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Differences in chromosome number between germ line and soma in the genus *Forda* (Homoptera: Aphididae)

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Abstract

The chromosomes of embryos within thelytokous females of *Forda* spp. were studied, in samples from galls on *Pistacia* in the Middle East, and from roots of Poaceae in the Middle East, Europe and North America. The nuclei of oogonial cells, oocytes and early cleavage stages have consistently more chromosomes than the nuclei of dividing cells in the somatic tissues of young embryos from the same mothers. Elimination of the extra germ line chromosomes apparently occurs in late cleavage. In *F. marginata* Koch the germ line chromosome number varies from 25 to 40 in different populations and the somatic cell number varies from 17 to 20; in *F. formicaria* the germ line has 21–23 chromosomes and somatic nuclei have 18 or 20. In both species variation occurs between samples from galls on *Pistacia* as well as between populations on roots of Poaceae. The numbers and relative sizes of the eliminated chromosomes also differ between populations. Comparable phenomena in other insects are briefly discussed.

Introduction

The pemphigine aphids *Forda marginata* Koch and *F. formicaria* von Heyden have a biennial sexual phase on their primary host, *Pistacia* spp. in the Mediterranean area and Middle East, but maintain themselves solely by thelytokous parthenogenesis on the roots of the secondary host plants, Poaceae, in North and Central Europe and North America. Robinson and Chen (1969) recorded 2n = 28 and 2n = 20 as the chromosome numbers of *F. marginata* and *F. formicaria* respectively in Canada, and illustrated the karyotypes with idiograms. Blackman (1980) noted that cells with two different chromosome numbers could be found within single embryos of both species, and suggested that this might be due to differences between the germ line and the soma, as in certain Diptera. However, the situation was confused because only anholocyclic (permanently thelytokous) populations in North America and Northern Europe had been sampled, and these showed considerably karyotype variation between samples and marked structural heterozygosity, as is frequently the case in anholocyclic aphids.

Several samples of both species collected from *Pistacia* have now been karyotyped. The aphids were collected from galls containing colonies started by fundatrices which hatched from sexually-produced eggs. It has still not been possible to study meiosis, as this occurs at a difficult stage in Pemphiginae, when the sexual morphs of the aphid are still embryos within their immature parents (sexuparae) on the roots of the secondary host plants. However, clear preparations of oogonia cells, parthenogenetic oocytes and early embryonic stages have now been obtained, so that it is possible to report on the differences between germ line and soma and discuss further the karyotype variation in these aphids.

Material and methods

Field collected aphids were preserved in 3 parts methanol: 1 part glacial acetic acid. Collection data

Table 1. Collection data and chromosome numbers of *Forda marginata.*

Locality	Date	Host plant	Collector	No. of chromosomes	
				Soma	Germ line
Canada	not stated	?grass	Robinson & Chen, 1969	–	28
Knowle, UK	3.xi.74	grass	R. G. Paul	17	–
Knowle, UK	15.ii.75	grass	R. G. Paul	17	25
Arcadia, California	23.v.75	*Bromus carinatus*	H. G. Walker	18	26
Arcadia, California	23.v.75	*Poa annua*	H. G. Walker	18	
Arcadia, California	30.ix.75	*Bromus carinatus*	H. G. Walker	18	29
Arcadia, California	2.x.75	*Bromus carinatus*	H. G. Walker	18	–
Staines, UK	20.iii.77	grass	R. G. Paul	17	32
Staines, UK	20.iii.77	grass	R. G. Paul	18	25
Arcadia, California	7.iii.77	*Bromus carinatus*	H. G. Walker	18	–
Catania, Sicily	20.x.77	*Pistacia terebinthus*	S. Barbagallo	–	27
Mt. Carmel, Israel	4.viii.78	*Pistacia palaestina*	D. Wool	20	26
Nicosia, Cyprus	12.v.81	grass	V. F. Eastop	18	–
Tehran, Iran	14.x.81	*Setaria* sp.	S. H. Hodjat	18	40
Har Meiron, Israel	9.v.85	*Pistacia palaestina*	D. Wool	20	–
Mt. Carmel, Israel	x.85	*Pistacia palaestina*	D. Wool	20	26

are listed in Tables 1 and 2. Squash preparations of ovarioles from immature and adult virginoparae were made according to methods described previously (Blackman, 1980). The dissected individuals were mounted on microscope slides to confirm identification. Preparations were examined initially, and sometimes photographed, by phase contrast; good preparations were stained with Giemsa and mounted

Table 2. Collection data and chromosome numbers of *Forda formicaria.*

Locality	Date	Host plant	Collector	No. of chromosomes	
				Soma	Germ line
Canada	not stated	?grass	Robinson & Chen, 1969	20	–
Englefield Green, UK	21.ix.74	grass	R. G. Paul	20	23
Kew, UK	27.ix.74	grass	V. F. Eastop	20	22
Silwood Park, UK	14.i.75	grass	R. G. Paul	20	22
Denham, UK	18.i.75	grass	R. G. Paul	20	22
Knowle, UK	26.i.75	*Festuca rufa*	R. G. Paul	20	22
Englefield Green, UK	7.ii.75	grass	R. G. Paul	20	–
Box Hill, UK	11.iii.75	grass	R. G. Paul	–	21
Hampstead, UK	11.iii.75	grass	R. G. Paul	–	22
Middlesex, UK	29.iii.75	grass	R. G. Paul	20	22
Isle of Arran, UK	10.vi.75	grass	R. G. Paul	20	–
Staines, UK	14.iii.76	grass	W. R. Dolling	20	–
Radcliffe, UK	15.v.76	grass	J. H. Martin	20	–
Faversham, UK	15.v.76	grass	W. R. Dolling	20	–
Catania, Sicily	20.x.77	*Pistacia terebinthus*	S. Barbagallo	20	–
Jerusalem, Israel	16.vii.78	*Pistacia palaestina*	D. Wool	20	–
Polis, Cyprus	17.vi.81	*Pistacia terebinthus*	V. F. Eastop	18	–
Kew, UK	27.iv.84	grass	A. Polaszek	–	22
Balham, UK	2.vi.84	*Poa annua*	J. H. Martin	20	–
Smolenice, Czechoslovakia	12.ix.85	*Bromus* sp.	R. L. Blackman	20	–

in DePeX for further examination and photography. Only those preparations with clear, well-spread chromosome sets giving unambiguous determinations of karyotype were recorded.

The spatial relationships of germaria, oocytes and young embryos in the ovarioles are destroyed in normal squashes, so some preparations were examined before squashing, the ovarioles being first mounted in a drop of distilled water under a cover-slip. Observation continued while 45% propionic acid was gradually introduced under the cover-slip with a micropipette, until the cells cleared and the preparation gradually flattened out. When all the water had been replaced by propionic acid, and the excess of this had evaporated, the slide was inverted on a filter paper and the preparation gently squashed. In this way it was possible to preserve the positions of the germarial cells, oocytes and blastoderm cells relative to one another in the ovariole, and thus make their identification easier.

Results

Forda marginata

All the ovarioles of *F. marginata* examined had already undergone at least one ovulation. The germarium differed in form and content from that of members of the subfamily Aphidinae (Blackman, 1978). It was bluntly conical in shape with a broad base, and the nuclei of the nurse cells were polyploid and irregularly shaped. The 13–14 presumptive oocytes were positioned in a zone at the base of the germarium, and each oocyte had all the chromatin of its nucleus compacted into a single, very dense body (Fig. 1). The nuclei of those oocytes nearest the base of the germarium, and presumably therefore the next to be ovulated, had rather less compact chromatin, and they were evidently at the start of decondensation. The earliest growth stages of the oocyte were not seen, but late in the growth phase the individual chromosomes, now in late prophase of the maturation division, could be observed. At maturation metaphase the chromosomes are strongly condensed and well-separated, so that they are easily countable (Fig. 2). Maturation metaphases were found in three samples of *F. marginata*, one from galls on *Pistacia*,

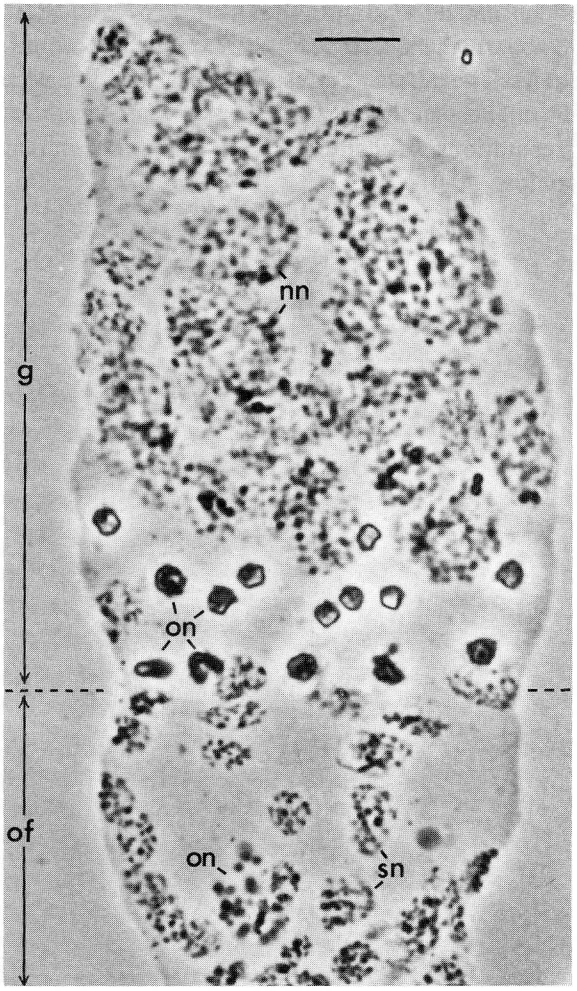

Fig. 1. Germarium (*g*) and first ovarian follicle (*of*) of an ovariole of *Forda marginata*, with the follicle containing a prematuration oocyte. *nn*, nurse cell nuclei; *on*, oocyte nuclei; *sn*, ovariole sheath cell nuclei. Phase contrast. Scale bar represents 5 μm.

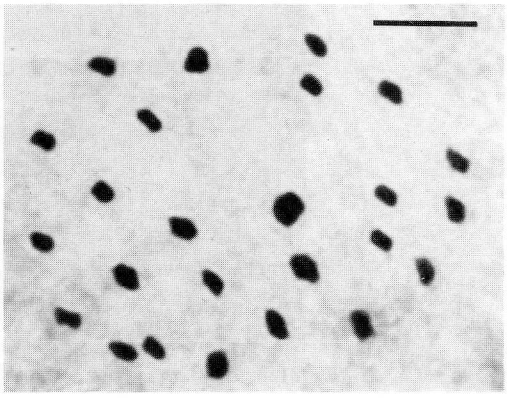

Fig. 2. Maturation metaphase of oocyte of *Forda marginata* with 25 chromosomes. Scale bar represents 5 μm.

which had 26 chromosomes, and two from grass roots, which had respectively 25 and 29 chromosomes. The number corresponded in each case with the higher of the two numbers found in squash preparations of embryos from the same parent aphids.

The parthenogenetic eggs of aphids have a single, equational division with formation of one polar body. The cleavage divisions that follow show the same number of chromosomes at metaphase as in the maturation division (Fig. 3), at least until the fourth or fifth cleavage division. However, the divid-

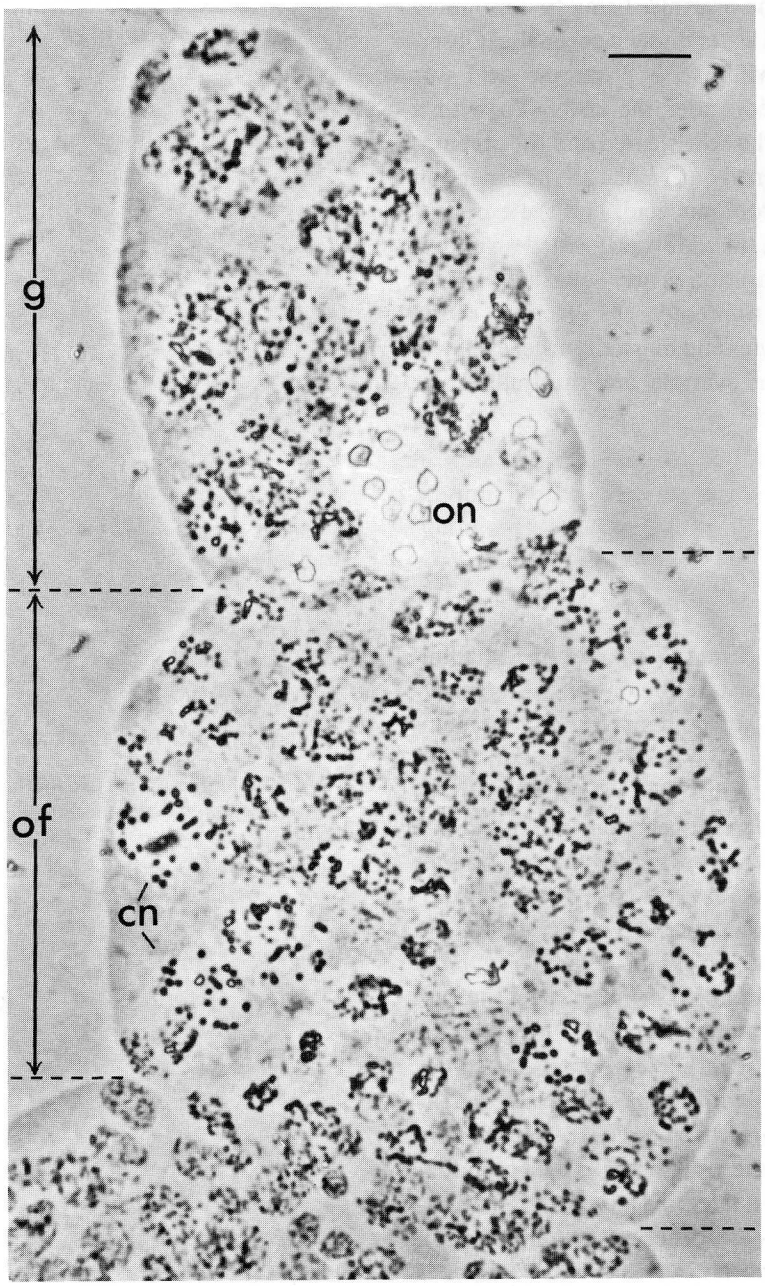

Fig. 3. Germarium (*g*) and first ovarian follicle (*of*) of an ovariole of *Forda marginata*, with the follicle containing an early embryo with cleavage nuclei (*cn*) showing 26 chromosomes. The nuclei of pregrowth oocytes at the base of the germarium (*on*) are refractory to light due to their high density. Phase contrast. Scale bar represents 5 μm.

ing cells in young embryos prior to gastrulation commonly have a reduced chromosome number and a distinctly different karyotype (e.g. Fig. 4a). A little later in embryonic development, but still well before limb bud formation, groups of synchronously dividing oogonia with the germ line karyotype can be seen (Fig. 5), and sometimes the oogonial cells have dividing somatic cells adjacent to them in squash

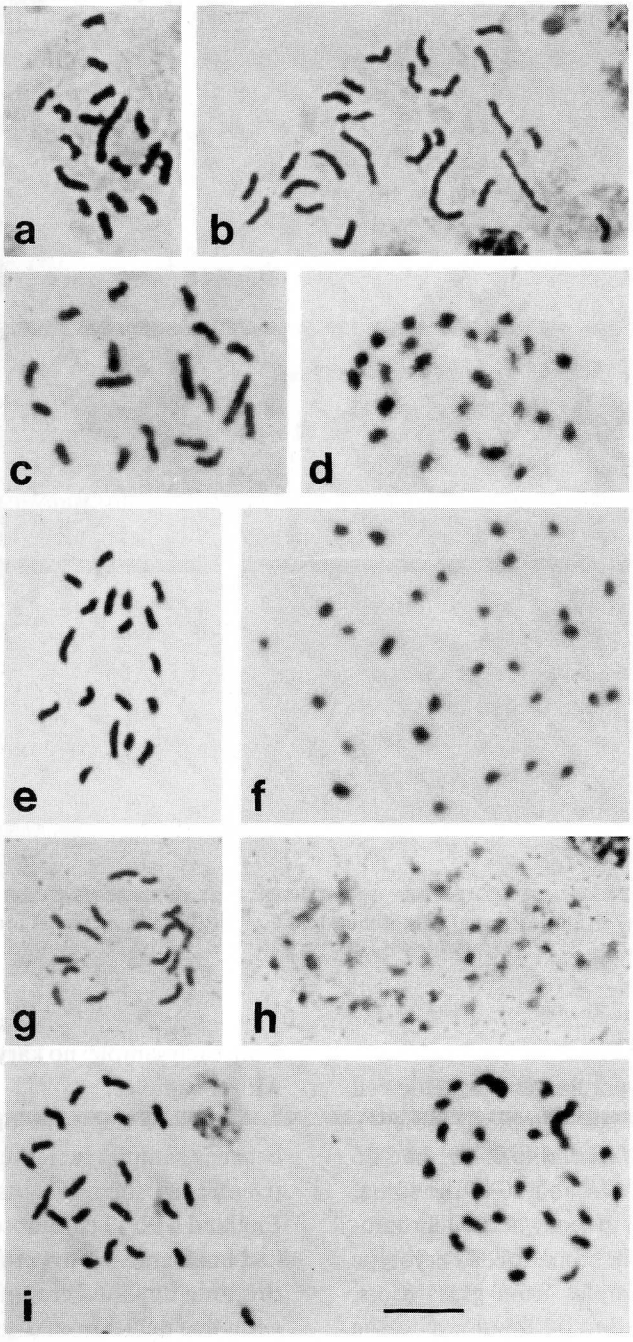

Fig. 4. Karyotypes of *Forda marginata*; somatic cell nuclei on the left, germ line karyotypes on the right: (a), (b), 17/25-chromosome form from England; (c), (d), 18/25-chromosome form from England; (e), (f), 18/29-chromosome form from California, USA; (g), (h), 18/40-chromosome form from Iran; (i) 20/26-chromosome form from Israel. (d), (f) and (h) are prematuration oocytes at prometaphase, and the nucleus on the right in (i) is an oogonial prophase. Scale bar represents 5 μm.

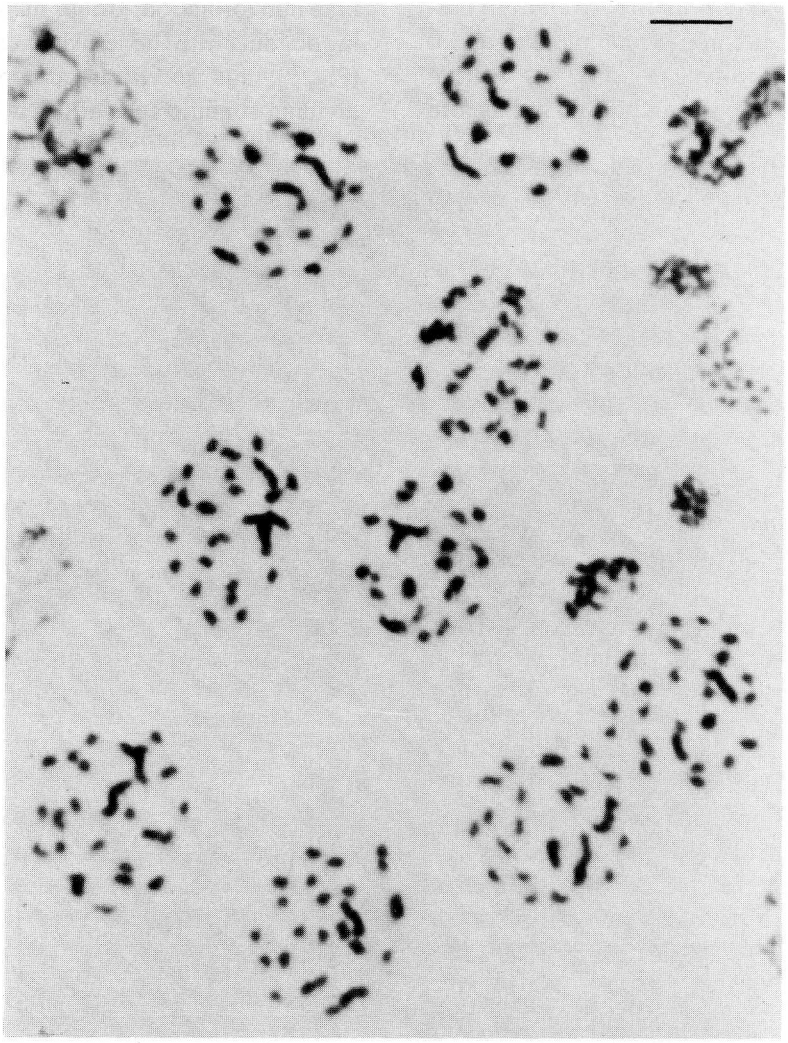

Fig. 5. A group of synchronously dividing oogonial cells of the 20/26 form of *Forda marginata* at prometaphase. Scale bar represents 5 μm.

preparations (Fig. 4i).

The numbers of both germ line and somatic cell chromosomes varied between samples (Table 1). Samples from *Pistacia palaestina* in Israel had 26 chromosomes in the germ line and 20 in the soma, but one from *P. terebinthus* in Sicily had 27 chromosomes in germ line nuclei, the somatic cell karyotype being indiscernible. In samples from grass roots, most of which are presumed to be anholocyclic, the somatic cell nuclei had either 17 or 18 chromosomes and the germ line number varied from 25 to 40. In most cases 2–3 individual aphids were karyotyped from each sample; no karyotype variation was found within samples.

As well as the variation in chromosome number between samples there were clear differences in relative chromosome lengths (Fig. 4). The form from England with 17 chromosomes in somatic cell nuclei had one element longer than any other (Fig. 4a), and this long unpaired chromosome was also evident in germ line nuclei from the same sample with 25 chromosomes (Figs. 2, 4b). Samples with 18 chromosomes in somatic cell nuclei from England, California and the Middle East were all of similar karyotype

(Figs. 4c, 4e, 4g), but the corresponding germ line nuclei were widely variable with 26–40 chromosomes, some of which were heterochromatic in prometaphase oocytes (Figs. 4d, 4f, 4h). The 20 chromosomes in somatic cell nuclei of samples from Israel were all of similar length, but germ line nuclei with 26 chromosomes from the same embryos had two chromosomes conspicuously longer than the rest, and in oogonial cells these longer elements were heterochromatic (Fig. 4i).

Forda formicaria

This species showed greater consistency of karyotype than *F. marginata* (Table 2). Somatic cell divisions showed 20 chromosomes with the sole exception of one sample from galls on *Pistacia terebinthus* in Cyprus, which had 18 chromosomes. In the 20-chromosome form at prometaphase a group of 5–8 elements is commonly associated with a single large nucleolus (Fig. 6a).

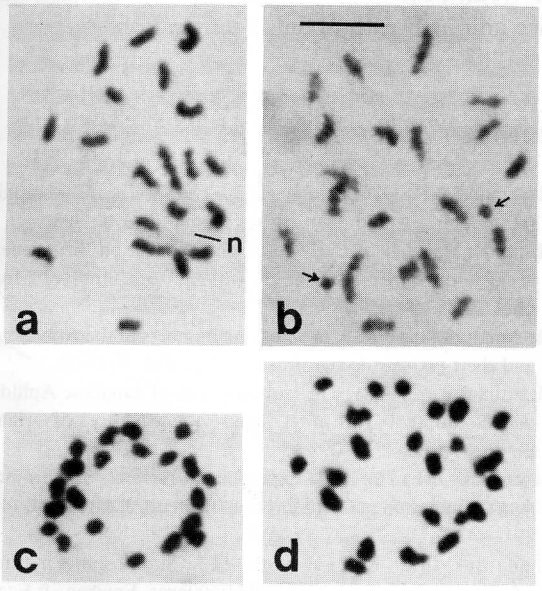

Fig. 6. Karyotypes of *Forda formicaria*: (a) prometaphase somatic cell nucleus with 20 chromosomes, several being associated with a nucleolus (*n*); (b) late prophase of germ line cell with 22 chromosomes, having two small fragments additional to the somatic cell complement (arrowed); (c) oogonial metaphase with 21 chromosomes; (d) oogonial metaphase with 23 chromosomes. Scale bar represents 5 μm.

The germ line chromosome number is usually 22, but samples from anholocyclic populations with 21 and 23 chromosomes have been collected in England (Figs. 6b, c, d). In oogonial metaphases it is not possible to identify how these karyotypes differ from the somatic 20-chromosome nuclei, but late prophase nuclei with 22 chromosomes show two small heterochromatic fragments which are additional to those present in the 20-chromosome complement (Fig. 6b, *cf.* Fig. 6a). No germ line chromosome sets were visible in the preparations made from *F. formicaria* from *Pistacia*.

Other Forda *species*

Two samples from Iran provisionally assigned to the species *F. hirsuta* Mordvilko, one from roots of *Cyperus* and the other from roots of *Triticum*, had 18 chromosomes in embryonic somatic cells; the number of chromosomes in the germ line was unrecorded. Blackman (1980) recorded the germ line chromosome number of *F. dactylidis* Borner (=*pawlowae* Mordvilko) as 30, but the single karyotyped specimen was misidentified; Dr. V. F. Eastop has re-examined it and considers it to be closer to *F. riccobonii* (Stefani). The somatic chromosome number from embryos of this aphid was 18, as recorded by Khuda-Bukhsh and Pal (1983) for *F. riccobonii*.

Discussion

The phenomenon of additional germ line chromosomes which are eliminated from the soma is known in three groups of Diptera; Cecidomyidae (where the additional elements are known as E chromosomes), Sciaridae (where they are termed L chromosomes), and the chironomid subfamily Orthocladinae (all reviewed by White, 1973, pp. 516–541). In these groups the extra chromosomes are eliminated at various times, depending on the group and species, between the 3rd and 7th cleavage divisions. The process of elimination has not been observed in *Forda*, but the first few cleavage divisions definitely occur before there is any elimination of the extra ele-

ments. In Cecidomyidae the E chromosomes fail to move to the poles at anaphase of the elimination division, probably due to a defect in the functioning of their centromeres (Geyer-Duszynska, 1959). Aphids, like all Hemiptera, have holocentric chromosomes. The scale insects, which are probably the sister group to the aphids, are noted for their aberrant chromosome behaviour; in Diasphididae the entire paternal chromosome set is heterochromatinised and then eliminated during the cleavage divisions. In aphids, elimination phenomena have previously only been described in spermatogenesis (Blackman, 1976).

Although the male karyotype of *Forda* has yet to be observed, the phenomena described in this paper could have some association with sex determination. Most aphids have XX/XO sex determination, and the X chromosomes are often recognisable even in female somatic cells by their allocyclic behaviour, and because the principal nucleolar organisers are usually situated at or near the ends of the X chromosomes. Multiple X chromosomes occur in some aphids, however, including certain Pemphiginae (Blackman, 1986). In *Forda* spp., several elements of the female karyotype may appear more heterochromatic than the rest, and some of these may be grouped around a single nucleolus in somatic cell prophase nuclei. The additional chromosomes present in the germ line differ in number and size between populations but a common feature is their heterochromaticity in prophase nuclei. Without knowledge of the male karyotype, however, the X chromosomes remain unidentified, and understanding of the nature and significance of the elimination process must await further study, especially of sex determination and events during spermatogenesis.

The taxonomy of the genus *Forda* is difficult and depends heavily on the form of the galls on the primary host *Pistacia*, so that identification of populations on the roots of Poaceae is problematic, especially in the Middle East where the group is indigenous and most of the species occur. Populations having the same shape of gall on *Pistacia* are not necessarily conspecific, and the sample of *F. formicaria* group from *P. terebinthus* in Cyprus with 18 chromosomes in somatic cell nuclei possibly is of a different species from the 20-chromosome populations in similar galls on *Pistacia* spp. in Israel and Sicily. Populations on grass roots may be anholocyclic even in areas where *Pistacia* is available as primary host, so variation in chromosome number between such populations is not necessarily indicative of the existence of separate taxa, unless the extreme view is taken that each clone of a parthenogen must be regarded as a separate taxon because of its genetic isolation from the rest. Clarification of the taxonomic and genetic relationships within the group requires greater knowledge of life cycles and host plants of populations in relation to their karyotypes and morphologies.

Acknowledgements

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