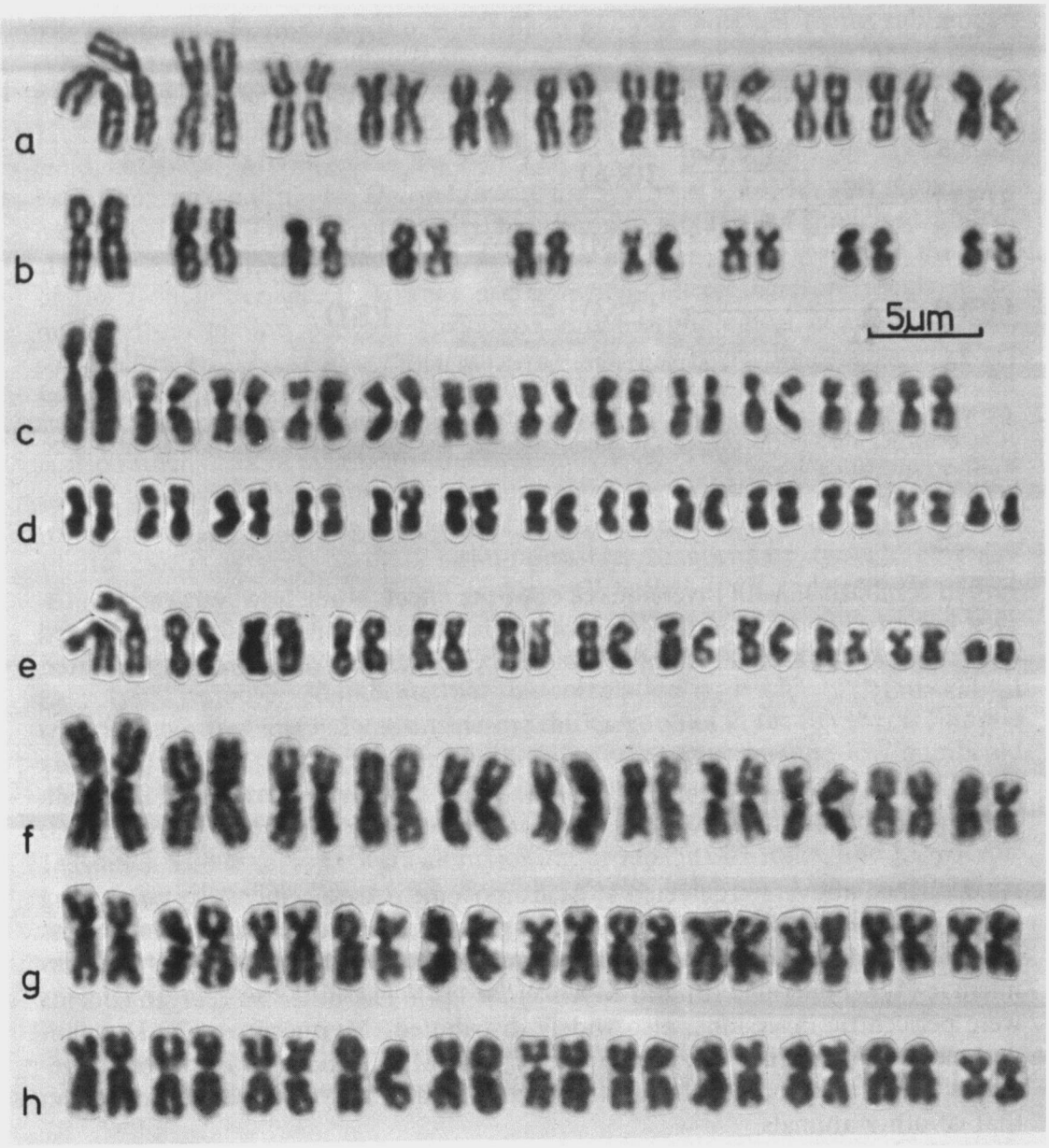

Fig. 21 a–h. Karyotypes of Myrmicine ants (III). *Meranoplus sp. 5* (2n = 22). **b** *Mayriella abstinens* (2n = 18). **c** *Crematogaster sp. 1* (2n = 24). **d** *C. sp. 2* (2n = 26). **e** *Strumigenys friedae* (2n = 24). **f** *Colobostruma sp. 1* (2n = 22). **g** *Orectognathus versicolor* (2n = 22). **h** *O. darlingtoni* (2n = 22)

three karyotypes. In this model, the *sp. 2* karyotype is considered ancestral, and one centric fission, one centric fusion, and two pericentric inversions are involved in this sequence of events generating the *sp. 4* karyotype via that of *sp. 3*. Both the other karyotypes remain possible as ancestral, but we believe that these alternatives are far less likely because both involve the derivation of chromosomes with terminal, or nearly terminal centromeres, a type of pericentric inversion (M, SM or ST→A) which we will argue below must be extremely

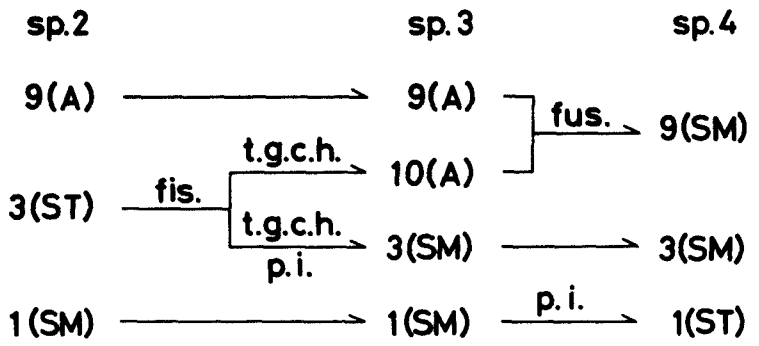

Fig. 22. Suggested pathways of karyotype change relating three *Xiphomyrmex* species. Chromosomes that do not differ from one karyotype to the next are omitted. Abbreviations: *fus.* centric fusion, *fis.* centric fission, *t.g.c.h.* tandem growth of constitutive heterochromatin, *p.i.* pericentric inversion, *A* acrocentric, *ST* subtelocentric, *SM* submetacentric. The karyotypes are illustrated in Fig. 20. Further explanation in text

rare in comparison with inversions of opposite effect. Note, however, that whichever karyotype was ancestral, one or two centric fusions must have occurred under our model, such a fusion, or fusions, being the only ones strongly indicated in this study.

6. Chromosome Rearrangements Detected by Comparative Karyotype Analysis

As described above, we detected 107 chromosome rearrangements by comparing the karyotypes of closely-related species. The 57 Robertsonian rearrangements occurred in all subfamilies studied: Myrmeciinae (23), Dolichoderinae (1), Formicinae (17), Ponerinae (2) and Myrmicinae (14). The other 50 rearrangements were pericentric inversions, also widely distributed: Myrmeciinae (7), Dolichoderinae (2), Formicinae (7), Ponerinae (25) and Myrmicinae (9). Both rearrangements seem therefore to be as important in ant karyotype evolution as in that of other animals.

Discussion

1. Correlation between Morphological and Karyotype Evolution

Karyotype evolution is often analysed on the basis of morphological evolutionary trends by assuming that a morphologically primitive species is also karyotypically primitive, i.e., has a karyotype closer to the ancestral one than does a related but morphologically advanced species (e.g., see Crozier, 1975). However, we now find that the primitive subfamilies Myrmeciinae and Ponerinae include both species with low and others with high chromosome numbers, leading us to revise our basic approach to this problem.

The family Formicidae (ants) is usually divided into ten living subfamilies (Wilson, 1971). The following ranges of chromosome numbers are now known for seven of those subfamilies: Poneroid complex: *Ponerinae*, n=3, 4, 6, 8, 11, 12, 14, 18–26, 30, Dorylinae n=15, *Myrmicinae* n=4, 9–26, 28 and Myrmecioid complex: *Myrmeciinae* n=4–6, 11, 15, 16, 19, 25, 26, 30, 33, 40–42, Pseudomyrmecinae n=16, *Dolichoderinae* n=5–9, 11, 13, 14, 16, and *Formicinae* n=8–10, 13–27. The karyotypically best-known subfamilies (italicised above) all show a significant range of chromosome number and, except for the case of the Dolichoderinae, this range is very wide. There therefore seems to be no good correlation between karyotype and morphological evolution at the subfamily level. The various subfamilies that are karyotypically well-known seem to have been following independent but parallel evolutionary trajectories.

The correlation between chromosome number and phylogenetic position is also rather weak in taxa below the subfamily level. Thus, even though differing ranges of chromosome number are known for formicine genera (e.g., *Formica* with n=26, 27, *Lasius* with n=14, 15, and *Camponotus* with n=9, 10, 13, 14, 16–26), it is very hard, if not impossible, to correlate these differences with phylogenetic position. Fossil evidence (Wheeler, 1965) indicates that these genera were present as separate entities back at least to Oligocene times. They have, therefore, evolved separately for at least 38 million years. The *Myrmecia* karyotypes we observed indicate that this correlation is also likely to be difficult to establish intragenerically. Considering the karyotypes in the *Myrmecia pilosula* and *fulvipes* groups for example, both pairs of siblings include low-numbered and high-numbered karyotypes. Because of these complications, we will here discuss ant karyotype evolution only on the basis of the chromosomal evidence. Inasmuch as our survey covers all major ant groups in Australia, and karyotypic knowledge of the world ant fauna is also fairly well spread over subfamilies, we believe our conclusions are therefore fairly general. Naturally, the possibility remains that further unusual karyotypes may be found, but we suggest that the principles we establish here would probably hold for these too.

2. *Three Hypotheses for Karyotype Evolution in Ants*

The known distribution of haploid numbers in ants is shown in Figure 23. This histogram embodies the data in Table 2 and Crozier's (1975) lists. The lowest number known is n=3 (*Ponera scabra*) and the highest n=42 (*Myrmecia brevinoda*), with the remaining numbers distributed almost continuously between these extremes. The modal number is n=11 and the median n=15. Only some 3% of all extant ant species have been karyotyped, so that the frequency distribution shown may be biased. However, we note that, apart from the dramatic increase in range due to the *Myrmecia* karyotypes reported here, the large number of karyotypes observed in this study have led to a histogram still very similar in all characteristics to that shown by Crozier (1975). We therefore have a firm basis for erecting and evaluating hypotheses about ant karyotype evolution. Ant karyotype evolution can be assumed to have followed the same rules since the group arose, because if there had been changes in directionality

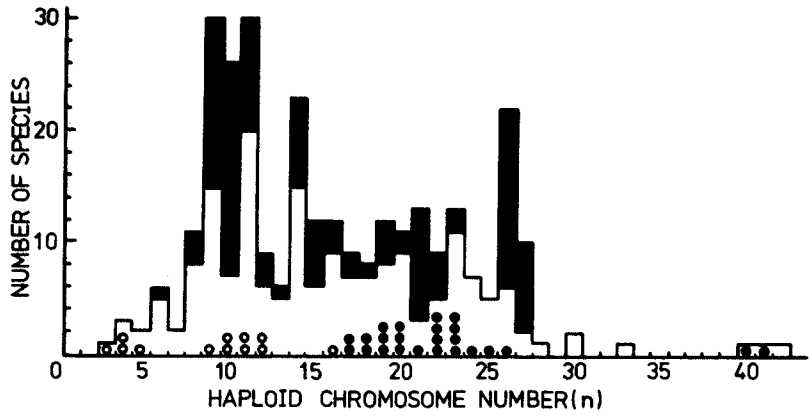

Fig. 23. Frequency distribution of chromosome numbers in ants. Solid circles: karyotypes with Robertsonian polymorphisms. Open circles: karyotypes with translocation polymorphisms. Black column: data from all ant species karyotyped. White column: data adjusted according to the genus karyotype concept (Crozier, 1975), with a chromosome number counted only once when a genus included more than one species with the same chromosome number

these would have had to affect all the various lineages independently, and there is no evidence for this.

While the ancestral ant chromosome number could be hypothesized to fall anywhere in a wide continuum (at least 3–42), the principles we seek can best be clarified by erecting only three hypotheses. In hypothesis 1, the primordial species had a high number ($n \approx 40$) and ant karyotypes have tended to evolve towards lower numbers (the "fusion" hypothesis). In hypothesis 2, the ancestral number was low ($n \approx 3$) and numbers have tended to increase (the "fission" hypothesis). A third ("modal" hypothesis) has the ancestral number coincident with the present mode.

3. Chromosome Rearrangements Contributing to Ant Karyotype Change

As indicated above, the following rearrangements have been found in ants: (1) Robertsonian rearrangements, (2) pericentric inversions, (3) saltatory changes of constitutive heterochromatin, (4) simple reciprocal translocations, (5) complex translocations accompanied by the loss of genetic material, (6) supernumerary (B-) chromosomes, and (7) chromosome deletions. Types (1), (5), (6) and (7) change chromosome number, whereas (2), (3), (4) and (5) lead only to variation in arm lengths.

Rearrangements (5), (6) and (7) above seem, from our study and previous works, to be rare ones in ant karyotype evolution, and we know of no good evidence for polyploidy playing any role in ant evolution. We therefore infer that chromosome number variation in ants is due primarily to Robertsonian rearrangements, as in many other animals. Note that we use "Robertsonian rearrangement" as a general term to cover the processes of centric fusion, centric fission, and centric dissociation. Robertsonian rearrangements fall into

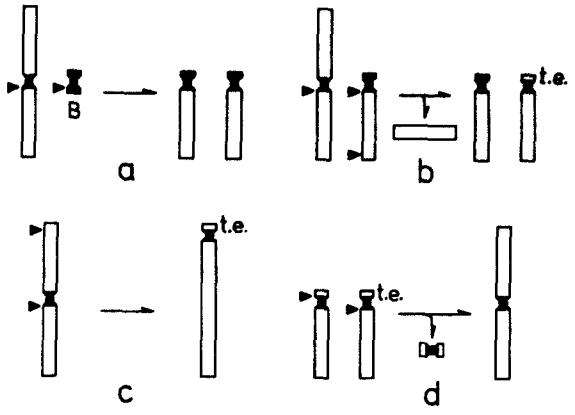

Fig. 24a–d. Problematic rearrangements in ant karyotype evolution. **a** Centric dissociation (Schema II in White, 1973). **b** Centric dissociation (Schema I). **c** Pericentric inversion (M, SM or ST→A or T). **d** Centric fusion accompanying loss of euchromatin. *B*; supernumerary (B-) chromosome. *t.e.* "terminal euchromatic cap". For details see text

two types with respect to the directionality they give to numerical changes: centric fusions reduce chromosome numbers while both centric fissions and centric dissociations increase chromosome number. Some indirect evidence suggests that centric dissociation can be excluded as a significant type of rearrangement in ant evolution. White (1973) gives two alternative mechanisms for centric dissociation, both of which involve the formation of acrocentrics. In one, there is a reciprocal translocation between a metacentric and a minute donor chromosome (a supernumerary- or B-chromosome) which provides a centromere (Fig. 24a). The other scheme involves a three break rearrangement, with one break in a metacentric and two in an acrocentric (Fig. 24b). If the first dissociation mechanism predominated during ant evolution, then a sizeable number of B-chromosome polymorphisms would be expected in ants, especially in species with Robertsonian polymorphisms. We know of only three ant species (all of which are placed in the same subfamily, the Myrmicinae) with B-chromosome polymorphisms: *Leptothorax spinosior* (Imai, 1974), *Aphaenogaster rudis* (Crozier, 1975) and *Podomyrma adelaidae* (this report). B-chromosomes have not been found in those species with Robertsonian polymorphisms. If the second mechanism occurred frequently in ant chromosomal evolution, ant acrocentrics should very often have terminal euchromatic "caps" or "blocks" to their heterochromatic short arms (Fig. 24b). Such caps have not been found; all acrocentrics observed have *totally heterochromatic short arms.* We therefore conclude that centric dissociation has played a minor role, if any, in ant evolution, Robertsonian change being primarily or wholly due to centric fusions and centric fissions.

Among the arm-size-changing rearrangements, our data indicate that tandem growth in constitutive heterochromatin and pericentric inversion are the most important in ant karyotype evolution.

Regarding the "growth" of heterochromatin, there is much evidence that the pericentromeric region, including the minute short arms of acrocentrics,

is substantially different from other sections of the chromosome, and it is generally thought to be composed of constitutive heterochromatin which includes a substantial repetitive DNA component (e.g., Pardue and Gall, 1970; Hsu and Arrighi, 1971; Yunis and Yasmineh, 1971). In this connection, Bradshaw and Hsu (1972) and Imai (1975, 1976) suggest that the short arms of mammalian acrocentrics may undergo tandem "growth" in a manner consistent with the saltatory replication hypothesis of Britten and Kohne (1969). As mentioned above, our data for ants also suggests such tandem growth, although the possibility of a "secondary" elimination of heterochromatic segments has also to be borne in mind. Gene duplication mechanisms possibly important in the saltatory growth of constitutive heterochromatin include unequal crossing-over (Bridges, 1936), errors during replication (Keyl, 1965), and integration of amplified DNA copies into chromosomes (Ritossa, 1973). Although there is some evidence that the heterochromatic segments of *Drosophila melanogaster* contain a few major loci (Cooper, 1959), most of the constitutive heterochromatin resulting from saltatory growth seems to be genetically inert (see, for example, Bostock, 1971; Flam, 1972; Hsu, 1975). If such heterochromatin is indeed genetically inert, as generally supposed, then the gain and loss of heterochromatic segments would be relatively unimportant to the organisms concerned compared with changes in euchromatin.

Pericentric inversions can be operationally divided into two types of opposite directionality, those that move the centromere closer to one end of the chromosome, and those that move it nearer to the middle. For our subsequent argument, we focus on those inversions that either result in or eliminate acrocentrics. Dividing chromosomes into two groups, we can denote meta-, submeta-, and subtelocentrics as M, SM and ST, acrocentrics and telocentrics as A and T, and distinguish four inversion types: p.i. (M, SM or ST→A or T), p.i. (A or T→M, SM or ST), p.i. (A or T→A or T) and p.i. (M, SM or ST→M, SM or ST).

As discussed below, centric fusions and p.i. (M, SM or ST→A or T) have predominated if the "fusion" hypothesis is correct, as against centric fissions and p.i. (A or T→M, SM or ST) if the "fission" hypothesis holds. Growth of constitutive heterochromatin can occur under either hypothesis, but particularly facilitates the course of events under the "fission" hypothesis. It is therefore important to determine which of these rearrangements have occurred during ant evolution if we are to evaluate these—and the "modal"—hypotheses. Direct evidence is lacking, because we can only detect Robertsonian changes and pericentric inversions without regard to the particular rearrangements that give rise to them. Even if some cases could be unambigously elucidated, we would still know little about the overall trend, because all four changes, including centric fusion and centric fission, have occurred in animal karyotype evolution (White, 1973; John and Freeman, 1975).

An ultimate solution to the problem may come from either or both of adequate phylogenetic analyses (e.g., using allozymic, immunological or protein-sequencing techniques) or the quantitative chromosome measurement approach already initiated for mammals (Imai, 1975, 1976). This latter approach has barely begun for ants, and a phylogenetic approach not based on morphological

criteria has yet to be initiated. We can, therefore, here only approach the problem of determining the predominant derectionality of chromosome change in ants indirectly, by considering (1) the likely directionality of pericentric inversions, (2) the range of chromosome numbers in other key groups, particularly hymenopterans, and (3) the non-random distribution of Robertsonian and translocation polymorphisms in ants.

4. On the Likely Overall Directionality of Pericentric Inversions

"Fusion Hypothesis". Under the fusion hypothesis, the ancestral ant karyotype had some 40 pairs of acrocentrics and evolution proceeded thereafter with a predominance of fusions leading to chromosome number reduction. Now, if all 40 of the primordial acrocentrics underwent centric fusions, the resulting karyotype would have had 20 M, SM or ST chromosomes, which would have had to undergo p.i. (M, SM or ST→A or T) before further centric fusions could occur. The lowest ant haploid number is that of *Ponera scabra* (n=3); for this number to have been derived from the primordial karyotype with n= 40 A, a minimum of 37 centric fusions and 34 pericentric inversions would have been required. Because at least some increases in chromosome number would be expected to have occurred in the lineage yielding the 3-chromosome karyotype, even if fusions predominated strongly, the number of fusions, and pericentric inversions involving acrocentrics would have been significantly more frequent than in our minimum estimate. Now, the short arms of acrocentrics formed by p.i. (M, SM or ST→A or T) should be sealed by minute euchromatic segments, which we can call "terminal euchromatic caps" (Fig. 24c). However, almost all of the ant acrocentrics we observed have *totally heterochromatic short arms* (e.g., Figs. 2a, c, 4b–d), and none had heterochromatic short arms with terminal euchromatic caps. There is a similar lack of such caps in those chromosomes with large heterochromatic arms (e.g., Figs. 4e, 5c and f), which probably arose from the minute heterochromatic short arms of acrocentrics through tandem growth of consitituive heterochromatin.

It might be argued that the terminal euchromatic caps expected are somehow always undetectably minute. It is therefore fruitful to consider the likely attrition of genetic material under the fusion hypothesis. When two acrocentrics with terminal euchromatic caps are converted into a metacentric by a centric fusion, their short arms and included loci are eliminated in the form of minute fragments (Fig. 24d). If we take the size of these terminal euchromatic caps to be 0.1–0.6% of the total genome [which Imai (1975) found to be the acrocentric short arm size in mammals], then 3.4–20.4% of the initial genome would have been eliminated by the time the 3-chromosome karyotype was derived. We suggest that genetic losses of this magnitude would be highly deleterious for ants—or any other organisms, though there are rare cases suggesting that such genetic losses have occurred in some ants, e.g., *Rhytidoponera metallica* and *Myrmecia pilosula*. Such a loss of loci could only be avoided if we assume that the undetectable terminal euchromatic caps contain only telomeres, but this seems unlikely.

Indeed, even if the terminal euchromatic caps contain only telomeres, there are still statistical considerations strongly indicating that pericentric inversions converting acrocentrics to other types greatly outnumber those causing the reverse change (Maruyama and Imai, 1974). The argument is simple. Pericentric inversions are two break rearrangements, with one break each in the long and the short arms of the chromosome. If breaks occur at random along the chromosome, and if each inverted chromosome has the same probability of survival, the short arms will usually gain in length from such exchanges. This effect will become stronger as the centromere approaches the end of the chromosome, and should be extremely powerful for acrocentric chromosomes. Therefore, if fusions predominated over fissions (or dissociations) in ant evolution, so that most ant acrocentrics arose following p.i. (M, SM or ST→A or T), then there must be selection favouring pericentric inversions producing acrocentrics (Fig. 24c), because acrocentrics seem more frequent than expected when the statistical argument is taken into account. We could not determine the directionality of most of the pericentric inversions found in this study, but the C-banding and comparative karyotype analyses suggest that p.i. (A or T→M, SM or ST) occurred frequently in ant evolution, being indicated for 10 out of 56 inversions (17.9%) found in the genera *Myrmecia, Camponotus* and *Iridomyrmex* (e.g., Fig. 9). Inversions neither producing nor breaking down acrocentrics, i.e., p.i. (M, SM or ST→M, SM or ST), were of course also found (e.g., Fig. 8a). Our data therefore do not support there having been a statistically-unexpected production of acrocentrics. Nor, for ants (and many other groups), do there seem to be *a priori* grounds for postulating the necessary selective mechanism.

"*Fission Hypothesis*". Under the fission hypothesis, we assume that the primordial ant karyotype had n=3 and that numbers increased thereafter chiefly through the joint action of centric fissions and the tendency of pericentric inversions to convert acrocentrics into other types of chromosomes. Under this model, centric fission converts a M, SM or ST chromosome into two telocentrics, tandem growth of constitutive heterochromatin converts the telocentrics to acrocentrics, pericentric inversions convert the acrocentrics to other types, and the cycle commences again resulting ultimately in four chromosomes corresponding to the original metacentric. Naturally, centric fusions will occur on occasion, but the overall directionality of the process is towards increase of chromosome number. To convert 20 M, SM or ST chromosomes to 40 acrocentrics, a minimum of 37 centric fissions, 58 heterochromatin growths, and 34 pericentric inversions breaking down acrocentrics [p.i. (A or T→M, SM or ST)] would have been required. As discussed above, our C-band analyses suggest that saltatory change in the amount of constitutive heterochromatin occurs frequently in ant evolution, that many acrocentrics have totally heterochromatic short arms, and that most pericentric inversions involving acrocentrics do indeed act to break them down rather than create them. We note that there is a strong tendency for those ant acrocentrics that are involved in Robertsonian polymorphisms to have minute or no short arms, compared with other acrocentrics [see, for example, Imai and Kubota (1972) and the present Fig. 1]. This relationship suggests the production of telocentrics via centric fission. If,

on the other hand, the observed Robertsonian polymorphisms arose following centric fusions, this suggests that centric fusions only occur between acrocentrics with minute short arms. This conclusion seems rather improbable, as acrocentrics with totally heterochromatic short arms should even, or perhaps especially where these are fairly long, be at least as likely to undergo fusion as acrocentrics with minute such arms.

The telomere concept has generally been a highly significant factor in the widespread acceptance of the fusion hypothesis and variants of it (White, 1973), as against hypotheses such as our fission hypothesis. Under the telomere concept, the ends of chromosomes must be "sealed" by telomeres for the chromosomes to be capable of survival, and these telomeres cannot arise *de novo*. The concept as stated thus poses difficulties for the fission hypothesis outlined here. One difficulty arises with centric fission itself, in which a break occurs through a centromere. Such chromosomes will certainly not survive if the resulting centromeres are too small to function adequately, but we suggest that the centromeric region is as capable of duplication as any other part of the chromosome, so that many fissions will not meet this bar. Indeed there is clear evidence (see John and Freeman, 1975) that in many metacentrics the centromere is a functionally duplicate region. The telomeric bar would also rule against our suggested pathway for the excision of terminal heterochromatin (Fig. 3). However, if we assume that ends due to new breaks will often be stable when formed, that is that telomeres *can* arise *de novo,* then the telomeric difficulty is obviated. Cavalier-Smith (1974) has suggested that telomeres are in fact palindromic base sequences. While Cavalier-Smith considered only terminally-located palindromic telomeres, there is evidence that numerous palindromes are present, probably evenly dispersed, in the chromosomes of phylogenetically-diverse organisms (Anonymous, 1974; Church et al., 1974; Thomas et al., 1973; Wilson and Thomas, 1974; Schmid et al., 1975). Such palindromes could well be "dormant telomeres", even if they fulfill some other function such as regulatory regions (Wallace and Kass, 1974). While we are well aware that these findings are still preliminary, and that there may be significant differences in chromosome organization between different groups (imposing differing constraints on chromosome evolution), we feel that the idea of "dormant telomeres" stimulated by the molecular findings is consistent with previous experiments on chromosome ends (see White, 1973).

"Modal Hypothesis". Under the modal hypothesis, the ancestral karyotype is assumed to have had that haploid number that is modal today. Thus, eleven would be assumed to be the ancestral haploid number for ants (Fig. 23). Similar assumptions have been made for *Drosophila* (Patterson and Stone, 1952), for Orthoptera (White, 1973), and for mammals (Matthey, 1973). This hypothesis differs from the fission and fusion hypotheses in that, under both of the other hypotheses, the mode is the "wave of advance" towards lower or higher numbers of lineages following an overall directionality in karyotype change, whereas under the modal hypothesis karyotypes are diverging in both directions from the ancestral position. Even under the modal hypothesis, it can readily be shown that fissions have been far more frequent in ant evolution than fusions. To

minimize the distorting effects of genera in which speciation has occurred without changes in chromosome number, consider the "genus-karyotype" curve shown in Figure 23. When this curve is analysed under the assumption that the ancestral karyotype had n=11, we find that there have been at least 113 fusions and at least 1033 fissions in ant evolution. Unless there are selective constraints favouring haploid numbers close to eleven, this 10:1 ratio of fissions:fusions suggests that, in time, there should be a shift of the mode upwards. Thus, if selective homeostatic effects are absent, the modal hypothesis is therefore one involving history: there has not been enough time for the prevailing processes of chromosomal change to have shifted the mode. Note, however, that the bulk of the known karyotypes *have* been shifted upwards – the median haploid number is 15.

5. Chromosome Number Ranges in Other Groups, Particularly Hymenopterans

The haploid numbers of hymenopterans other than ants range between 5 and 26 (Crozier, 1975). Some of the *Myrmecia* species covered in this study have therefore by far the highest numbers in the Hymenoptera. The highest numbered karyotype, that of *M. brevinoda* (n=42), must therefore have been derived from those of other Hymenoptera by centric fission, granted that, with the possible exception of the bees (see Crozier, 1975), polyploidy and centric dissociation seem unlikely to have contributed significantly to hymenopteran evolution. If fission predominated in the origination of the ancestral ant karyotype, it would seem remarkable that there should have been a subsequent reversal in the direction of karyotype evolution. One could perhaps propose a modification of Cope's Law (Simpson, 1953; Stanley, 1973) under which chromosome numbers tend to decrease but new groups arise only from species with high numbers, but this argument does not seem compelling to us at present. We suggest, on the contrary, that the rules of the game of chromosomal evolution remain relatively stable, at least within major groups, and that the low numbers in other Hymenoptera render it unlikely that the highest ant haploid numbers are ancestral.

With many families still unstudied, it is still possible that very high haploid numbers might be found in hymenopterans other than ants. Let us then consider the ranges of haploid number in other orders. The known ranges of haploid number for various groups are (White, 1973): Diptera, n=2–10, Odonata n=3–15, Lepidoptera n=7–33 (omitting the thin "tail" of this frequency distribution which could conceivably be due to polyploidy), Heteroptera n=3–25, and Orthoptera n=4–12. The range for Hymenoptera is n=3–42, which spread is due to the ants (Fig. 23). Although there is a wide range between the highest chromosome numbers, the lowest numbers are quite similar. We suggest that this pattern indicates, not that high numbers are ancestral, but that the primordial insect karyotype had n=2–3, and that the speed of karyotype evolution differs from group to group, as well as, possibly, the strength of the expected directionality. If instead we postulated that the ancestral insect haploid number

was high, we face the difficulty that the same pattern with regards to highest and lowest numbers that occurs within the class Insecta applies also to comparisons between higher taxa—e.g., mammals have n=3–42 (White, 1973). Todd (1970) suggested that mammal chromosome numbers tend to increase rather than decrease, but bases his argument on phylogenetic reasoning whereas this is unimportant in our analysis.

The natural conclusion to be drawn from these arguments is that the primordial eukaryote might have had but a single pair of chromosomes (n=1), with numbers increasing thereafter. The alternative, that eukaryote evolution began with a high number, seems less likely to us but remains a possibility. If our comparative arguments in this section are correct at any level, then they provide additional support for the fission and modal hypotheses, as formulated above, and do not support the fusion hypothesis.

6. The Non-random Distribution of Robertsonian and Translocation Polymorphisms in Ants

In Figure 23 we show the haploid numbers of karyotypes in which Robertsonian and translocation heterozygotes have been found. The known Robertsonian polymorphisms occur only in higher-numbered species ($n \geqq 17$) and translocation heterozygotes only in lower-numbered species ($n \leqq 16$). There is no overlap in the two distributions (Fig. 23). However, despite the fact that numerical variation is very restricted in low-numbered species, there is a great deal of observable differentiation between the karyotypes of related species due to complex rearrangements, as can be seen by comparing *Pheidole sp. 24* (2n=18, Fig. 19e) with *Ph. sp. 23* (2n=20, Fig. 19d), and *Iridomyrmex sp. 8* (2n=14, Fig. 13l) and *I. sp. 9* (2n=12, Fig. 13m). On the other hand, above the median haploid number chromosome numbers vary markedly between, and sometimes within, species. In this range of haploid numbers, karyotype change has been due chiefly to Robertsonian changes, as in *Camponotus* (2n=32, 38, 46 and 48, Figs. 15 and 16), and *Rhytidoponera* (2n=34–39, 41–48, 50 and 52, Figs. 1 and 17d–i; Crozier, 1969). The differing patterns of karyotype differentiation correlate with the observed distributional differences between Robertsonian and translocation heterozygosities. We suggest that these unexpected patterns may best be interpreted by postulating that numerical change has been, and probably is, occurring rapidly in the high-numbered karyotypes but slowly in the low-numbered ones. Thus, it seems plausible that Robertsonian rearrangements, the principal mechanism leading to numerical change in ants, must occur most frequently in those lineages undergoing the most numerical change. Now, translocations are generally considered to be very rare rearrangements in animal karyotype evolution, and our data seem consistent with this belief. We suggest that translocations arise at a constant, although low, rate in all lineages, but that, in those lineages undergoing rapid numerical change, subsequent Robertsonian changes (fissions) soon eliminate any evidence in the form of continued observable heterozygosity for the translocations. In slowly-changing lineages, however, the evidence of translocations will persist longer. We thus feel that

these asymmetries in the distributions of Robertsonian and translocation polymorphisms are consistent with the fission but not the fusion hypothesis. Under the fusion hypothesis, which assumes that it is the higher-numbered karyotypes that have been relatively static with respect to numerical change, these are the karyotypes that should have the highest levels of translocation heterozygosity. The reverse is true.

7. Fusion and Fission in Ant Karyotype Evolution: Final Remarks

Whether fissions as well as fusions occur in animal evolution has been discussed by a number of authors. John and Hewitt (1966, 1968), John and Freeman (1975) and Imai and Kubota (1975) all conclude that there are no valid arguments against both processes occurring. In this paper we have examined the question of the relative rates of occurrence of the two rearrangements using various lines of evidence, such as the expected great rarity of acrocentric configurations among chromosomes being shaped by pericentric inversions and the different distributions of Robertsonian and translocation polymorphisms. The fission hypothesis is in better accord with our data than is the fusion hypothesis. The modal hypothesis also fits the data, but this hypothesis, under our interpretation, is similar to the fission hypothesis and is operationally hard to distinguish from it. Our data indicate that the principle rearrangements in ant evolution have been centric fission, centric fusion (but occurring less frequently than fission), pericentric inversions (usually moving the centromere away from the chromosome ends), and tandem growth of constitutive heterochromatin.

Although we believe that centric fissions outnumber fusions in ant evolution, the latter certainly do occur. Well-documented examples have been found in the case of *Pheidole nodus* (Imai and Kubota, 1975) and the *Xiphomyrmex* species reported here (Fig. 20f–h). Another possible case, requiring phylogenetic analysis for verification, is that of the Western forms of *Rhytidoponera metallica*, which, with $n=12$, may have been derived from the Eastern form with $n=17$–23.

Under our model, centric fusions will usually occur following centric fissions. Following a fission, there can be no further change until a short arm has been generated by the growth of heterochromatin. Pericentric inversions are then likely to convert the chromosomes from acrocentrics to other types. Fusion, if it is to occur, will do so before this conversion takes place, but will often lose the "race". However, although such fusions will often only reconstitute the original chromosome, it is possible to envisage fusion between arms not originally associated. It is tempting to speculate that such new linkage rearrangements might be an important source of evolutionary novelties.

Finally, we emphasise that we have above but sketched a complex model that one of us (H.T.I) will develop in greater detail in further publications. We are well aware that karyotype evolution depends on a number of parameters, and that the various constraints involved may differ in relative importance from group to group. Thus, where duplication mechanisms leading to growth of heterochromatic and centromeric regions operate slowly, the ratio of fusions to fissions will be higher than where such mechanisms operate rapidly. It

is therefore possible for fusions to predominate, but this would imply a change in the "ground rules" of karyotype evolution in the lineage concerned compared with its ancestors. One of us (R.H.C.) suggests that a corollary of these arguments is that chromosome number modes may sometimes neither represent "waves of advance" towards one end of the spectrum or the other, nor indicate historical persistence of the ancestral number, but rather represent equilibria. These equilibrium points would be determined by the relative rates of occurrence of fissions and fusions, and not by selection acting directly on chromosome size, number, or arm ratio. The overall rate of fixation of rearrangements, and hence the speed of karyotype evolution, is of course modified by selection and such factors as population size and social structure (A.C. Wilson et al., 1975). However, rather than supporting the presence of such an equilibrium in ants, our data indicate a probable continuing predominance of centric fissions.

We believe that the models we have considered, and especially our fission hypothesis, are applicable to groups other than ants, and hope that in presenting these models we have assisted in the formulation of a new paradigm for animal karyotype evolution.

Acknowledgements. We thank E.J. Brough, P.J.M. Greenslade, R.B. Halliday, M. Kubota, L. Lehoczky, C.J. Orton, K. Stante, and P.S. Ward for assistance in obtaining material, B.B. Lowery for identifying the *Myrmecia* species, the New South Wales National Parks and Wildlife Service for permission to collect in national parks, and B. John and G.L. Gabor Miklos for helpful discussion. H.T. Imai's visit to Australia was supported by a Grant-in-Aid (No. 49-ko-203) from the Ministry of Education, Japan.

References

Anonymous: Palindromes in eukaryotic DNA. Nature (Lond.) **248**, 733–734 (1974)

Bostock, C.: Repetitious DNA. In: Advanc. Cell Biology (D.M. Prescott, L. Goldstein and E. McConkey, eds.) **2**, 153–223 (1971)

Bradshaw, W.W., Hsu, T.C.: Chromosomes of Peromyscus (Rodentia, Cricetidae) III. Polymorphism in Peromyscus maniculatus. Cytogenetics **11**, 436–451 (1972)

Bridges, C.B.: The bar "gene" a duplication. Science **83**, 210–211 (1936)

Britten, R.J., Kohne, D.E.: Implications of repeated nucleotide sequences. In: Handbook of molecular cytology (A. Lima-de-Faria, ed.), pp. 37–51. Amsterdam: North-Holland Publ. Comp. 1969

Brown, W.L.: Revisionary notes on the ant genus Myrmecia of Australia. Bull. Mus. comp. Zool. Harvard **111**, 1–35 (1953)

Brown, W.L.: A review of the ants of New Zealand (Hymenoptera). Acta Hymenopterologica **1**, 1–50 (1958)

Brown, W.L., Taylor, R.W.: Superfamily Formicoidea. In: The insects of Australia (I.M. Mackerras ed.), pp. 951–959. Melbourne: Melbourne Univ. Press 1970

Cavalier-Smith, T.: Palindromic base sequences and replication of eukaryote chromosome ends. Nature (Lond.) **250**, 467–470 (1974)

Church, R.B., Ryskov, A.P., Georgiev, G.P.: Structure of nuclear pre-mRNA. VI. "Reverse repeats" in animal DNA and their hybridization with double-stranded regions of pre-mRNA. Molek. Biologiya **8**, 503–509 (1974)

Clark, J.: The Formicidae of Australia. I. Subfamily Myrmeciinae. Melbourne: C.S.I.R.O. 1951

Cooper, K.W.: Cytogenetic analysis of major heterochromatic elements (especially Xh and Y) in Drosophila melanogaster, and the theory of "heterochromatin". Chromosoma (Berl.) **10**, 535–588 (1959)

Crozier, R.H.: An acetic acid dissociation, air-drying technique for insect chromosomes, with aceto-lactic orcein staining. Stain Technol. **43**, 171–173 (1968a)
Crozier, R.H.: Cytotaxonomic studies on some Australian Dolichoderine ants (Hymenoptera: Formicidae). Caryologia (Firenze) **21**, 241–259 (1968b)
Crozier, R.H.: Chromosome number polymorphism in an Australian ponerine ant. Canad. J. Genet. Cytol. **11**, 333–339 (1969)
Crozier, R.H.: Karyotypes of twenty-one ant species (Hymenoptera; Formicidae), with reviews of the known ant karyotypes. Canad. J. Genet. Cytol. **12**, 109–128 (1970a)
Crozier, R.H.: Pericentric rearrangement polymorphism in a North American dolichoderine ant (Hymenoptera: Formicidae). Canad. J. Genet. Cytol. **12**, 541–546 (1970b)
Crozier, R.H.: Hymenoptera. Animal cytogenetics (B. John, ed.), vol. 3. Insecta 7. Berlin, Stuttgart: Gebrüder Borntraeger 1975
Crozier, R.H.: Genetic differentiation between populations of the ant Aphaenogaster "rudis" in the southeastern United States. Genetica (den Haag) **46**, (in press)
Flamm, W.G.: Highly repetitive sequences of DNA in chromosomes. Int. Rev. Cytol. **32**, 1–51 (1972)
Forel, A.: Formicides australiens reçus de MM. Froggatt et Rowland Turner. Rev. Suisse Zool. **18**, 1–94 (1910)
Hsu, T.C.: Chromosome structure a possible function of constitutive heterochromatin: The body-guard hypothesis. Genetics **79**, 137–150 (1975)
Hsu, T.C., Arrighi, F.E.: Distribution of constitutive heterochromatin in mammalian chromosomes. Chromosoma (Berl.) **34**, 243–253 (1971)
Imai, H.T.: Proposal of a new criterion for the classification of mammalian chromosomes. Ann. Rep. Nat. Inst. Genetics (Japan) No. **23**, 50–52 (1973)
Imai, H.T.: B-chromosomes in the myrmicine ant, Leptothorax spinosior. Chromosoma (Berl.) **45**, 431–444 (1974)
Imai, H.T.: Evidence for non-random localization of the centromere on mammalian chromosomes. J. theor. Biol. **49**, 111–123 (1975)
Imai, H.T.: Further evidence and biological significance for non-random localization of the centromere on mammalian chromosomes. J. theor. Biol. **61** (in press 1976)
Imai, H.T., Kubota, M.: Karyological studies of Japanese ants (Hymenoptera, Formicidae). III. Chromosoma (Berl.) **37**, 193–200 (1972)
Imai, H.T., Kubota, M.: Chromosome polymorphism in the ant, Pheidole nodus. Chromosoma (Berl.) **51**, 391–399 (1975)
John, B., Freeman, M.: Causes and consequences of Robertsonian exchange. Chromosoma (Berl.) **52**, 123–136 (1975)
John, B., Hewitt, G.M.: Karyotype stability and DNA variability in the Acrididae. Chromosoma (Berl.) **20**, 155–172 (1966)
John, B., Hewitt, G.M.: Patterns and pathways of chromosome evolution within the Orthoptera. Chromosoma (Berl.) **25**, 40–74 (1968)
Keyl, H.-G.: Duplikationen von Untereinheiten der chromosomalen DNS während der Evolution von Chironomus thummi. Chromosoma (Berl.) **17**, 139–180 (1965)
Levan, A., Fredga, K., Sandberg, A.A.: Nomenclature for centromeric position on chromosomes. Hereditas (Lund) **52**, 201–220 (1964)
Maruyama, T., Imai, H.T.: Karyotype evolution as a stochastic process. 45th Annual Meeting Zool. Soc. Japan, Sapporo 1974
Matthey, R.: The chromosome formulae of eutherian mammals. In: Cytotaxonomy and vertebrate evolution (A.B. Chiarelli and E. Capanna, eds.), pp. 531–616. London: Academic Press 1973
Pardue, M.L., Gall, J.G.: Chromosomal localization of mouse satellite DNA. Science **168**, 1356–1358 (1970)
Patterson, J.T., Stone, W.S.: Evolution in the genus Drosophila. New York: Macmillan 1952
Ritossa, F.: Crossing-over between X and Y chromosomes during ribosomal DNA magnification in Drosophila melanogaster. Proc. nat. Acad. Sci. (Wash.) **70**, 1950–1954 (1973)
Schmid, C.W., Manning, J.E., Davidson, N.: Inverted repeat sequences in the Drosophila genome. Cell **5**, 159–172 (1975)
Simpson, G.G.: The major features of evolution. New York: Columbia University Press 1953
Stanley, S.M.: An explanation for Cope's rule. Evolution (Lawrence, Kans.) **27**, 1–26 (1973)

Thomas, C.A., Pyeritz, R.E., Wilson, D.A., Dancis, B.M., Lee, C.S., Bick, M.D., Huang, H.L., Zimm, B.H.: Cyclodromes and palindromes in chromosomes. Cold Spr. Harb. Symp. quant. Biol. **38**, 353–370 (1973)

Todd, N.B.: Karyotypic fissioning and canid phylogeny. J. theor. Biol. **26**, 445–480 (1970)

Wallace, B., Kass, T.L.: On the structure of gene control regions. Genetics **77**, 541–558 (1974)

Wheeler, W.M.: Ants. Their structure, development and behavior. New York: Columbia Univ. Press 1965

White, M.J.D.: Animal cytology and evolution, 3rd ed. London: Cambridge University Press 1973

Wilson, A.C., Bush, G.L., Case, S.M., King, M.-C.: Social structuring of mammalian populations and rate of chromosomal evolution. Proc. nat. Acad. Sci. (Wash.) **72**, 5061–5065 (1975)

Wilson, D.A., Thomas, C.A., Jr.: Palindromes in chromosomes. J. molec. Biol. **84**, 115–144 (1974)

Wilson, E.O.: The insect societies. Cambridge, Massachussetts: Harvard University Press 1971

Yunis, J.J., Yasmineh, W.G.: Heterochromatin, satellite DNA and cell function. Science **174**, 1200–1209 (1971)

Received October 6, 1976 / Accepted October 10, 1976 by B. John
Ready for press October 30, 1976