# The Last Twenty Years of Parasitic Hymenoptera Karyology: An Update and Phylogenetic Implications

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Abstract.—A survey of karyological data in the parasitic Hymenoptera shows a considerable uniformity in chromosome numbers within higher taxonomic groupings, with most chalcidoids having n = 5-6 and most cynipoids, ichneumonoids and those very few species of Diapriidae and Scelionidae examined to date having n = 10-12. The Encyrtidae and Eurytomidae differ from other chalcidoids examined to date in having higher n values (8–11). The braconid subfamilies Aphidiinae (n = 4-7), Charmontiinae (n = 5) and Exothecinae (n = 6) are shown to have lower values than do other braconids. New data are presented for 32 species, including the first records of chromosome numbers for the Eupelmidae, Bethylidae, Diapriidae, Ormyridae, the ichneumonid subfamilies Banchinae, Pimplinae, and Tryphoninae, and the braconid subfamilies Aphidiinae, Charmontinae, Exothecinae, Macrocentrinae, Meteorinae, and Miracinae. Chromosome number data are discussed in relation to current views on Hymenoptera phylogeny.

#### **INTRODUCTION**

There has long been a tendency for taxonomists to ignore many potential sources of systematic evidence in favour of more traditional morphological, and nowadays molecular, data. Whilst this lack has not been ubiquitous amongst taxonomists, where it has occurred it may have been due to a number of reasons including lack of understanding of novel characters, lack of facilities for investigating them and lack of availability of information about such characters. In this respect, it is not surprising that chromosome number and other karyological features have played little role in considerations of hymenopteran phylogeny, and this is especially true for the parasitic families. Reasons for this are manifold. Hymenopteran chromosomes have traditionally been studied in immature stages in which dividing cells are common, but larvae of parasitic wasps are perhaps especially difficult to identify and obtain except when species are in culture. Recent developments in the study of hymenopteran chromosomes (Imai *et al.* 1988, Baldanza *et al.* 1991b), have opened the possibility of greatly expanding our knowledge of parasitic wasp karyology. Further, it is now possible to obtain good results working with adult tissues such as ovaries (Gokhman 1985, 1990a), and such protocols have allowed, for the first time, an examination of chromosome number and structure in natural populations.

Karyological studies have, nevertheless, played an important role in the systematics and species level taxonomy of various groups of the non-parasitic Hymenoptera including, sawflies, ants, bees and social wasps (principally Polistinae) and these have been reviewed in detail elsewhere (see for example, Kerr 1972, Kerr and da Silveira 1972, Naito 1982, Imai et al. 1977, Moritz 1986, Pompolo and Takahashi 1987, 1990, Hoshiba, Matsuura and Imai 1989, Costa et al. 1993). Wide variation in *n* is well known among the ants (Formicidae) and ranges from 1 to 47 (Imai and Taylor 1989, Imai et al. 1990), but is modal at 11 (Imai et al. 1988). Indeed, the greater part of this range can even be found within the single ant genus *Myrmecia*. However, within the parasitic Hymenoptera there appears to be be rather more consistency, and for most families the observed range of variation is far narrower.

Outside of the Hymenoptera, both chromosome numbers, structure and size have all been found useful in phylogenetic reconstruction, usually but not exclusively in combination with other morphocharacters, and this has been particularly true of plant systematics. For example, George & Geethamma (1992) have recently proposed a phylogeny of jasmines based on chromosome numbers and assumed polyploidy events. Among the insects, chromosome numbers have been used extensively in the systematic treatment and phylogeny of the Hemiptera (Blackman 1980, Emeljanov & Kirillova 1989, 1991). Kuznetsova (1985), for example, has concluded that the homopteran subfamily Orgeriinae is monophyletic based on an autosomal fusion giving 2n=27 (in males) compared with the plesiomorphic value of 2n=29 (in males), and similarly that the tribe Almanini of the Orgeriinae is monophyletic on the basis of an autosome-sex chromosome fusion (giving 2n=26).

In this paper we review the current state of knowledge of the karyology of parasitic wasps and, in addition, provide new data for approximately 32 taxa including the first records of chromosome numbers for the Eupelmidae, Bethylidae, Diapriidae, Ormyridae, the ichneumonid subfamilies Banchinae, Pimplinae, and Tryphoninae, and the braconid subfamilies Aphidiinae, Charmontinae, Doryctinae, Exothecinae, Macrocentrinae, Meteorinae, and Miracinae.

## MATERIALS AND METHODS

New karyotypic data were obtained using ovarian tissues of adult wasps which were either collected from the wild at Silwood Park, Berkshire, U.K., during early September 1995, or obtained from laboratory cultures. Chromosome preparations were obtained according to the schedule described in Appendix I. Voucher specimens for Ichneumoninae are deposited in the collection of Moscow State University, those for other taxa are in the Natural History Museum, London.

## **RESULTS AND DISCUSSION**

The last review of chromosome number in the parasitic Hymenoptera was by Crozier (1975) at which time values were known for only about twenty species (with data published before 1930 not included for the reason of insufficient reliability); his data are summarized in Figure 1. Since then, chromosomes of nearly 140 additional species have been studied (Gokhman 1994), and for this review we have investigated 32 more in order to help fill in a number of gaps and to confirm some previous findings. These data are presented in Table 1 and summarized in Figure 2. The great majority of published chromosome numbers come from studies on the ichneumonid subfamily Ichneumoninae by Gokhman (1985, 1987, 1989, 1990a, 1990b, 1991a, 1991b, 1993a), though they also include a substantial body of evidence especially relating to various families of Chalcidoidea. Within the better studied parasitoid families (i.e. Ichneumonidae, Cynipidae, Torymidae) chromosome numbers were found to be relatively stable, and were generally uniform at the generic level. This contrasts markedly with observations for many aculeate Hymenoptera which often show striking chromosomal variability even within genera (Imai and Taylor 1989, Imai et al. 1990).

The apparent bimodality in haploid number of parasitic Hymenoptera apparent in Crozier's small sample (Fig. 1) now appears to be well founded (Fig. 2). However, the exact modal numbers are slightly different (n = 5 and 11 compared with n = 5 and 10 of Crozier), probably due to the strong bias in favour of the ichneumo-



Fig. 1. Histogram of haploid chromosome numbers for parasitic Hymenoptera, data modified after Crozier (1975) to take into account subsequent taxonomic changes.

nid subfamily Ichneumoninae in the present enlarged data set. As will be apparent from the figures, the peak in haploid values around 5 largely comprises members of the Chalcidoidea and there is only slight overlap between these and the other peak centred around 11. The same is true, though less pronounced, when the data are plotted for genera rather than species (Figure 3: each *n* value in a genus being represented only once in the histogram). Members of three of the chalcidoid families investigated to date, the Eurytomidae (Eurytoma), Encyrtidae (6 species in three genera) and Aphelinidae (one species, Pteroptrix (= Archenomus) orientalis Silvestri, out of 5 investigated; see below) differ from other chalcidoids in having haploid numbers ranging between 8 and 11, whilst members of the braconid subfamilies Aphidiinae (Aphidius, Diaretiella, Ephedrus, Praon), Charmontinae (Charmon) and Exothecinae (Rhysipolis) are atypical in having haploid numbers of 4 to 7, 6 and 5 respectively, compared with other ichneu-

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monoids whose haploid n values range from 8 to 17.

# GENOMIC SIZE AND ITS IMPLICATIONS

Rasch et al. (1975, 1977) calculated the haploid genomic size of the braconid wasps, Habrobracon juglandis and H. serinopae both to be 0.15–0.16  $\times$  10<sup>-12</sup>g DNA and that of the pteromalid, Nasonia (as Mormoniella) vitripennis to be 0.33–0.34  $\times$ 10<sup>-12</sup>g. These values translate (using an average molecular weight of a base pair as 660) to base pair numbers of approximately  $1.4 \times 10^8$  and  $3.0 \times 10^8$  base pairs for the ichneumonoid and the chalcidoid respectively. More recently, Bigot et al. (1991) using DNA reassociation kinetics calculated the haploid genomic sizes of the ichneumonid Diadromus pulchellus and the chalcidoid, Eupelmus vuilleti, as  $1-2 \times$  $10^9$  base pairs and  $10 \times 10^9$  base pairs respectively. Unfortunately, no data for the numbers of chromosomes are available for Eupelmus vuilleti but we have investigated

Table 1. Chromosome number in parasitic wasps

Taxon	·	2n*	Reference(s) <sup>†</sup>
Diaprioidea		F. 6. 1	and an and the state of the state of the
Diapriidae			
Belyta depressa Thomson	8	16	present papertt
Cinetus lanceolatus Thomson	10	20	present paper
Scelionoidea			
Scelionidae			
Telenomus fariai Lima	10	20	Dreyfus & Breuer 1944
Chalcidoidea			
Aphelinidae			
Aphelinus mali Haldeman	5	10	Viggiani 1967
Aphytis mytilaspidus (Le Baron)	5	10	Rössler & De Bach 1973
Archenomus orientalis Silvestri	11	22	Baldanza <i>et al.</i> 1991a
Encarsia berlesei Howard	5	10	Baldanza <i>et al.</i> 1991b
Encarsia pergandiella Howard	6	12	Hunter et al. 1993
Chalcididae			
Brachymeria intermedia Nees	3	6	Hung 1986
B. lasus Burks	5	10	Hung 1986
B. ovata Say	5	10	Hung 1986
Dirhinus himalayanus Westwood	5	10	Amalin et al. 1988
Encyrtidae			
Ageniasnis fuscicollis Dalman	c. 10	c. 20	Silvestri 1908: Martin 1914
Conidosoma huussoni Mavr	12	24	Silvestri 1914
C gelechige Howard	11	22	Hegner 1915
C gelechine Howard	10	20	Patterson 1921
C truncatellum (Dalman)	10	20	Hunter & Bartlett 1975
(= ?floridanum Ashmead)	10	-0	Hunter & Duritett 1970
C floridanum Ashmead	8	16	Leiby 1922: Patterson 1917, 1921.
C. flor aunan Asiancad	0	10	Patterson & Porter 1917
C floridanum Ashmead	11	22	Strand & Ode 1990
Anognagurus longzi (DeSantis)	10	20	Diiken 1991
Fulophidae	10	20	Dijken 1991
Cirrospilus diallus Walkor	6	12	present paper
Colochimeus florus Walker	6	12	Diiketra 1986
Melittohia chaluhii Ashmead	5	10	Schmieder 1938
Tatrastichus gigas Burks	6	10	Coodpasture 1974
T megachilidic Burks	6	12	Coodpasture 1974
Fundmidae	0	12	Goodpasture 1974
Anastatus satalanisus Polivar	5	10	procent paper
Macronaura macicularis (Potzius)	5	10	present paper
Europeuro descutoris (Retzius)	5	10	present paper
Eurytoinidae	0	10	present paper
European antiference Ashmand	9	10	Coodposture 1974
Lougopidag	10	20	Goodpasture 1974
Leucospidae	(	10	Candrasture 1074
Ceucospis affinis Say	0	12	Goodpasture 1974
Omyridae	20	210	
Drangen ali dag	:0	:12	present paper
Automaticae	7	14	
Anisopteromatus catanarae (Howard)	7	14	present paper
Coelopistnia extenta (vvalker)	5	10	present paper
Dioracnys sp.	5	10	Goodpasture 1974
Lariophagus aistinguendus Foerster	5	10	Gershenzon 1968
Musciaijurax zaraptor Legner	5	10	Goodpasture 1974
Nasonia vitripennis (Walker)	5	10	Gershenzon 1946, 1968; Pennypacker 1958; Whiting 1960, 1968; Wahr- man & Zhu 1993

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#### Table 1. Continued

Taxon	n*	2 <i>n</i> *	Reference(s)†
Nasonia vitripennis (Walker)	5 + 0 - 1B	10	Nur et al. 1988; Werren 1991
Nasonia vitripennis (Walker)	6	12	Goodpasture 1974
Pteromalus puparum L.	5	10	Guhl & Dozortseva 1934, Dozortseva 1936
P. venustus Walker	5	10	McDonald & Krunic 1971
Torymidae			Contraction of the second s
Monodontomerus clementi Grissell	6	12	Grissell 1973b: Goodpasture 1975a
M. montivagus Ashmead	6	12	Goodpasture 1975a
M. obscurus (Westwood)	4	8	Goodpasture 1975a
M. obscurus (Westwood)	6	12	McDonald & Krunic 1971
M. saltuosus Grissel	5	10	Grissell 1973b; Goodpasture 1975a
Torymus baccharidis Huber	6	12	Goodpasture & Grissell 1975
T. californicus (Ashmead)	6	12	Goodpasture & Grissell 1975
T. capillaceus Huber	6	12	Goodpasture & Grissell 1975
T. koebelei Huber	5	10	Goodpasture & Grissell 1975
T. occidentalis Huber	6	12	Goodpasture & Grissell 1975
T. tubicola Osten-Sacken	6	12	Goodpasture & Grissell 1975
T. umbilicatus Gahan	5	10	Goodpasture & Grissell 1975
T. vesiculi Moser	6	12	Goodpasture & Grissell 1975
T. warreni (Cockerell)	6	12	Goodpasture & Grissell 1975
Trichogrammatidae			1
Trichogramma chilonis Ischii	5	10	Hung 1982
T. deion Pinto & Oatman	5	10	Stouthamer & Kazmer 1994
T. dendrolimi Matsumura	5	10	Liu & Xiong 1988
T. evanescens Westwood	5	10	Hung 1982
T. nubialale Ertle & Davis	5	10	Hung 1982
T. pretiosum Riley	5	10	Hung 1982; Stouthamer & Kazmer 1994
T. spp. (7 strains)	5	10	Fukada & Takemura 1943 (cited by Hung 1982)
Cynipoidea			0 ,
Cynipidae			
Andricus curvator Hartig	10	20	Dodds 1938; Sanderson 1988
A. fecundator Hartig	10	20	Dodds 1938
A. kollari Hartig	10	20	Hogben 1920; Sanderson 1988
A. quercuscalicis Burgsdorf	10	?20	Sanderson 1988
Aulacidea hieracii Bouche	10	20	Dodds 1938
Biorrhiza pallida Olivier	10	20	Dodds 1938
Callirhytis palmiformis Ashmead	10	20	Goodpasture 1979b
Cynips divisa Hartig	10	20	Sanderson 1988
Diastrophus nebulosus Osten-Sacken	10	20	Hegner 1915 <sup>1</sup>
Diplolepis elganteriae Hartig	9	27(3n)	Sanderson 1988
D. nervosum Curtis	9	18	Sanderson 1988
D. rosae L.	9	18	Henking 1892; Stille & Dävring 1980;
			Sanderson 1988
D. rosae L.	?12	?24	Schleip 1909
D. spinosissimae Girault	9	18	Sanderson 1988
Dryocosmus kuriphilus Yasumatsu	10	20	Abe 1994
Dryophanta erinacea Mayr	c. 12	24	Wieman 1915
Neuroterus laeviusculus Schenck	10	20	Sanderson 1988
N. numismalis Fourcroy	10	20	Dodds 1938
N. quercusbaccarum L.	10	20	Doncaster 1910, 1911, 1916; Dodds 1938
Trigonaspis megaptera Panzer	10	20	Dodds 1938
Xestophanes potentillae Retzius	10	20	Dodds 1938

# Table 1. Continued

Taxon	<i>n</i> *	2 <i>n</i> *	Reference(s)†
Eucoilidae			
Pseudeucoila bochei Weld	10	20	Jungen cited in Crozier 1975
Ichneumonoidea			
Braconidae			
Aphidius rhopalosiphi De Stefani Perez	7	14	present paper
Biosteres carbonarius (Nees)	14	28	present paper
Charmon cruentatus Haliday	5	10	present paper
Cotesia glomeratus (L.) (as Apanteles)	12	24	Hegner 1915 <sup>1</sup>
Dacnusa sp.	17	34	present paper
Diaeretiella rapae McIntosh	6	12	present paper
Ephedrus sp.	?7	?14	present paper
Habrobracon hebetor Say	10	20	Torvik-Greb 1935
H. juglandis Ashmead <sup>2</sup>	10	c. 20	Torvik-Greb 1935; Speicher 1937; Rasch et al 1977
H juglandis Ashmead <sup>2</sup>	11	22	Whiting 1927
H nectinonhorae Watanaho	10	20	Inaba cited in Makino 1959
H. serinopag Ramakrishna	10	20	Rasch et al 1977
Heterospilus prosonidis Viorock	17	20	Rascil et ul. 1977
Macrocentrus thoracisus (Noos)	17	26	present paper
Mataorus parsicolor Mosmool	13	20	present paper
Meteorus curator Thunhora	0	20	present paper
Meteorus gyrator Thunberg	10	20	present paper
Miner an	10	20	present paper
Dhaanaama nansimilis Baran	10	20	Primes & Stage (sited has Creation 1077)
Praenocarpa persimilis Papp	17	34	Prince & Stace (cited by Crozier 1977)
Praon abjectum Haliday	4	8	present paper
Knystpolis aecorator (Handay)	6	12	present paper
Ichneumonidae			C 11 4005
Aethercerus discolor Wesmael	11	22	Gokhman 1985
Ae. aispar Wesmael	12	24	Gokhman 1991
Ae. nitidus Wesmael	11	22	Gokhman 1990a
Ae. ranini Gokhman	11	22	Gokhman 1991
Agrothereutes extrematus (Cresson)	10	20	Koonz 1939
Aoplus pulchricornis (Gravenhorst)	13	26	Gokhman 1990b
Aptesis puncticollis (Thomson)	8	16	Gokhman 1990a
Baeosemus dentifer Gokhman	11	22	Gokhman unpubl. obs.
Baranisobas ridibundus (Gravenhorst)	11	22	Gokhman 1990b
Chasmias motatorius (Fabricius)	17	34	Gokhman 1985
Coelichneumon cyaniventris (Gravenhorst)	13	26	Gokhman 1990a
C. sugillatorius (L.)	13	26	Gokhman 1990a
Colpognathus celerator (Gravenhorst)	11	22	Gokhman unpubl. obs.
Cratichneumon viator (Scopoli)	14	28	Gokhman 1985
Diadromus prosopius Holmgren	11	22	Gokhman 1990a
D. pulchellus Wesmael	11	22	Hedderwick et al. 1985
D. subtilicornis (Gravenhorst)	11	22	Gokhman 1990b
D. troglodytes (Gravenhorst)	11	22	Gokhman 1990a
D. varicolor Wesmael	11	22	Gokhman pers. obs.
Dicaelotus sp. nr. parvulus (Gravenhorst)	11	22	Gokhman 1990a
D. pumilis (Gravenhorst)	11	22	present paper
Diphyus latebricola (Wesmael)	12	24	Gokhman 1990a
D. raptorius (L.)	12	24	Gokhman 1990a
Dirophanes callopus (Wesmael)	9	18	Gokhman 1987
D. fulvitarsis (Wesmael)	10	20	Gokhman 1990a,b
D. invisor (Thunberg)	10	20	Gokhman 1987
Dyspetes arrogator Heinrich	10	20	present paper

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# Table 1. Continued

Taxon	n*	2 <i>n</i> *	Reference(s)†
Ephialtes manifestator (L.)	15	30	present paper
Eurylabus torvus Wesmael	10	20	Gokhman 1987
Gelis sp.	13	26	present paper
Glypta lapponica Holmgren	9	18	present paper
Heterischnus nigricollis (Wesmael)	11	22	Gokhman 1990b
H. truncator (Fabricius)	11	22	Gokhman unpubl. obs.
Homotherus locutor (Thunberg)	11	22	Gokhman 1990b
Ichneumon albiger Wesmael	12	24	Gokhman 1990a
I. amphibolus Kriechbaumer	12	24	Gokhman 1990b
I. bucculentus Wesmael	12	24	Gokhman 1993a
I. confusor Gravenhorst	12	24	Gokhman 1985
I. crassifemur Thomson	12	24	Gokhman 1985
I. croceipes Wesmael	12	24	Gokhman 1990b
I. extensorius L.	12, 13	24, 26 <sup>3</sup>	Gokhman 1993a
I. cf. extensorius L.	11	22	Gokhman 1990a
I. formosus Gravenhorst	11	22	Gokhman 1990a
I. gracilentus Wesmael	12	24 (25)	Gokhman 1993a
I. gracilicornis Wesmael	11	22	Gokhman 1990a
I. ingratus Hellén	12	24	Gokhman 1990b
I. inquinatus Wesmael	13	26	Gokhman 1993a
I. insidiosus Wesmael	12	24	Gokhman 1990a
I. lugens Gravenhorst	12	24	Gokhman unpubl. obs.
I. melanotis Holmgren	12	24	Gokhman 1990a
I. minutorius Desvignes	12	24	Gokhman 1987
I. molitorius L.	12	24	Gokhman 1990a
I. nereni Thomson	11	22	Gokhman 1990a
I. sarcitorius L.	11	22	Gokhman 1990a
I. stramentarius Gravenhorst	12	24	Gokhman 1987
I. submarginatus Gravenhorst	10	20	Gokhman 1990a
I. subquadratus Thomson	12	24	Gokhman 1990b
I. suspiciosus Wesmael	12	24 (26)	Gokhman 1993a
I. validicornis Holmgren	12	24	Gokhman unpubl. obs.
Lissonota sp.	11	22	present paper
Mastrus smithu (Packard)	13	26	Koonz 1936
Oronotus binotatus (Gravenhorst)	11	22	Gokhman 1987
Orthocentrus sp.	14	28	Gokhman 1990a
Orthopelma mediator (Thunberg)	11	22	Hogben 1920
Patrocloides chalybeatus (Gravenhorst)	8	16	Gokhman 1993a
Phaeogenes melanogonos (Gmelin)	11	22	Gokhman 1990b
Ph. nigridens Wesmael	11	10	Gokhman 1990b
Ph. semioupinus (Gravenhorst)	9	18	Gokhman unpubl. obs.
Ph. spiniger (Gravenhorst)	11	10	Goknman 1990a
Polysphincia tuberosa Gravenhorst	9	10	Calibration water blacks
Stavishnauman substar (Schrank)	9	18	Gokham 1085
Sucaonia sicaria (Crayonhorat)	14	20	Cokham 19000h
Suspasis alboauttata (Cravenborst)	11	22	Coldman 199900
S scutellator (Gravenhorst)	11	22	Cokhman unnuhl ohs
Tucherus australogeminus Cokhman	11	22	Gokhman 1991
T. bellicornis (Wesmael)	10 11	20 (21)	Gokhman 1989
T. dilleri Ranin	11	20 (21)	Gokhman 1989
T. fuscicornis (Wesmael)	11	22	Gokhman 1990b
T. ischiomelinus (Gravenhorst)	9	18	Gokhman 1991
(cruteratorot)			

Ι	ab	le	1.	Continued

Taxon	n*	2 <i>n</i> *	Reference(s)†
T. ophthalmicus (Wesmael)	11	22	Gokhman 1990a
T. osculator (Thunberg)	11	22	Gokhman 1989
T. suspicax (Wesmael)	11	22	Gokhman 1987
Venturia canescens (Gravenhorst)	11	22	Speicher 1937
Virgichneumon digrammus (Gravenhorst)	17	34	Gokhman 1990a
V. faunus (Gravenhorst)	11	22	Gokhman 1990a
Vulgichneumon saturatorius (L.)	9	18	Gokhman 1987
Chrysidoidea			
Bethylidae			
Epyris niger Westwood	14	28	present paper
Laelius utilis Cockerell	10	20	present paper

\* In papers which only quote *n* or 2*n*, the other value has been surmised and is given in italics.

<sup>+</sup> Data appearing in works before 1930 should be considered with great caution as most resulted from histological rather than cytological protocols, involving sectioned material rather than squash preparations and also often involving fixation techniques not well suited for the study of chromosomes, though some of these earlier findings are clearly correct including the oldest one (Henking 1892).

tt For some new data we were not able to obtain an unambiguous chromosome number but our best approximation is presented (data indicated in table with a "?") as in some cases these still provide potentially valuable information.

<sup>1</sup> Hegner (1915) did not provide a definitive statement on chromosome number and the data here come from his rather stylized figures; such data need therefore to be considered with extreme caution.

<sup>2</sup> Some workers consider *H. juglandis* to be a junior synonym of *H. hebetor*, however, this is not yet absolutely confirmed and therefore we prefer to keep these records separate.

<sup>3</sup> A single, probably aneuploid, female specimen with 25 chromosomes has also been found.



Fig. 2. Histogram of haploid chromosome numbers of parasitic Hymenoptera, data from Table 1 based on *n* values of each species.



Fig. 3. Histogram of haploid chromosome numbers of parasitic Hymenoptera, data from Table 1, each *n* value occurring in a genus being represented only once.

two other eupelmid species in the genera Macroneura and Anastatus, which were both found to have haploid numbers of 5 in common with Nasonia. Although the results of Rasch et al. (1975, 1977) and of Bigot et al. (1991) don't quite agree in the order of magnitude of base pairs they estimate, probably due to differences in procedure, they did both show the chalcidoid to have a larger genome than the ichneumonid. Thus, if these values are roughly representative of other members of their superfamilies, then the chromosomes of most chalcidoids would be expected to have between four and twenty times as much DNA on average than chromosomes of ichneumonids.

# CHROMOSOME NUMBER IN RELATION TO HYMENOPTERA PHYLOGENY

In karyological studies it is common practice to interpret modal chromosome numbers as representing the initial (ancestral) number, though to many cladists this

would be interpreted as the application of the much decried commonality principle. The 'common equals primitive' association is of course probabilistic rather than deterministic (Watrous and Wheeler 1981, Frohlich 1987, Quicke 1993). Some further insight into whether modal chromosome number is likely to reflect the ancestral number can come from the comparison of modal and median numbers. If these numbers coincide, and the whole distribution may be approximated to a normal one, then the data provide no evidence of a directed change, though it must be remembered that there is always the possibility that an evolutionary change in chromosome number early in the evolution of the group could lead to the same distribution. In the Ichneumonoidea and the Cynipoidea both the median and modal chromosome numbers are the same 11 and 10 respectively; in contrast, for example, ants (Formicidae) have a modal number of 11 but the median is 15. However, cladistic analyses based on independent character systems, via outgroup comparisons, provide the most reliable means of determining plesiomorphic chromosome numbers and, where possible, this is the rational that we have employed.

According to currently accepted views of Hymenoptera phylogeny, the sawflies ('Symphyta') form a paraphyletic group with respect to the Apocrita with the latter being the sister group of the Orussidae, and the Apocrita+Orussidae in turn most probably being the sister group of the Xiphydriidae (Königsmann 1977, Rasnitsyn 1980, 1988, Gibson 1985). Unfortunately, chromosome numbers are not known either for the Orussidae or for the Xiphydriidae, although they are known for members of two other sawfly families with claims for a close relationship with the Apocrita, viz the Siricidae and Cephidae (Königsmann 1977, Basibuyuk & Quicke 1994, 1995). In the Cephidae n ranges from 9 to 22-26 (Mackay 1955, Crozier and Taschenberg 1972), whilst in the Siricidae, according to Sanderson (1932, 1970), the haploid number varies between 8 and 18. As regards other, less derived sawflies, haploid chromosome number ranges from 5 to 22 in the Tenthredinoidea, with three quarters of species having an *n* value ranging between 7 and 10 (Naito 1982). Taking the Siricidae and Cephidae as the two sawfly families closest to the ancestral lineage of the Apocrita (i.e. putative sister groups of the Apocrita + Orussidae) for which chromosome numbers are available, it seems reasonable to conclude that the plesiomorphic haploid chromosome number in the latter was at least 8 and possibly rather higher.

Within the Apocrita there is a picture emerging from independent investigations of phylogenetic relationships (Rasnitsyn 1988, Johnson 1988, Gibson 1985, Mason 1983, Quicke *et al.* 1993, 1994, Heraty *et al.* 1994) that the group divided relatively early in its history into a lineage giving rise to the Ichneumonoidea+Aculeata and a second comprising the bulk of the taxa currently regarded as 'Microhymenoptera' including Chalcidoidea, Cynipoidea, Scelionoidea, Diaprioidea and Proctotrupoidea s.s. (Fig. 4).

Our data show that the modal *n* value in the Ichneumonoidea, the probable sister group of the Aculeata, is 11. Further, our limited data for the less derived aculeates of the family Bethylidae (Epyris and Laelius; Fig. 10), whilst demonstrating some degree of variation in haploid number between 10 and 14, when considered together with data for other aculeates suggest the ancestral aculeate may have had a haploid number around 11, as was also concluded by Hoshiba, Matsuura and Imai (1989). Similarly, available values for three other parasitoid superfamilies, the Diaprioidea (Fig. 5), Scelionoidea and Cynipoidea, are similar. According to Rasnitsyn's (1988) phylogenetic hypothesis (see Fig. 4), the Scelionoidea are putatively the sister group of the Chalcidoidea s.l., and thus taking the former as the outgroup, the plesiomorphic haploid chromosome number for the Chalcidoidea may be postulated as being approximately 10. Therefore the data collectively support the hypothesis that the small values of n(from 3 to 7) found in the majority of Chalcidoidea are likely to be apomorphous. Unfortunately, there are no well founded views of relationships within the Chalcidoidea (Trjapitzin 1978, LaSalle 1987, Boucek 1988a, Woolley 1988, Noyes 1990, Gibson 1990), largely perhaps because of the considerable plasticity in adult morphology displayed by many of the families, which may result because of the undoubtedly polyphyletic natures of some family level taxa. Trjapitzin (1978) made few proposals about higher level relationships, and only suggested two possible groupings, his 'pteromaloid' group comprising Pteromalidae, Tanaostigmatidae, Eupelmidae and Encyrtidae, and a 'tetracampoid' group comprising Tetracampidae, Eulophidae, Elasmidae and Aphelinidae. A relationship between the Aphelinidae



Fig. 4. Chromosome number and evolution of the parasitic Hymenoptera, with haploid values overlain on a cladogram in which superfamilial relationships are those proposed by Rasnitsyn (1988) but with relationships within the Chalcidoidea based on current karyological evidence for clarity. Ranges and (in parentheses) modal values.

and the Encyrtidae has also been proposed by a number of workers, but Gibson (1986) and Woolley (1988) considered that the supposed synapomorphies might be better regarded as resulting from convergence. LaSalle (1987) upheld Trjapitzin's view that the Tanaostigmatidae, Eupelmidae and Encyrtidae form a monophyletic group, citing two putative synapomorphies. Boucek (1988b) suggested that four families, the Chalcididae, Eurytomidae, Torymidae and [some] Pteromalidae were relatively 'ancient', partly because of their 5 segmented tarsi, compar-

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Fig. 5–10. Photomicrographs of chromosomes of adult female Hymenoptera revealed by Giemsa staining of ovarian tissue. 5, *Belyta depressa* (Diapriidae), meiosis, diakinesis in mature egg (2n = 16); 6, 7, *Anastatus catalonicus* (Eupelmidae), 6, meiosis, diplotene figures in developing oocyte, 7, metaphase mitosis (2n = 10); 8, *Diaretiella rapae* (Braconidae, Aphidiinae), metaphase mitosis (2n = 12); 9, *Meteorus versicolor* (Braconidae, Meteorinae), mitosis, one cell at pro-metaphase and one at metaphase (2n = 16); 10, *Laelius utilis* (Bethylidae), metaphase mitosis (2n = 20).

atively large size and thoracic structure. and he agreed with Trjapitzin and LaSalle that at least the Eupelmidae and Encyrtidae might form a closely related group. However, this view was not supported by Gibson (1989, 1990) who considered both that the Eupelmidae might not be monophyletic and that the characters used to unite them with the Encyrtidae and Tanaostigmatidae are "... either primitive features or apparently were derived independently several times".

The present data could suggest that there has been an approximate halving of chromosome numbers within the Chalcidoidea, from around a modal number of 10 as shown by the Eurytomidae and Encyrtidae to 5 or 6 in Aphelinidae (but see below), Chalcididae, Eulophidae, Leucospidae, Ormyridae, Pteromalidae, Torymidae and Trichogrammatidae. It should be noted that within the Aphelinidae, whilst most taxa examined have haploid values of 5, Pteropterix orientalis has an n value of 11 (referred to as Archenomus orientalis by Baldanza et al. 1991a). However, P. orientalis is an highly derived species within the Coccophaginae, being either a sister group of Coccophagoides or representing a derived branch within Encarsia itself (A. Polaszek personal communication). In either case, the high haploid chromosome number in this taxon is clearly derived with respect to the values of 5 (and 6) displayed by the other aphelinids studied (collectively representing both the Coccophaginae and Aphelininae), and these lower values may therefore be taken as representing the ancestral range of values for the family as a whole. The *n* value of 11 in Pteropterix orientalis, being nearly twice that found in the other aphelinids investigated, is further suggestive that this taxon could have originated through a polyploidy event.

The karyological data summarized here lend some support to Boucek's view that the Eurytomidae are a relatively ancient and underived family of Chalcidoidea but they do not agree well with the hypothesis of a close relationship between Eupelmidae and Encyrtidae (Trjapitzin 1978, La-Salle 1987, Boucek 1988a, Woolley 1988, Noves 1990) which have haploid numbers of 5 (Figs 6, 7) and 8-11 respectively, because if a modal haploid chromosome number of 5 represents a synapomorphy, then this relationship would require either two separate reductions (from c. 10) or a reversal. However, a close relationship between Eupelmidae and Encyrtidae is not universally accepted (Gibson 1989, 1990), and chromosome number should be incorporated in future cladistic analyses of the superfamilies as an independent character.

Potentially significant variation occurs within the Braconidae and the Ichneumonidae. In the former family, several subfamilies have haploid numbers between 8 and 11, for example, the Braconinae (Habrobracon), Meteorinae (Meteorus; Fig. 9), and Miracinae (Mirax). However, rather higher numbers from 12 to 17 are found in the Doryctinae (Heterospilus), Alysiinae (Phaenocarpa, Dacnusa) and Opiinae (Biosteres), whereas substantially lower numbers (n = 4 to 7) are found in the Aphidiinae (Aphidius, Diaretiella (Fig. 8), Ephedrus, Praon), in the exothecine genus *Rhysipolis* (n = 6), and in the unrelated Charmontinae (*Charmon*; n = 5). The possession of low values within the four genera of Aphidiinae examined provides a potential synapomorphy for the group, which otherwise seems an heterogeneous assemblage in which members are united by few characters other than biology, and more taxa will need to be examined before this can be confirmed. The low *n* values found in Rhysipolis might be an autapomorphy, and it would be interesting to know something about chromosome numbers in the apparently closely related Clinocentrini and other Rogadinae s.s.. The haploid number of 5 found in Charmon was especially surprising as the subfamily seems to be close to the Macrocentrinae

(Quicke and Achterberg 1990, Quicke et al. 1994) for which we obtained a value of 13.

Within the Ichneumonidae, most data available up until now were for the subfamily Ichneumoninae but a few chromosome numbers for the Campopleginae (Venturia), Cryptinae (Agrothereutes, Mastrus), Orthocentrinae (Orthocentrus), and Orthopelmatinae (Orthopelma) had also been published. The overwhelming majority of the species had haploid chromosome numbers modal at 11. We extended this data set by making chromosomal preparations also for the Banchinae (Glypta, Lissonota), Pimplinae (Ephialtes and Polysphincta), and Tryphoninae (Dyspetes). These additional data generally support the earlier findings, although the ephialtine genus *Ephialtes* had an *n* value of 15, rather higher than appears typical for the family, while the polysphinctine genus Polysphincta had the more typical, even low, haploid value of 9.

In addition to chromosome number. chromosome size and structure have been used extensively for cytotaxonomic purposes in other groups of organisms. Discussion of size and centromere position in the parasitic Hymenoptera is currently severely hampered by the relative paucity of data; in fact, in many illustrations, and especially among the earlier ones, centromeres are hardly (if at all) discernable. However, if we look at the karyotypes of the best studied groups (i.e. Ichneumonidae, Torymidae, Cynipidae), bi-armed (metacentric in the broad sense) chromosomes predominate in most cases (Figs 7-10). Some Hymenotera, for example several Diplolepis species (Sanderson 1988), may also have numerous acrocentric chromosomes. Even less can be said about chromosome size, though in general in the parasitic Hymenoptera it is inversely related to chromosome number. Much more by way of comparative and quantitative data will have to be assembled before it will be possible to use these features to

any great extent in studies of Hymenoptera systematics.

# INTRA- AND INTERSPECIFIC VARIATION AND IMPLICATIONS FOR HYMENOPTERA SYSTEMATICS

Only with the advent of techniques for examining chromosome number and morphology in adult Hymenoptera has it become possible to study intraspecific variation in nature and thus even to reveal the presence of hitherto unsuspected cryptic species or species complexes. As will be apparent from Table 1, chromosome number is often relatively consistent within a single genus. For example, in the Ichneumonidae, the 5 species of *Diadromus* for which chromosome numbers are available all have a haploid value of 11. Even for the large genus *Ichneumon*, 18 of the 25 species examined have n = 12.

Although chromosome numbers are usually considered as differentiating characters, serving to help distinguish between closely related forms, they may also be used in an integrative fashion, providing evidence for uniting related forms if the chromosome number represents a synapomorphy. For example, the discovery that all members of the cynipid genus *Diplolepis* have n=9, whereas the haploid values for all members of the other cynipid genera investigated to date for which reliable figures are available is 10, provides additional evidence for the monophyly of *Diplolepis*.

Chromosome numbers are fixed in the great majority of species of parasitic wasps whose populations have been studied in detail in the field. Two possible exceptions, however, are the ichneumonines *Icheumon extensorius* and *I. suspiciosus*, both of which were revealed by Gokhman (1993a) to comprise individuals with two different diploid numbers, 24 and 26. Since specimens with the intermediate chromosome number were not found (with one possible exception in *I. extenso-rius*) the possibility that these represent

Family Ichneumonidae

Ichneumonidae

Ichneumonidae

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Species	Chromosomal characteristics	Reference
ethecerus dispar Wesmael ethecerus ranini Gokhman	2n = 24 $2n = 22$	Gokhman 1991a
cherus australogeminus Gokhman	2n = 22	Gokhman 1991a
cherus ischiomelinus (Gravenhorst)	2n = 18	
neumon extensorius L.	$2n = 24, 26^{1}$	Gokhman 1993a
<i>meumon suspiciosus</i> Wes- mael	2n = 24, 26	Gokhamn 1993a
pidosoma ''truncatellum (Dalman)''²	n = 10, 2n = 20	Hunter & Bartlett 1975
<i>pidosoma floridanum</i> (Ash- mead)	n = 11, 2n = 22	Strand & Ode 1970

Table 2. Sibling species in the parasitic Hymenoptera detected by karyological features

	C. floridanum (Ashmead)	n = 8, 2n = 16	Leiby 1922; Patterson 1917 1921; Patterson & Porter 1917
Pteromalidae	Nasonia vitripennis (Walker) Nasonia vitripennis (Walker)	n = 5 + 0 - 1B, 2n = 10 n = 6, 2n = 12	Nur <i>et al.</i> 1988 and others Goodpasture 1974
Torymidae	Torymus californicus (Ash- mead)	n = 6(6M), 2n = 12	Goodpasture & Grissell 1975
	<i>Totrymus warreni</i> (Cocker- ell) <sup>3</sup>	n = 6(5M + 1A), 2n = 12	
Torymidae	Monodontomerus obscurus (Westwood) <sup>4</sup>	n = 4	Goodpasture & Grissell 1975
	Monodontomerus obscurus (Westwood) <sup>4</sup>	n = 6, 2n = 12	McDonald & Krunic 1971

Abbreviations: A = acrocentric; M = metacentric (in a broad sense).

<sup>1</sup> A single, probably aneuploid, female specimen with 25 chromosomes has also been found.

<sup>2</sup> Apparently belongs to C. floridanum.

<sup>3</sup> Apart from *T. californicus*, the second chromosome pair in *T. warreni* has secondary constrictions.

<sup>4</sup> Apparently belongs to M. laticornis Grissell & Zerova.

two sibling species rather than chromosomal races, seems much more likely, though the reverse cannot at present be discounted. Despite intensive effort, in neither case was it possible to detect any external morphological criteria to permit delineation of these putative taxa in the absense of karyological evidence. Further, in one instance, a chromosomal polymorphism in terms of C-banding pattern has been detected in the ichneumonine species, Dirophanes invisor (Thunberg). The Cbanding patterns of the two homologous chromosomes of the second pair of metacentrics do not differ from one another in some individuals, but in others, this pair

is obviously heteromorphic, one member of the pair being substantially longer than the other, and its segment of pericentric heterochromatin is also much more developed (Gokhman 1993b).

Several other recently discovered examples of apparent interspecific variation within other parasitic Hymenoptera are summarized in Table 2 and discussed below in more detail. The torymid chalcidoids *Torymus californicus* and *T. warreni* were considered to form a single species by Grissell (1973a). However, karyological analysis has shown that despite the fact that these two wasps have the same chromosome number (2n = 12), *T. warreni* has

a pair of acrocentric chromosomes and has secondary constrictions on the second largest pair of submetacentrics, whilst in T. californicus all the chromosomes are biarmed and the second pair has no constrictions (Goodpasture and Grissell 1975). Thus these two may well be best interpreted as sibling species. Other cases of possible karyologically-detected sibling species in the Chalcidoidea are more problematical. Goodpasture (1975a) and Mc-Donald and Krunic (1971) reported n = 4and n = 6 respectively for apparently the same torymid species, Monodontomerus obscurus. The most likely explanation for this difference is that one of these works involved a misidentified species with the specimens examined by McDonald and Krunic actually belonging to the very similar species, M. laticornis Grissell & Zerova, described 14 years later (Zerova and Grissell 1985). Two new ichneumonid species of the subfamily Ichneumoninae, Tycherus australogeminus and Aethecerus ranini, were originally detected on the basis of karyo-

morphological differences were also found (Gokhman 1991a; see also Table 2). Application of karyology may also be important in laboratory cultures as available evidence suggests that, at least in some instances, strains that were believed to belong to a single species may in fact represent more than one, with different laboratories working on different entities. For example, Hunter and Bartlett (1975) working with what they referred to as Copidosoma truncatellum, reported it as having a haploid number of 10. C. truncatellum was subsequently partly synonymized with C. floridanum, but Strand & Ode (1990) reported n = 11 for apparently the same species. Several earlier workers had reported the haploid number for C. floridanum (as Paracopidosomopsis floridanus) to be 8 (Leiby 1922, Patterson 1917, 1921, Patterson and Porter 1917), but their findings have to be treated with considerable caution as the techniques for fixa-

logical evidence, but in each case reliable

tion and preparation used in pre-1930 studies are often unreliable. Also in the genus Copidosoma, Hegner (1915) reported n = 11 and Patterson (1921) reported n =10 for C. gelechiae. Again these data may not be fully reliable for technical reasons. However, it is harder to interpret the apparent conflict in reported numbers for the widely studied pteromalid, Nasonia vitripennis. Many workers (e.g. Gershenzon 1946, 1968, Pennypacker 1958, Whiting 1960, 1968) have reported an *n* value of 5, but Goodpasture (1974) working on the University of California at Davis culture found n = 6. As with the case of the ichneumonines discussed below, intensive morphological investigation of these and other strains by Goodpasture failed to reveal any differences. Therefore, the possibility that the Davis culture had developed as a unique chromosomal race must be considered. Such variants are not uncommon in cultures of other organisms. However, it should be noted that Darling and Werren (1990) recently discovered two cryptic species of Nasonia in North America, and the karyological results could also reflect a sibling species complex.

The discovery of sibling species that can only reliably be separated by karyotype may pose a considerable nomenclatural problem, since the current Code of the Zoological Nomenclature requires new taxa to be differentiated from existing ones. As it is not normally possible to obtain karyological data from the type specimens of the species that have already been described, it would not possible to give scientific names to both of them (Gokhman 1993a) unless it were possible (for instance through geographic distribution) to infer the karyotype of the described taxon.

Intraspecific karyotypic variation may also be of interest for population cytogenetics. This type of variation is favoured in Hymenoptera by some characteristics of their genetic system, which allows the sur-

vival and comparatively high viability of aneuploids (Imai et al. 1984). The data obtained, for example, for the ichneumonid Tycherus bellicornis suggest the long-term persistence of a chromosomal population polymorphism, probably induced by a translocation and subsequent non-disjunction of chromosomes (Gokhman 1989). Occasional aneuploid specimens were also found in Ichneumon extensorius and I. gracilentus. Perhaps the most interesting case of numerical chromosomal polymorphism is described by Nur et al. (1988) and Werren (1991) and occurs in the pteromalid, Nasonia vitripennis. This type of polymorphism implies the existence of a particular B chromosome. Being transmitted paternally into the diploid zygotes, this chromosome eliminates all other chromosomes of the paternal set from the zygote, thus converting the originally diploid zygote into a haploid one. Therefore this B chromosome may be considered as the most selfish genetic element ever known.

## FUNCTIONAL IMPLICATIONS

Reduced chromosome numbers will generally be associated with reduced levels of recombination (Vorontsov 1966, White 1973). Under many circumstances, a reduction in recombination will be maladaptive. However, situations that favour high levels of inbreeding, for example, either parasitisation of aggregated hosts or gregarious parasitism combined with mating near the emergence site, may lead to selection in favour of low intrinsic levels of genetic diversity. Such situations may, for example, favour parthenogenesis or in the case of the parasitic Hymenoptera, thelytoky. Similarly, under such circumstances, high levels of recombination, or large numbers of separate linkage groups, will not be favoured, and consequently, mutations leading to reduced recombination will not necessarily be so deleterious. Such circumstances will permit a reduction in chromosome number more readily than will situations favouring high levels

of genetic diversity. Consideration of the data currently available for parasitic Hymenoptera may be relevant in this respect. For example, in the Aphidiinae the chromosome number is markedly lower than in the rest of the Braconidae. Aphidiines parasitise aphids which in turn often form clonal patches. In chalcidoids, many species are also gregarious or attack clustered hosts, perhaps in part as a result of their small size and dispersal capabilities. It would be difficult to quantify this, but the possibility exists that inbreeding may be more common in the Chalcidoidea as a whole than in the Ichneumonoidea or Cynipoidea (Askew 1968). Information on recombination levels in parasitic Hymenoptera is extremely limited to date (Crozier, 1975), being based on linkage data (Habrobracon hebetor) and chiasmata per bivalent arm (Aphytis mytilaspidus). Further studies taking into account cross-over and levels of heterozygosity in parasitic wasps with different biologies and different chromosome numbers and morphologies might provide additional evidence in this respect.

## FURTHER PERSPECTIVES

Though we have managed for the first time to obtain cytogenetic information for several major groups of parasitic Hymenoptera, the overwhelming majority of these insects still remains untouched by karyological investigation, and this includes some entire superfamilies such as the Stephanoidea, Megalyroidea, Trigonalyoidea, Evanioidea and Ceraphronoidea (Fig. 4). The data available at present, however, suggest that the new chromosomal evidence may be a substantial help in future phylogenetic and taxonomic studies. As regards the higher level phylogeny of the parasitic Hymenoptera, new karyological evidence is especially needed for various groups of Proctotrupoidea sensu lato and of Chalcidoidea, especially of such apparently underived taxa as the Mymaridae, Tetracampidae and Rotoitidae (Noyes 1990) and the pteromalid subfamily Cleonyminae (Boucek personal communication).

Recent investigations (e.g. Gokhman 1991b, 1994, Costa *et al.* 1993, Odierna *et al.* 1993) also show that other karyological data, especially those obtained using differential chromosome staining (e.g. Cbanding), can still provide useful information even in the absence of differences in chromosome number. More detailed morphological investigations are therefore particularly likely to be of use in future species level work.

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# APPENDIX I. METHODOLOGY FOR STAINING CHROMOSOMES IN ADULT PARASITIC WASPS

General comments. Chromosomes can be stained in many adult parasitic wasp females especially in recently eclosed individuals using ovarian tissue. As always in insects, care should be taken to count multiple cells so that the occasional polyploid cell can be recognised and discounted. In common with Crozier (1975) we recommend counting at least ten individual metaphase plates though occasionally this may not be possible. If fewer plates are available, one may gain extra confidence in the results if it is possible to identify through size and morphology, particular pairs of chromosomes (see for example, Fig. 9). Usually, mitotic divisions are most evident, but in some individuals and taxa, meiosis may also be observed, sometimes with very clear spreads. The number of plates may also be increased if the wasp is fed on honey water containing colchicine for a few hours before preparation, though beyond about 5 hours one stands the risk of increasing the proportion of polyploid cells. The general method described below is modified after that of Imai et al. (1988).

Method. Metasomas of adult female wasps are dissected in hypotonic sodium citrate solution with colchicine (Solution A). Ovaries are incubated in this solution at room temperature (c. 25°C) for 20 minutes (optimal times for different taxa may vary slightly). Following incubation, ovaries are transferred to a thoroughly cleaned microscope slide, excess citrate solution pipetted off and the slide is gently flushed with Fixative I taking care not to wash off the ovaries. Whilst still moist with Fixative I, the ovaries are disrupted (e.g. using fine mounted needles) and their cells spread gently over the middle part of the slide. One or two drops of Fixative II are then applied to the centre of the area of spread cells and the more aqueous phase which is displaced to the edge of the slide is blotted off. The same procedure is then performed with Fixative III. The slide is then air dried before staining for at least 20 minutes. Excess stain should be washed off with distilled water and the slide examined dry or under emersion oil (do not apply mounting media). Slides can be restained if the initial result was insufficiently intense, or they can be destained with alcohol in the reverse situation.

#### Solutions

(A) Hypotonic sodium citrate with colchicine 1g Na citrate.2H<sub>2</sub>O

5mg colchicine 100 ml distilled water

- (B) Fixative I
  3 parts Ethanol
  3 parts Glacial acetic acid
  4 parts distilled water
  (C) Fixative II
- 1 part Ethanol 1 part Glacial acetic acid
- (C) Fixative III Glacial acetic acid
- (D) Stain
   2 ml Giemsa solution
   50 ml 0.089M Na<sub>2</sub>HPO<sub>4</sub>
   50 ml 0.066M KH<sub>2</sub>PO<sub>4</sub>



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