Genetica 77: 189–216, 1988



# Cytological investigations of parthenogenesis in gall wasps (Cynipidae, Hymenoptera)

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Received 13.11.1987 Accepted 4.6.1988

**Abstract**

The chromosome number was determined in eleven heterogonous species of gall-wasps on oaks and in four species which reproduce by constant parthenogenesis on wild roses. One of the latter group proved to be a natural triploid. Maturation was studied in representative forms from synapsis through diakinesis and the abortive first division to the second division in the laid egg. Chromosome regulation in the developing egg, the production of parthenogones of different sex and the role of the sperm are discussed briefly.

## Introduction

In the majority of gall wasps the life cycle usually involves two generations, an overwintering one consisting of females only and a summer generation of males and females in about equal numbers. The females of the two generations differ in their choice of oviposition site on the host plant and often differ morphologically, the parthenogenetic females often being larger and sometimes wingless. In a few species, the life cycle involves only a unisexual generation, the bisexual phase having been suppressed. A well-illustrated account of the life cycle of British gall makers is available (Darlington, 1968). Where alternation of generations occurs in heterogonous species, the bisexual generation gives rise by zygogenesis to an agamic generation of two classes of parthenogenetic females which produce either all-male haploid or all-female diploid broods. A typical life cycle of a heterogonous species is shown in Figure 1.

Androphore eggs must undergo reduction to give haploid males and, if gynaephore eggs also reduce, they must restore the diploid number in order to generate females. The manner of regulation may have different genetic consequences which influence the type of female produced in the agamic generation. The investigations described here relate to the chromosomes and their behaviour during oogenesis and maturation of the eggs in both generations of the life cycle.

Earlier cytological investigations undertaken by Doncaster (1910, 1911 and 1916), Hogben (1919, 1920) and Dodds (1938, 1939) were incomplete and inconclusive.

The present cytological investigation in Cynipidae aims at elucidation of the complete maturation process in two generations of heterogonous species and comparison of these with each other and with the process in a wholly thelytokous species. The results of the investigation of the number and morphology of the chromosomes in eleven species of gall-wasps are presented first, followed by observations on meiotic phenomena during oogenesis in nine species and maturation in two heterogonous and two thelytokous species.

### *The species investigated*

The species of cynipids which have been investigated in one or both phases of the life cycle are given in Table 1.

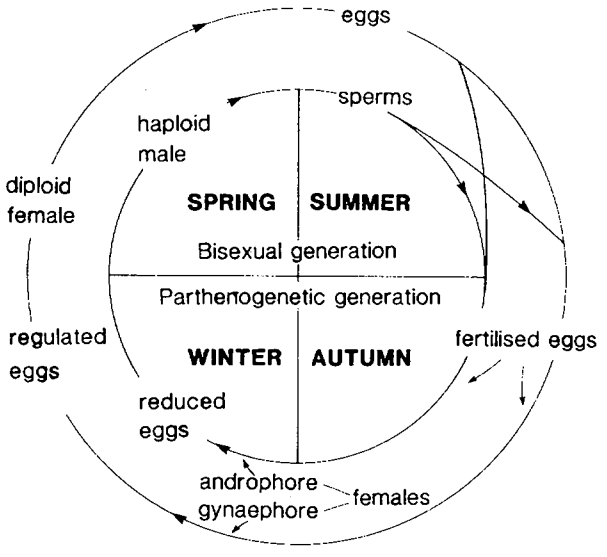


*Fig. 1.* Diagram of a typical heterogonous life cycle in *Biorhiza pallida-aptera* on the leaves and roots of oak, showing alternation of a bisexual spring generation with a parthenogenetic winter generation consisting of male-producing androphores and female-producing gynaephores. The diagram indicates that the androphores and gynaephores derive from different females but they may have the same mother.

### *Cytological technique*

The stages most useful for the study of spermatogonia and spermatocytes are the full-grown larva and white pupa; for oogonia, synapsis and follicle cell mitoses, the larva and white pupa; for diakinesis and the abortive maturation division, the pupa and teneral adult female.

When the gonads are very small, as in the young larva, they can be squashed on the microscope slide in lacto-acetic orcein or fixed on the slide for a few seconds in alcohol/acetic acid (3/1, v/v) then loosened and washed off into a dish of fixative and left for an hour. After fixation the gonads may be stored in 70% alcohol and subsequently softened in 45% acetic acid before being squashed in orcein. When mounting older ovaries, the ovarioles must be well separated so that, on squashing, the egg strings retain the egg arrangement as far as possible. The sticky anteriorly directed pedicels help to keep the eggs in position in the strings and facilitate study of the progress of nuclear activity.

Clusters of eggs laid by *Biorhiza pallida-aptera* and *Diplolepis rosae,* are best fixed before dissection from the plant tissues, otherwise the entangled pedicels break and the egg contents are lost. Many eggs not examined soon after extraction were stored for a time in 70% alcohol. To allow the yolk to spread evenly in squash preparations the chorion has to be broken and, where this is resistant, the escape of the yolk is assisted by first softening the eggs in 45% acetic acid and by tapping and pushing the coverslip in such a way that the eggs are rolled over in the orcein and finally flattened. Under pressure the chorion usually bursts over the widest part of the egg and it is a matter of chance whether the first developmental nuclei are uncovered. In eggs which have reached the blastoderm stage the chorion bursts readily. This aceto-orcein squash technique, first used on Hymenoptera by Sanderson & Hall (1948) has proved satisfactory for chromosome study in many insects. The addition of lactic acid, introduced in human blood culture preparations prevents drying out when slides have to be kept for lengthy periods (see Sanderson & Stewart 1961).

The microscope used throughout these studies was a Reichert Zetopan fitted with a 'Polyphos' anoptric condenser, giving both phase positive and negative images. The photomicrographs were taken on fine grain Ilford film using a 1/12th objective and a ×5 ocular. All are squash preparations and are to the same scale unless otherwise stated.

## Cytological results

### *The chromosomes*

The karyotypes of eleven species have been studied (see Table 1). Apart from oogonial cells, the most accurate chromosome counts are obtained in the diakinetic stage of the oocyte with a facility rarely found in other groups of Hymenoptera. The chromosomes in cleavage nuclei tend to be long and intertwined and although they may be counted they rarely yield good photographs. Where male material is available spermatocytes in premetaphase clearly show the haploid number. As a working aid provisional idiograms of eight species, prepared from all available sources, are shown in Figure 5.

*Table 1.* The species of cynipids investigated in one or both phases of the life cycle*.
(a) Cyclic species of Cynipidae on *Quercus robus* and *Q. petraea***

| | Bisexual (gamic) form | Gall name | Parthenogenetic (agamic) form | Gall name |
|---|---|---|---|---|
| 1. | *Neuroterus baccarum* L. | currant | *Neuroterus lenticularis* | lentil |
| 2. | *Neuroterus vesicator* (Schlecht) | blister | *Neuroterus numismalis* (Geoff.) | silk button |
| 3. | [*Neuroterus albipes* Schenck] | Schenck's | *Neuroterus laeviusculus* (Schenck) | smooth spangle |
| 4. | [*Andricus circulans* Mayr.] | | *Andricus kollari* Htg. | marble |
| 5. | *Andricus curvator* Htg. | curved leaf | [*Andricus collaris* Htg.] | collared bud |
| 6. | [*Cynips verrucosa* Schlecht] | red wart | *Cynips divisa* Htg. | scarlet pea |
| 7. | *Biorhiza pallida* (Oliv.) | oak apple | *Biorhiza aptera* (Fabric.) | root |

(b) Acyclic species of Cynipidae on *Rosa canina* et al.

| | | | | |
|---|---|---|---|---|
| 8. | | | *Diplolepis rosae* (L.) | bedeguar |
| 9. | | | *Diplolepis eglanteriae* Hartig. | smooth pea |
| 10. | | | *Diplolepis nervosum* (Curt.) | spiked pea |
| 11. | | | *Diplolepis spinosissimae* Gir. | burnet |

* The earlier names used for the alternating generations are now usually combined but to avoid confusion only one specific name is used throughout the text and the taxonomic authority is henceforth omitted.
** Those in brackets were not obtainable for study of this phase of the life cycle.

1. *Neuroterus baccarum-lenticularis:* The haploid number, 10, found in first and second spermatocytes in the bisexual phase is shown in Figure 2f–h and the diploid number, 20, in ovarian cells in Figure 2a–e, with chromatid separation clearly apparent in Figure 2d. Haploid and diploid complements are shown in cleavage cells in Figure 8g–i. As the overwintering gall of the agamic phase *N. lenticularis* is plentiful its karyotype has been examined more thoroughly than in the other species. Although larval ovaries give good oogonial and somatic counts, the individuality of the chromosomes has also been studied in meiotic stages in developing oocytes of both generations. Only one chromosome, the small no. 9, is metacentric, all others being acrocentric, with numbers 5 and 6 (Fig. 5a) most readily recognisable.

2. *Neuroterus vesicator-numismalis:* The chromosome number is 20. Cytological examination of this species was difficult and no photographs were taken. The overwintering larvae are easily damaged during removal from the gall and their small size makes it necessary to squash the entire larva in order to locate the ovary. In the agamic female *N. numismalis* oocytes clearly show 10 bivalents, smaller in size than those of *N. lenticularis,* but showing the same range of form (Fig. 5b). Blister galls of the bisexual generation, *N. vesicator* are difficult to locate within the oak leaf and are so heavily parasitised that it was impossible to obtain enough male pupae for better study of chromosome morophology.

3. *Neuroterus albipes-laeviusculus:* The chromosomes of only *N. laeviusculus* were examined and all 10 except two of the smallest are acrocentric (Figs. 2i and 5c). Mitosis in follicle cells and prophase stages in oocytes was observed in larvae collected in Surrey

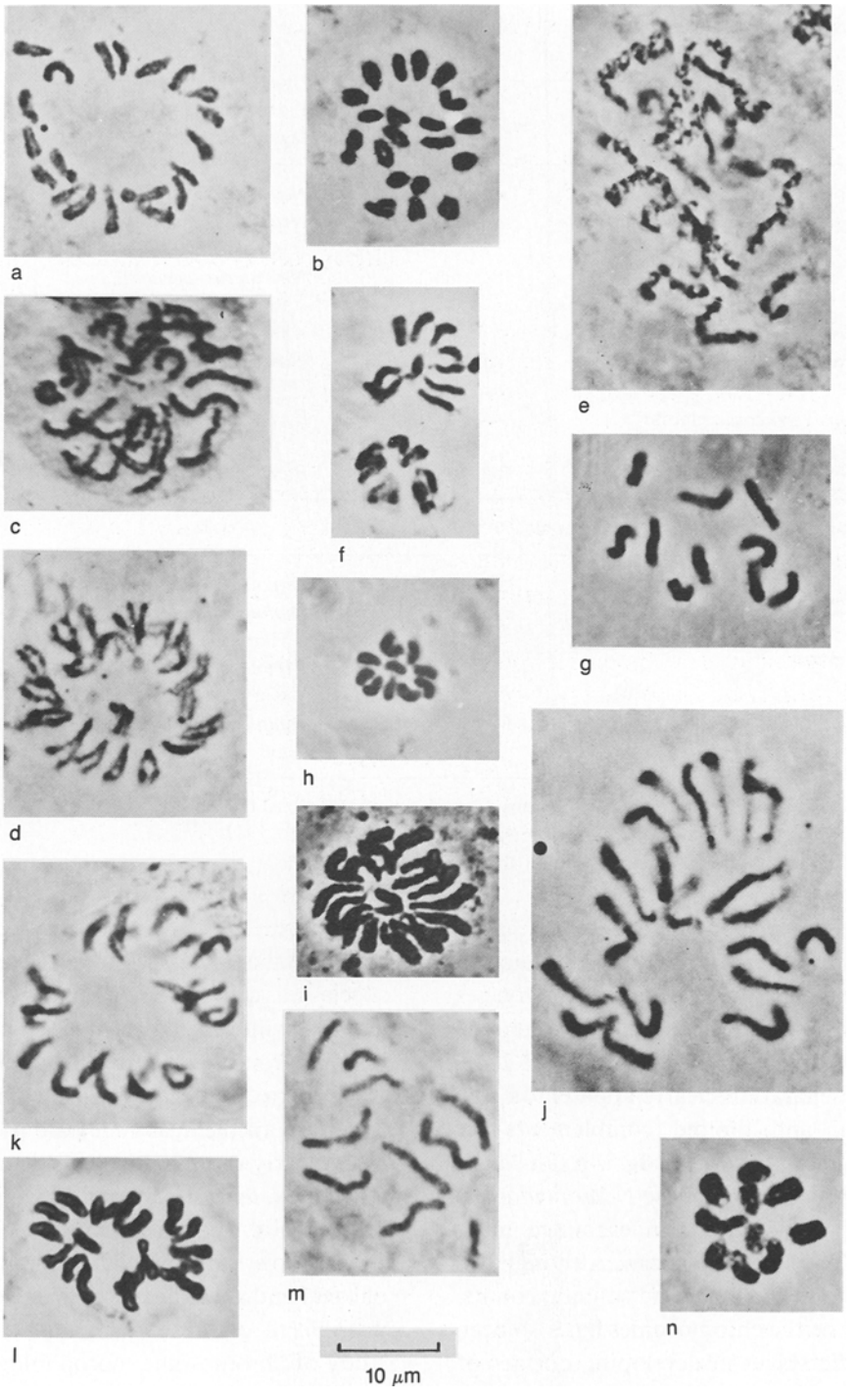


*Fig. 2.* Chromosomes of oak gall-wasps: (a–b) *Neuroterus lenticularis*, (a) oogonial metaphase plate showing chromatids, (b) somatic cell; – (c–h) *N. baccarum*, (c–d) female somatic cells, (d) showing chromatids, (e) female somatic prophase, (f) two spermatogonia with 10 chromosomes, (g) first spermatocyte with 3 acrocentric chromosomes, (h) second spermatocyte; – (i) *N. laeviusculus*, somatic plate with 20 chromosomes; – (j) *Andricus kollari*, somatic plate with 20 chromosomes; – (k) *Biorhiza aptera*, oogonial plate with 20 chromosomes; – (l–n) *B. pallida*, (l) female somatic cell with 20 chromosomes, (m) first spermatocyte prophase, (n) second spermatocyte.

but no oogonial or synaptic stages were available.

4. *Andricus kollari:* Oogonia and somatic cells have 20 chromosomes which appear to show somatic pairing (Fig. 2j). Satellites or trabants are distinct on some of the acrocentric chromosomes and only one pair are metacentric (Fig. 5g).

5. *Andricus curvator-collaris:* Spermatogonia and spermatocytes have 10 chromosomes and spermatogenesis follows the usual hymenopteran pattern with abortion of the first spermatocyte division followed by a vesicular interkinesis (Fig. 3a-e). In one female follicle cell (Fig. 3f) a precocious split in the chromosomes is clear. The 20 chromosomes shown enlarged in Figure 3g include one metacentric and nine acrocentric pairs (Fig. 5f). The overwintering generation, *A. collaris,* has not been examined.

6. *Cynips verrucosa-divisa:* The chromosome number in the agamic form is 20. The only stages examined were diakinesis in the oocyte and cleavage in the egg of *C. divisa* (Fig. 8j, k). Six chromosomes are metacentric, three acrocentric and one telocentric. No stages of *C. verrucosa* have been examined.

7. *Biorhiza pallida-aptera:* Spermatogonia and first spermatocytes have 10 chromosomes (Fig. 2m). In the fully grown spermatocyte a wide incompletely polarised spindle forms but no division takes place. The transition from the first spermatocyte to the next stage is not clear but the spindle aborts and the chromatin forms irregular masses and lines the persistent nuclear membrane (as is shown in the abortive stage in *Andricus curvator* (Fig. 3c). Dividing second spermatocytes with 10 chromosomes (Fig. 2n) lie in cysts of 32 cells and give rise to bundles of 64 spermatids which elongate into long-tailed sperms. In the female, 20 chromosomes can be counted in follicle cells (Fig. 2l) and 10 bivalents in the oocytes. The sexual females lay eggs in July but galls do not appear until the following April. Although the galls grow rapidly, the larvae of *B. aptera* remain very small and are still in the first instar at the end of May. One month later they are half-grown and by the end of July have reached the pupal stage with elongating ovaries. Although oogonial divisions are scarce, (Fig. 2k) somatic plates are plentiful and show precocious splitting of chromosomes. Of the 10 chromosomes, four are acrocentric (Fig. 5c).

8. *Diplolepis rosae:* The diploid chromosome number is 18. One pair is larger than the rest and may be clearly seen in oogonial, follicle and cleavage cells (Fig. 4a-c). All chromosomes except one of the smaller pairs are metacentric (Fig. 5d). The larvae of this unisexual, univoltine species develop in separate chambers within an extremely hard woody multilocular gall. In well-grown larvae the pear-shaped ovary may still contain rosettes of 8 or 16 oogonial cells connected by intercellular bridges. For a long period the larvae are mitotically inactive but just before pupation mitoses are common in follicle cells in the lengthening ovarioles.

9. *Diplolepis eglanteriae:* This species is a triploid with 27 chromosomes (Fig. 4d-f). One chromosome is larger than the others in the genome and only two of the nine appear to be acrocentric (Fig. 5h). Eight annual generations have been reared and a plentiful supply of all stages obtained.

10. *Diplolepis nervosum:* A photograph of the 18 chromosomes in a somatic cell of this locally scarce species in shown in Figure 4h.

11. *Diplolepis spinosissimae:* This gall-wasp has not been found locally in Angus but a dozen galls were collected on the banks of the Fraser River in British Columbia in 1979. Since emerged adults were not available for accurate identification, the data given here are based on material from well- grown larvae identified from galls only. Oogonial plates obtained show that all 18 chromosomes are metacentric and include one large pair (Fig. 4i). Synizesis occurs in postoogonial cells but no later stages were examined.

*Oogenesis*

Variation in the number of ovarioles and the egg potential in the different species is shown in Table 2.

A general outline of the origin and development

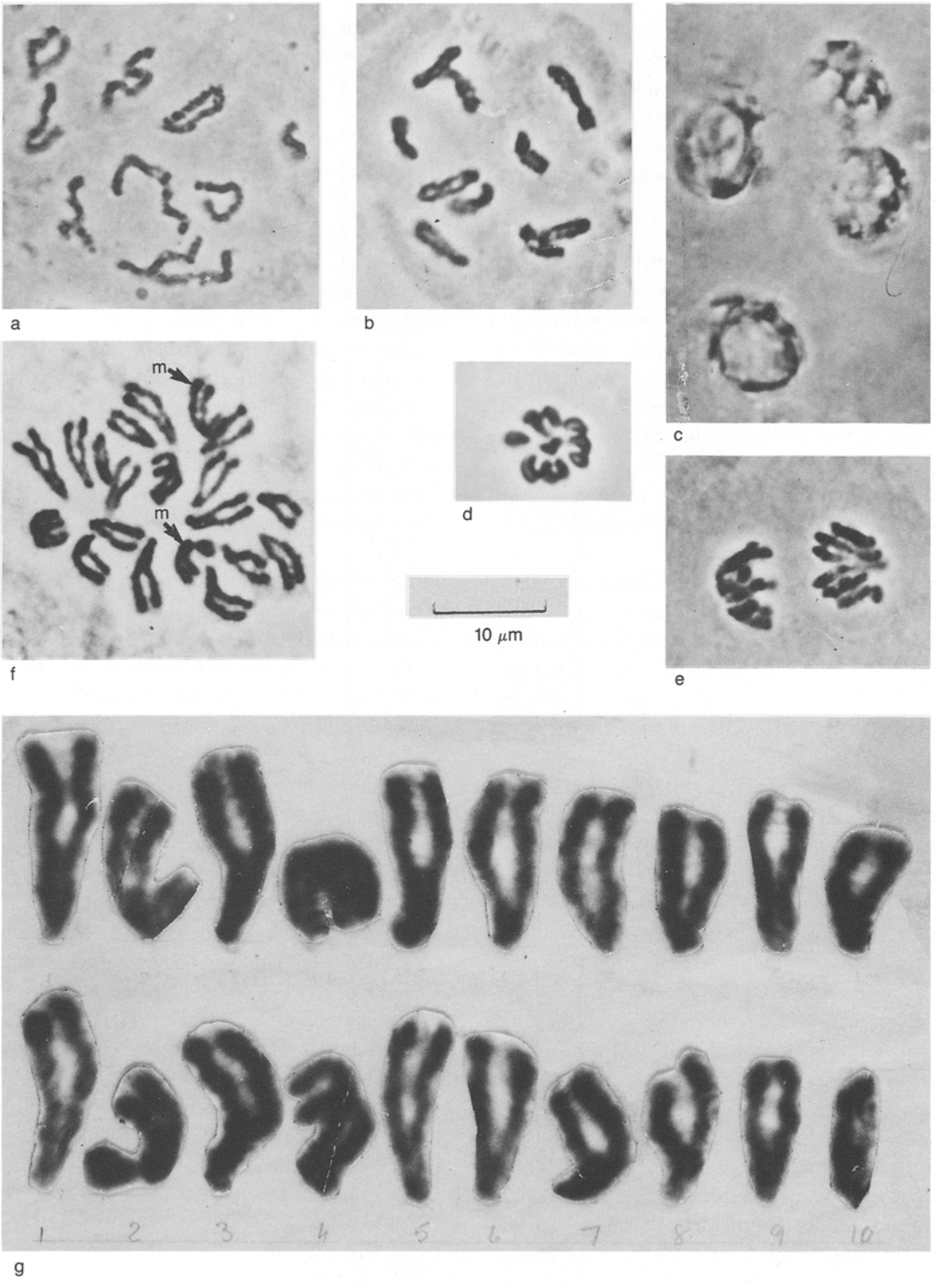


*Fig. 3.* Chromosomes of *Andricus curvator:* (a–c) males, (a) premetaphase I, 10 bivalents with single metacentric in centre, (b) same, metacentric at top right, (c) abortive stage in spermatocyte, (d–e) second spermatocyte metaphase (d) and telophase (e); – (f–g) female, (f) somatic cell showing 20 chromosomes, the two metacentrics are marked m; (g) the chromosomes of (f) enlarged and arranged according to size. The metacentric pair are the second largest.

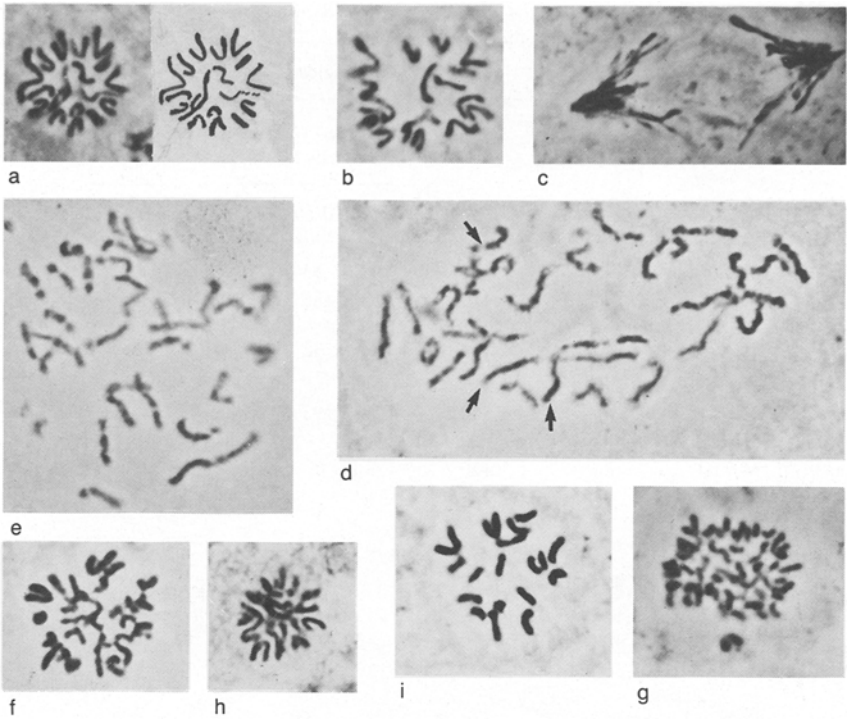


*Fig. 4.* Chromosomes of *Diplolepis* species: (a–c) *D. rosae*, (a) somatic plate (sketch shows the 18 chromosomes), (b) oogonium, two large chromosomes, (c) cleavage telophase showing the long pair of chromosomes; – (d–g) *D. eglanteriae*, the three longest chromosomes arrowed, (d) somatic prophase, 27 chromosomes, (e) ovariole tube cell, (f) oogonial metaphase, 27 chromosomes, (g) hexaploid tube cell; – (h) *D. nervosum*, somatic plate with 18 chromosomes; – (i) *D. spinosissimae*, oogonial plate with 18 chromosomes.

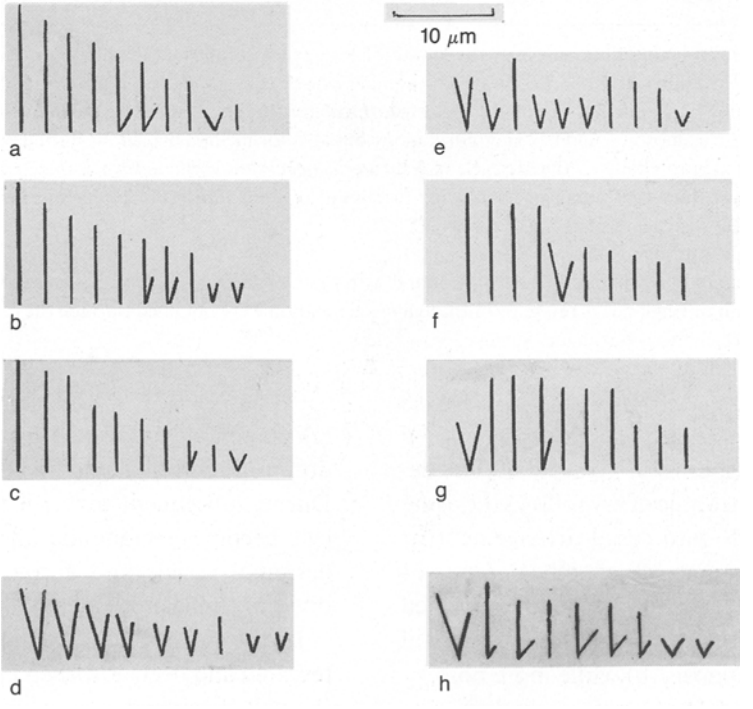


*Fig. 5.* Idiograms of (a) *Neuroterus baccarum-lenticularis*; (b) *N. numismalis;* (c) *N. laeviusculus;* (d) *Diplolepis rosae;* (e) *Biorhiza pallida-aptera*; (f) *Andricus curvator*; (g) *A. kollari*; (h) *D. eglanteriae.*

*Table 2.* Biological and cytological data relating to thirteen species of gall wasps*.

| Species | Biological | | | Cytological | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Emergence month, year | Number of ovarioles | No. eggs produced | No. eggs examined in diakinesis maturation | | | | Chromosome no. | | Synapsis |
| | | | | total | drawn | laid | extruded | male | female | |
| *Neuroterus baccarum* Oliv. | V-VII | 24 | 120 | 72 | 54 | 38 | - | 10 | 20 | + |
| – *lenticularis* Mayr. | III-IV | 24 | 120 | 1068 | 486 | 227 | 284 | | 20 | + |
| *N. vesicator* Schenck. | VI-VII | | | | | | | | | |
| – *numismalis* (Geof.) | IV | 16 | 100 | 80 | 39 | | | | 20 | |
| *N. albipes* Schenck. | VI-VII | | | | | | | | | |
| – *laeviusculus* Schenck. | III | 16 | 100 | 1700 | 193 | | | | 20 | |
| *Andricus kollari* (Htg.) | IV-IX | 48 | 500 | | | | | | 20 | + |
| *A. curvator* (Htg.) | V-VIII | 18 | 120 | | | | | 10 | 20 | + |
| – *collaris* (Htg.) | – | | | | | | | | | |
| *A. fecundator* (Htg.)** | III 2–3 | | | | | | | | 20 | |
| *A. quercuscalicis* (Burgsdorf.)*** | II | 96 | 1000 | | | | | | 20? | |
| *Cynips verrucosa* Schlech. | | | | | | | | | | |
| – *divisa* Stg. | VIII-III | 48 | 200 | 2 | | | | | 20 | + |
| *Biorhiza pallida* Fabr. | VII-VIII | 36 | 200 | | 30+ | 359 | | 10 | 20 | + |
| | | | | | | 194 V | | | | |
| – *aptera* (Oliv.) | XI-2 | 80 | 800 | | 35 | 2340 | | | | + |
| *Diplolepis rosae* (L.) | V-VII | 60 | 600 | | 42 | 1434 | | | 18 | + |
| *D. eglanteriae* (Htg.) | V | 64 | 400 | 100 | 25 | 160 | 32 | | 27 | -ve |
| *D. nervosus* Curt. | V+? | | | | | | | | 18 | + |
| *D. spinosissimae* Gir. | VI | | | | | | | | 18 | |

* Where the life cycle covers more than one year, the month of emergence is followed by the year number e.g. in *Biorhiza aptera* emergence occurs in November of the second year. The number of ripe eggs developed in an ovariole varies from five to ten, the larger number occurring in agamic females of *Andricus, Biorhiza* and *Diplolepis* species. Diakinetic stages were studied not only for verification of the chromosome number and morphology but also for meiotic behaviour during prophase. Maturation of the eggs was investigated in both phases of the life cycle in *Neuroterus baccarum-lenticularis, Biorhiza pallida-aptera,* in two species of *Diplolepis* and in extruded eggs obtained from dying females of *N. lenticularis* and *D. eglanteriae.* Incipient parthenogenesis occurred in some unfertilised eggs of *B. pallida* (194 V).

** Only one mature adult was available.

*** The species were all reared or collected in central Scotland with the exception of *Andricus quercuscalicis* which was recently introduced into the south of England. A few galls of the agamic generation were obtained through the courtesy of Mr. T. C. Winter of the Forest Research Station, Farnham, Surrey.

of the egg is described and the diagram in Figure 6 is applicable to most species of Cynipidae. The development of the polytrophic ovary follows the usual hymenopteran pattern with serial arrangement of oocytes separated by nurse-cell chambers. Cysts of oogonial cells (a) lie anterior to undifferentiated post-oogonial cells in the apical zone. These may still show synizesis and synapsis (b) while more posteriorly, within a group of 16–32 cells, a single oocyte with enlarged nucleus and homogeneous ooplasm (c) may be distinguished from nurse cells with endomitotic chromosomes in permanent prophase (d). During subsequent growth in the pupal stage, the oocyte becomes rectangular and has a relatively large germinal vesicle and a covering layer of actively dividing follicle cells (e, f).

By the end of pupal life the nurse cells have disintegrated and been absorbed into the oocyte in which the yolky ooplasm is now more homogeneous and transparent (h). The best stage for the study of diaki-

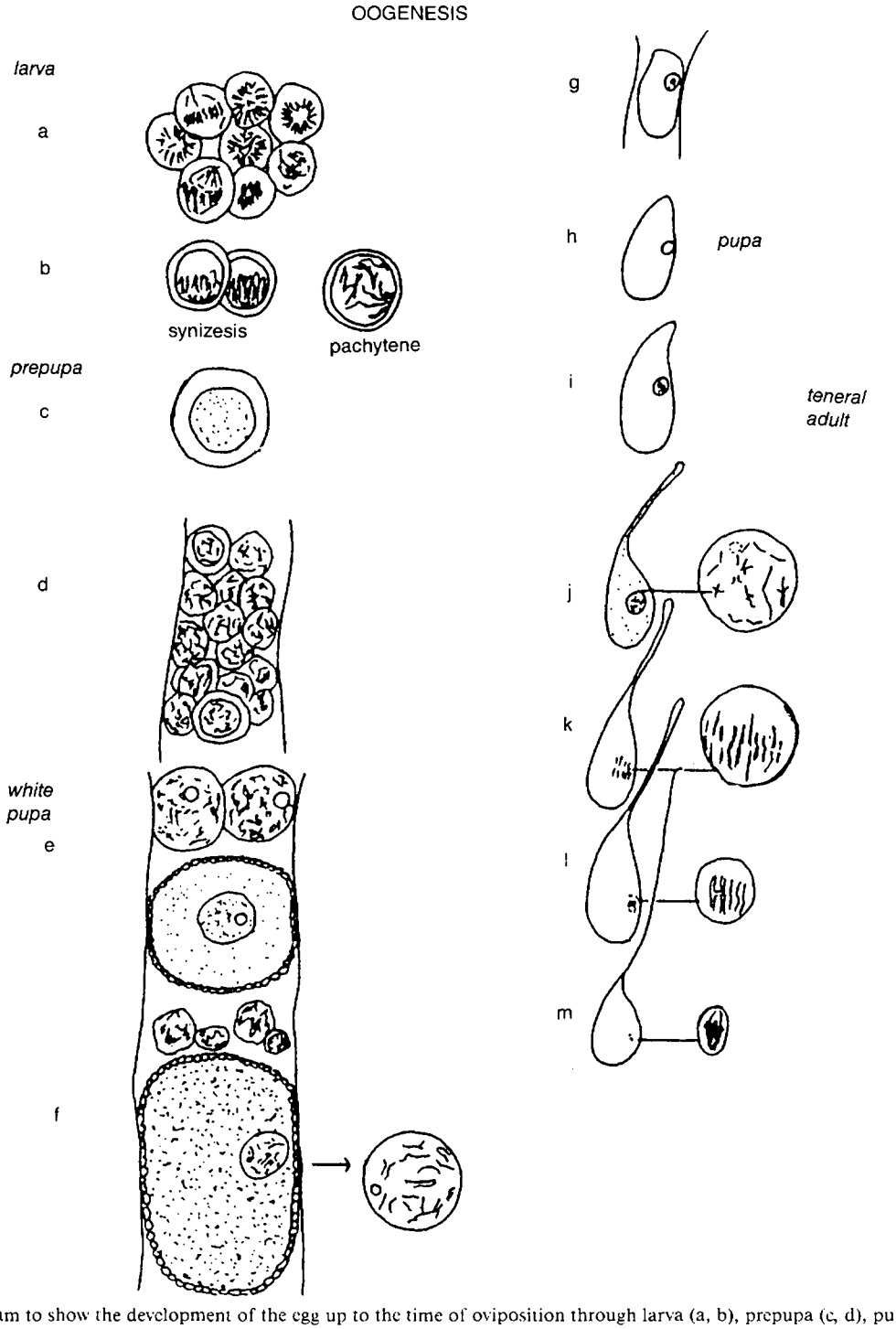


*Fig. 6.* Diagram to show the development of the egg up to the time of oviposition through larva (a, b), prepupa (c, d), pupa (e – h) and teneral adult (i – m). The left hand column shows chromosomes in cysts of oogonial cells (a), differentiation of the oocyte and nurse cells (c, d), the enlargement of the germinal vesicle (c – f) and meiotic stages from synapsis through pachytene (b) and the first diffuse period (d – e). The right hand column (to smaller scale) shows the development of the chorion (h) and pedicel (i) of the egg with details of chromosome behaviour from the second diffuse period (g – h) through the diakinetic stage (j) with end-to-end pairing to congression and alignment on the abortive first maturation spindle (k, l) and the resting fusiform nucleus in the ripe egg (m). Cf. text.

nesis is the soft-shelled oocyte (i, j) and for the abortive maturation spindle and resting fusiform nucleus (k, l, m) the fully formed eggs of the teneral adult or the newly emerged imago. The length of pedicel varies in different species according to the generation and the seasonal state of the host plant. Thus eggs deposited in winter buds are firm-shelled with long pedicels while summer eggs laid in leaves are thin-shelled with short pedicels. Three stages of meiosis-synapsis, diakinesis and congression are considered below:

*Synapsis:* Meiotic behaviour during oogensis is fairly consistent in both gamic and agamic females. Before oocytes and nurse cells differentiate, all cells go through synizesis where chromosomes form polarised loops at one side of the nucleus. In the oocytes this is followed by pachytene (b) in which the haploid number of paired chromosomes lie scattered within the nucleus. The nucleus now enlarges to form the germinal vesicle (c), the chromosomes lose their identity, rapidly become diffuse and are lost to view until they reappear later as irregular masses within the much enlarged germinal vesicle. Yolk globules appear in the ooplasm after the oocyte has acquired a layer of follicle cells (e) and, as more yolk is laid down, it is a matter of chance whether the chromosomes can be observed below the enveloping layer of follicle cells. Diplotene chromosomes are so faintly stained that they can only be discerned (and sometimes photographed) under phase optics. Each chromosome is a loosely spiralized filament which lies parallel to, but distant from its homologue (f). A second diffuse pale-staining period ensues during which the pedicel arises and the follicular envelope disappears as the chorion is laid down round the egg (h, i). While the chorion is still soft, the yolk is less dense and can be spread easily within the vitelline membrane in squash preparations. One or more plasmosomes lie freely in the nucleoplasm while the chromosomes are still pale and tenuous, but seem to be closely associated with one or more of the compact bivalents in a more advanced stage of diakinesis until they disappear during congression.

*Diakinesis:* In all the cyclical species examined, homologous chromosomes are elongated and loosely in contact with each other at one point only (j), usually terminally but in some species a few cruciform bivalents may have a median point of contact. No clear chiasmate configuration is apparent although an earlier diplotene condition is sometimes evident.

*Congression and formation of abortive spindle:* After diakinesis the nuclear membrane persists and a wide blunt spindle forms within it. On congression (k) the long arms of the homologues are directed axially and for a time appear to be in a state of premetaphase stretch. The bivalents do not deconjugate but shorten and fuse laterally on a much contracted abortive spindle (l). The nucleus of the fully formed egg then enters a resting stage in which the chromosomes lose their identity on the equatorial plate, the spindle shrinks and flattens at the poles and, still invested in the nuclear membrane, forms the fusiform nucleus (m). Thus the first maturation spindle aborts in metaphase and forms a resting nucleus which is activated to complete maturation after the egg is laid.

### *Meiotic prophase in nine species*

The following observations were made on the meiotic prophase in nine different species. They are discussed later.

1a. *Neuroterus lenticularis:* The growth of the germinal vesicle was followed from the early synapsis with the haploid number of loops (Fig. 7a) through pachytene and early diakinesis to congression of the bivalents on the spindle. In the diakinetic stage the homologues are paired end-to-end (Fig. 7b–d) and the 10 bivalents are loosely aligned in parallel on a wide spindle. Occasionally the homologues are more condensed and lie side by side as in Figure 7e. In several hundreds of nuclei examined and drawn in the study of chromosome individuality, the two acrocentric numbers 5 & 6 are always recognisable in both generations by their 'hockey stick' appearance (Figs. 5a and 7j). The 10 bivalents consolidate on the abortive spindle (Fig. 7f), then denucleinate within the nuclear membrane forming the fusiform

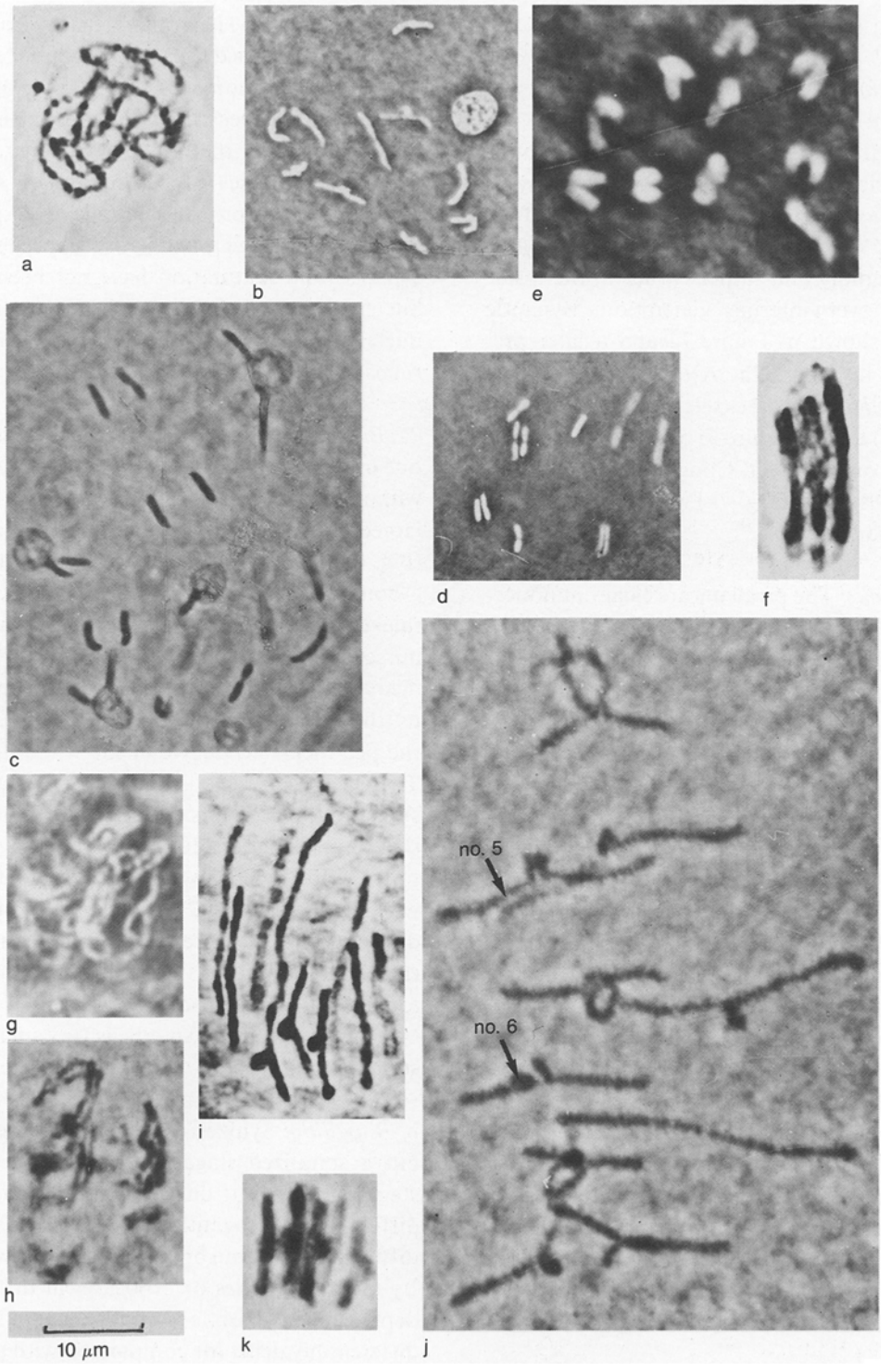


*Fig. 7.* Meiotic prophase in *Neuroterus baccarum-lenticularis:* (a – f) *N. lenticularis*, (a) synapsis, (b) early diakinesis showing single unattached plasmosome, (c) slightly later than (b), several plasmosomes attached to bivalents, (d) linear arrangement of bivalents (smaller scale), (e) unusual side-by-side alignment of bivalents, (f) abortive spindle in unlaid egg; – (g – k) *N. baccarum*, (g) synapsis, (h) later prediffuse stage, (i) congression: 10 bivalents, three showing lateral 'arms' and two showing chromatids, (j) enlargement to show loops on bivalents, acrocentric numbers 5 and 6 identified, (k) abortive spindle in unlaid egg.

nucleus which persists until the egg is laid.

b. *N. baccarum:* As in the agamic female there are 24 ovarioles in the two ovaries but the tubules are slimmer and the eggs more rounded with shorter pedicels. All stages found in *N. lenticularis* may be found in *N. baccarum* with the exception of the fusiform nucleus. With a shorter growth period, events occur more rapidly and rather earlier in the instar than in the overwintering generation. Synaptic pachytene is shown in Figure 7g and a later prediffuse stage in Figure 7h. After the spindle has formed, bivalents sometimes seem to have chiasmate loops (Fig. 7j) and in Figure 7i chromatids are visible in at least one bivalent. Congression is followed by an abortive spindle (Fig. 7k) which very soon becomes vesicular.

2. *N. numismalis:* The bivalents are clear but noticeably smaller than in *N. lenticularis*. Only the diakinetic stage was examined.

3. *N. laeviusculus:* There are only eight tubes in the ovary and the young oocytes are unusually long and narrow until they are fully formed. All prophase stages up to the abortive spindle were found (see Fig. 8a–f). In Figure 8a there is a hint of doubleness in some of the bivalents undergoing distance pairing. Later the chromosomes become rod-like and, at their most condensed stage, show terminal or near terminal conjugation (Fig. 8b, c). Polar and side views of the abortive stage are shown in Figure 8d–f.

4. *Andricus kollari:* The very large ovary has 24 ovarioles each with about 12 eggs separated by groups of about sixteen nurse cells. Ten distinct synaptic loops are shown in Figure 8g. During pupal life some of the oldest oocytes in the string may exhibit a most striking feature when viewed on a dark field. No distinct pairing of spiralized chromosomes has been seen but in diakinesis there are 10 bivalents, one of which forms an almost complete ring.

5. *A. curvator:* The earliest synaptic spiralized stages were found in the sexual females (Fig. 8h). In diakinesis one bivalent is ring-like (Fig. 8i) and another stirrup-shaped.

6. *Cynips divisa:* The synaptic stage was not found but the fully formed eggs in the agamic female show 10 bivalents in some of which a bridge is clear (Fig. 8k). This species is of interest in that prior to diakinesis during the mid-growth stage, 20 flocculent paired masses of chromatin are still visible (Fig. 8j). This is probably a stage corresponding to the spiralized stage found in other species. Stages of synapsis and maturation have not been examined but a few eggs in cleavage showed many haploid nuclei in developing males. The sexual form, *C. verrucosa* was not available for investigation.

7a. *Biorhiza aptera:* After the last oogonial division, one of a group of 16 cells differentiates as an oocyte with an increased amount of cytoplasm and an enlarged nucleus with synaptic chromatin loops (Fig. 9a). The other 15 cells in the group do not show a bouquet stage but become endomitotic with the thickened spiralized chromosomes characteristic of nurse cells. No spiralized stage could be found immediately after the diffuse stage in the oocyte nucleus, the chromosomes appearing next as bivalents in the pedunculated oocyte in the white pupal ovary. The germinal vesicle is unusually resistant to pressure and the bivalents are difficult to locate, but in the young teneral adult, when the eggs are fully formed, 10 discrete bivalents are seen to include four which are cruciform. The stage of congression is difficult to follow because the spindle breaks under the manipulative pressure required to burst the very tough chorion. The abortive spindle is of short duration and the fusiform spindle is very small, slim and stains poorly.

b. *B. pallida:* Synizesis and synapsis were recorded but a spiralized stage was not found. Diakinetic stages are of short duration and, when found, are difficult to photograph because the bivalents lie at different depths and break if squashed flat (Fig. 9g). By making a series of exposures at different focal depths, it was possible to obtain enlargements of the different bivalents for comparison with those of the agamic form. No significant difference could be detected between the bivalents of the two generations. The abortive spindle, shown in Figure 9h is followed by a fusiform nucleus.

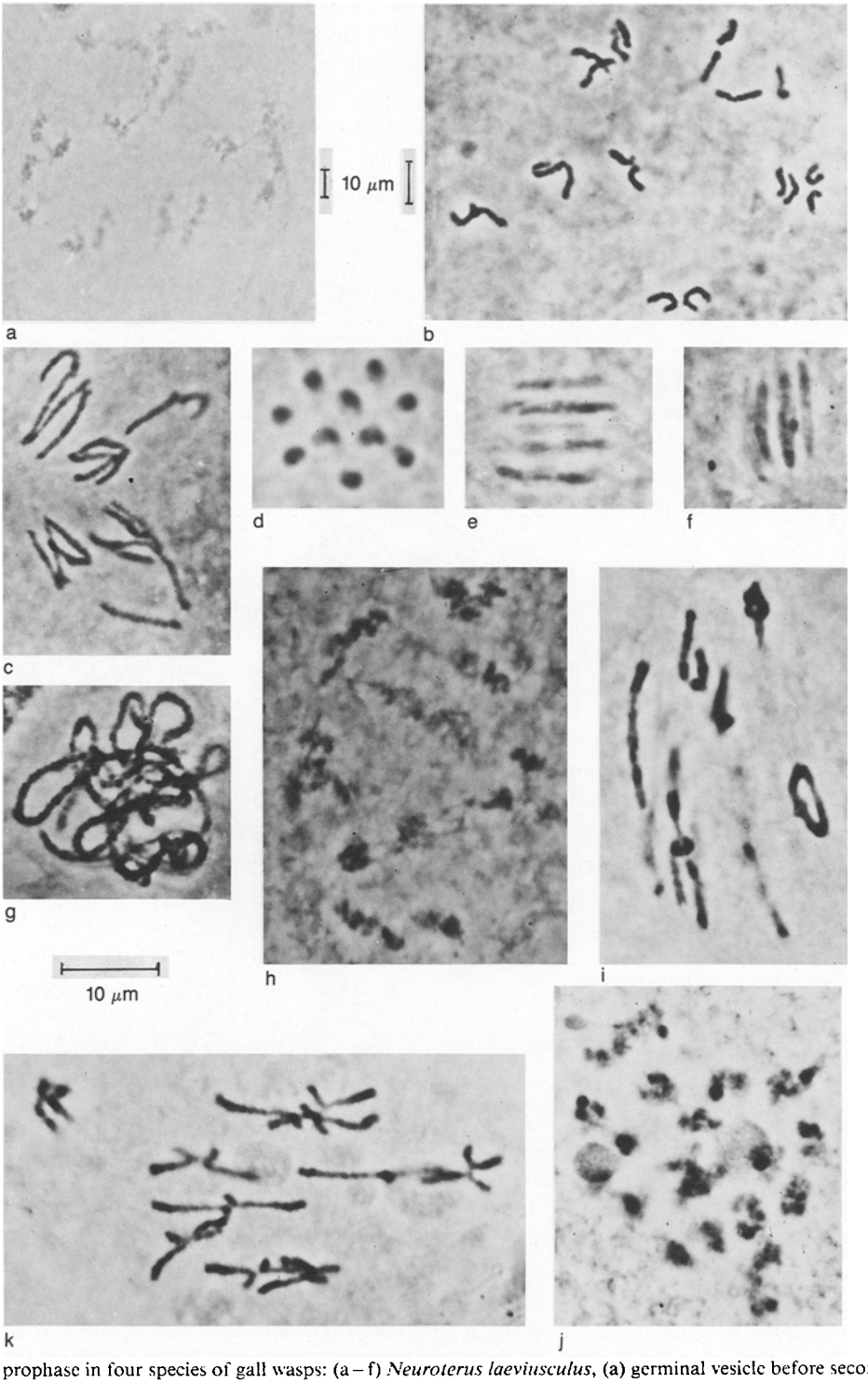


*Fig. 8.* Meiotic prophase in four species of gall wasps: (a – f) *Neuroterus laeviusculus*, (a) germinal vesicle before second diffuse period showing signs of doubleness in distantly paired chromosomes, (b – c) diakinesis, (b) 10 pairs of chromosomes loosely paired, (c) paired homologues form a V-precongression, (d) more advanced stage: bivalents more condensed, polar aspect of spindle within germinal vesicle, (e – f) two abortive spindles, one parallel to margin; – (g) *Andricus kollari*, synapsis – ten loops; – (h – i) *A. curvator*, (h) germinal vesicle with loosely paired double spirals, prior to second diffuse stage, (i) diakinesis, 10 bivalents, the metacentric pair form a ring; – (j – k) *Cynips divisa*, (j) early diplotene, seven pairs visible, (k) diakinesis, 10 bivalents, connecting bridge between homologues.

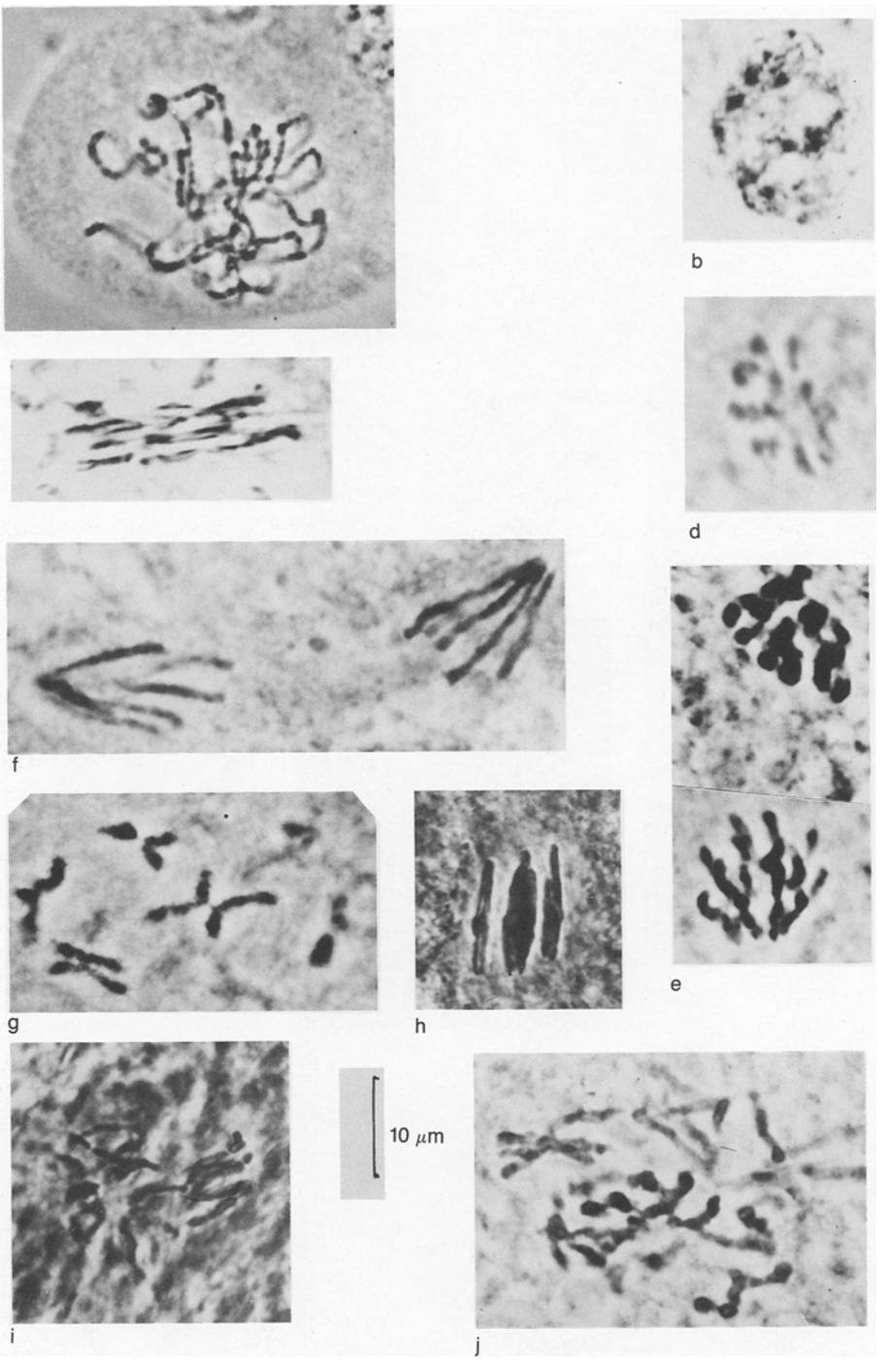


*Fig. 9.* Prophase and maturation in *Biorhiza pallida-aptera:* (a–f) *B. aptera*, (a) late synaptic stage in growing oocyte, (b) interphase nucleus in laid egg from ♀ 1 prior to spindle II formation, (c) Spindle II formed in egg laid 9 h by ♀ 2, a male-producer, (d) one of two nuclei in egg 20–28 h old, from ♀ 5, a male producer, 10 chromosomes, (e) single late anaphase in egg less than 40 h old, more than 10 chromosomes from ♀ 4, a probable female-producer, (f) haploid cleavage in egg 20–28 h old, from ♀ 5, a male producer; – (g–j) *B. pallida*, (g) diakinesis, not all bivalents in focus, 3 pairs showing bridging thread, (h) large abortive spindle in egg of emerged female, lateral fusion of bivalents, (i) peculiar apposition of two groups of chromosomes in egg 1.5–4.5 h old; left side group diploid, right side group looks haploid; in egg of mated female with a number of nuclei in yolk, (j) cleavage anaphase in egg from virgin female less than 18 h old, diploid.

8. *Diplolepis rosae:* Synizesis and synapsis occur in the ovarian apical cells from November to February. The paired chromosomes are visible in both early and late pachytene (Fig. 10a, b) and even after considerable growth of the oocyte (Fig. 10c). During pupation the nurse chambers disintegrate, the oocytes develop pedicels and form a string of closely packed eggs in which for a time the germinal vesicles can be detected only with difficulty. The post-pachytene chromosomes may appear as paired short-fibres (Fig. 10d) or as more condensed bodies (Fig. 10e). Later the pairs loosen further and become diffuse within a turgid germinal vesicle and reappear only after the chorion has formed and the pedicel elongates. After the diakinetic stage (Fig. 10f) which is of short duration and rarely seen, the condensed bivalents usually appear as nine double spheres or dumb-bells and may show a cruciform arrangement (Fig. 10g, h). During early congression (Fig. 10i), the chromosomes are not so stretched out along the spindle fibres as in cyclic types and rapidly form a dense band on an abortive spindle (Fig. 10j, k) and later, in the fusiform nucleus, are very tightly packed within a much shrunken nuclear membrane.

9. *Diplolepis eglanteriae:* Each ovary has 32 tubules which show no cells in synizesis in the terminal zone or in synapsis. After differentiation the nurse cells are readily distinguished from the oocytes by the presence of a very large plasmosome; they do not appear to increase their chromatin by endomitosis as in the diploid species. Following a leptotene stage with a large number of apparently single threads (Fig. 11a) the chromatin in the enlarging oocyte is at first flocculent and later thread like and only discernible with difficulty. After the prepupal moult the germinal vesicle in the elongating egg almost disappears but a rare 'prediakinetic' stage is shown in Figure 11b. The 'diakinetic stage' in the fully formed egg is more easily seen than the corresponding stage in the diploid *D. rosae* (Fig. 11d, e). The 27 univalent chromosomes are rounded and variable in size, the largest being present in triplicate (Fig. 11c). No distinctive abortive spindle forms but the chromosomes lose their individuality and form irregular clumps (Fig. 11f–h). Maturation follows rapidly after the clumped stage. In a batch of eggs taken from an unemerged female some had begun development and after 24 hours had reached the blastoderm stage.

*Maturation in the laid egg of four species*

1a. *Neuroterus lenticularis:* Although this species does not lay readily in captivity, eggs may be obtained from females which fail to lay and which, dying after two or three days, extrude the ovipositor and deposit batches of eggs during their death throes. In some cases these eggs may be deposited on a microscope slide and, if kept in a moist atmosphere may proceed to development as far as the embryonic stage. So far, only one other species, *Cynips divisa,* has been found to develop under these conditions.

Since eggs normally are laid singly, several females were set up to lay on a limited number of buds on a small oak twig. A total of 227 laid and 284 extruded eggs were fixed at intervals from 0.5–4 h for the study of maturation and up to 17 h for the study of cleavage. In eggs laid naturally, the fusiform nucleus swells to form a vesicular nucleus (Fig. 12a) and the first spindle forms after some 30 min (Fig. 12b). The chromosomes are never sufficiently distinct to be counted but the haploid number appears to be present in the anaphase in Figure 12c. The spindle with more than the haploid number of chromosomes in Figure 12d is perpendicular to the margin of the egg and, although somewhat larger than usual, could be presumed to be a maturation spindle since no other nuclei are present in the yolk, although the polarisation of the fibres is more characteristic of a cleavage nucleus. The anaphase spindle in Figure 12f appears to be haploid.

The spindles shown in Figure 12g lie parallel to the margin and, considering the age of the egg, are more likely to be late anaphase and telophase stages of the second cleavage division than an elongated maturation telophase. The relatively long chromosomes certainly have the appearance of cleavage chromosomes in haploid number. Where cleavage nuclei form a mosaic of haploid and diploid nuclei, it is possible to estimate the probable sex of the developing blastoderm. In 27 eggs from one female, 50% were predominantly diploid, 24% haploid and 26% had no countable plates. In 27 eggs from another

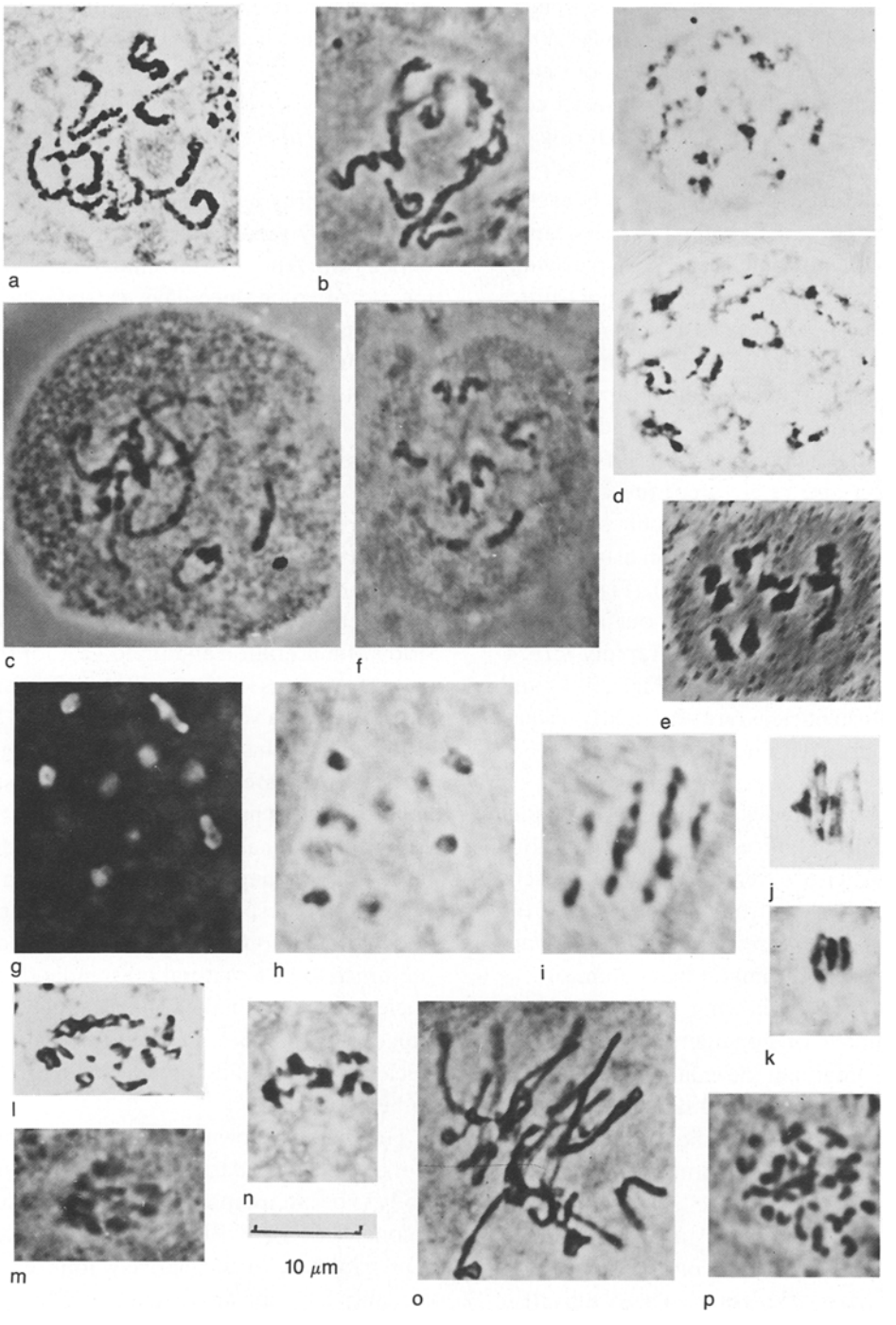


*Fig. 10.* Prophase and maturation in *Diplolepis rosae:* (a) Synapsis in larvae oocyte showing nine double loops; – (b – c) Pachytene stage, (c) in enlarging oocyte; – (d) Two post-pachytene germinal vesicles, beginning of first diffuse phase with separating homologues; – (e) Germinal vesicle in fully grown larva, chromatin in 9 blocks; – (f) Diakinesis in pupal oocyte, 6 bivalents in germinal vesicle; – (g) Later diakinesis in teneral adult, two ring bivalents on right; – (h) Diakinesis in emerged adult, 9 bivalents; – (i) Congression on spindle, bivalents not distinct; – (j) Blocked metaphase I, one lateral 'arm' showing; – (k) Abortive spindle with condensed chromosomes, magnification as in (i) and (j), from female emerged 8 h; – (l) Breakdown of interphase nucleus; – (m) Formation of metaphase II in egg 1.0 – 5.5 h old; – (n) One of two plates in a late anaphase with no other nuclei in the egg, about 9 double chromosomes (a regulatory phase?) – (o) One of 4 cleavage nuclei in egg 2 – 9 h old, diploid; – (p) Polyploid blastoderm cell.

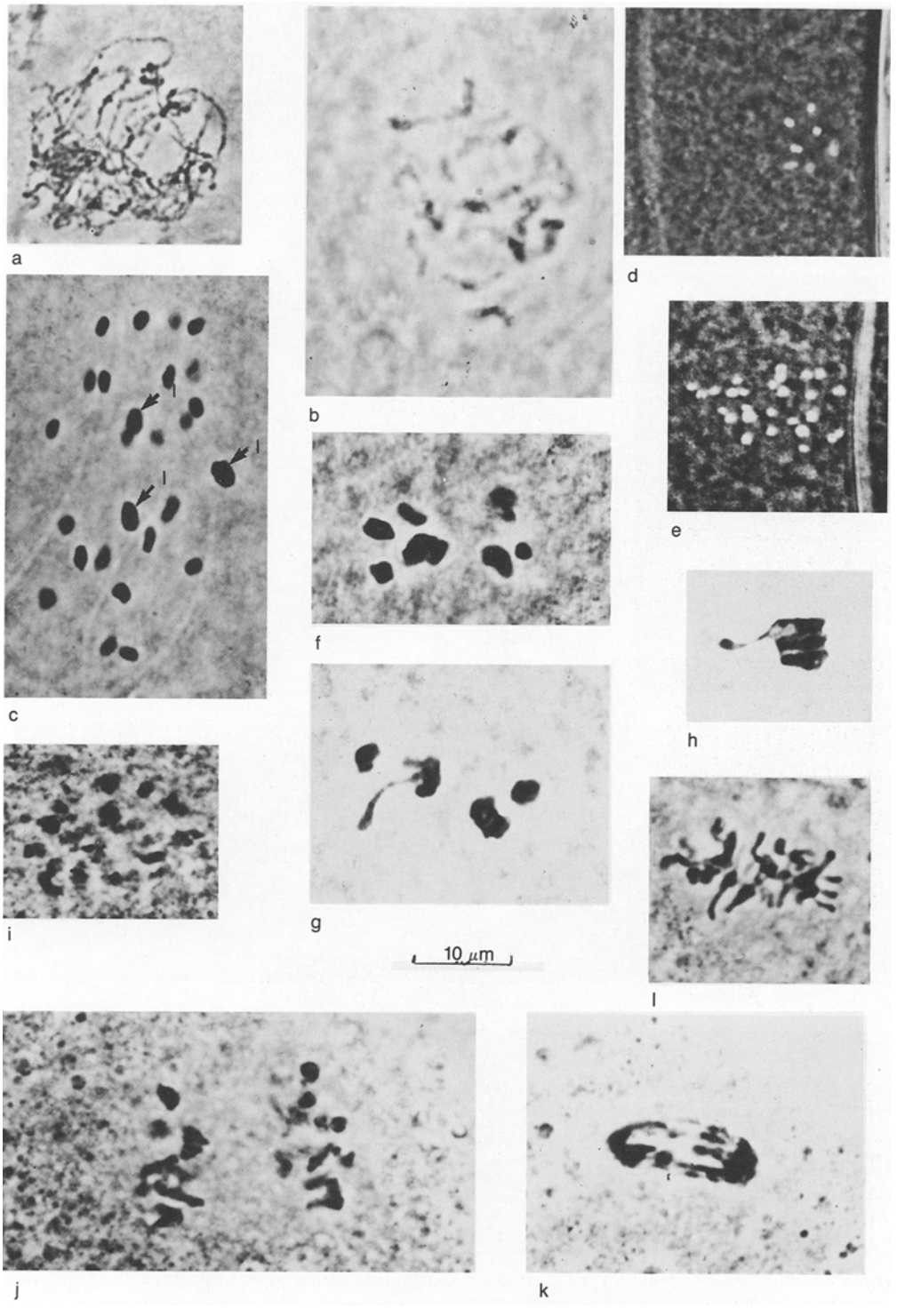


*Fig. 11.* Prophase, maturation and cleavage in the triploid *Diplolepis eglanteriae* (except d): (a) Leptotene in ovary of well grown larvae; – (b) Germinal vesicle in elongated oocyte, more than 20 chromosomes confined to central area; – (c) 'Diakinesis' stage, 27 chromosomes present (note 3 large, l); – (d) Diakinesis in *D. rosae* clearly showing seven of nine bivalents (lower magnification); – (e) triploid *D. eglanteriae* showing corresponding stage to (d) with 27 univalents; – (f–h) Stages in the formation of blocked abortive metaphases; – (i) Metaphase II plate, 22 chromosomes or more; – (j) Anaphase II, chromosomes large and apparently split, not all in focus; – (k) Anaphase II, small spindle; – (l) Second cleavage in egg.

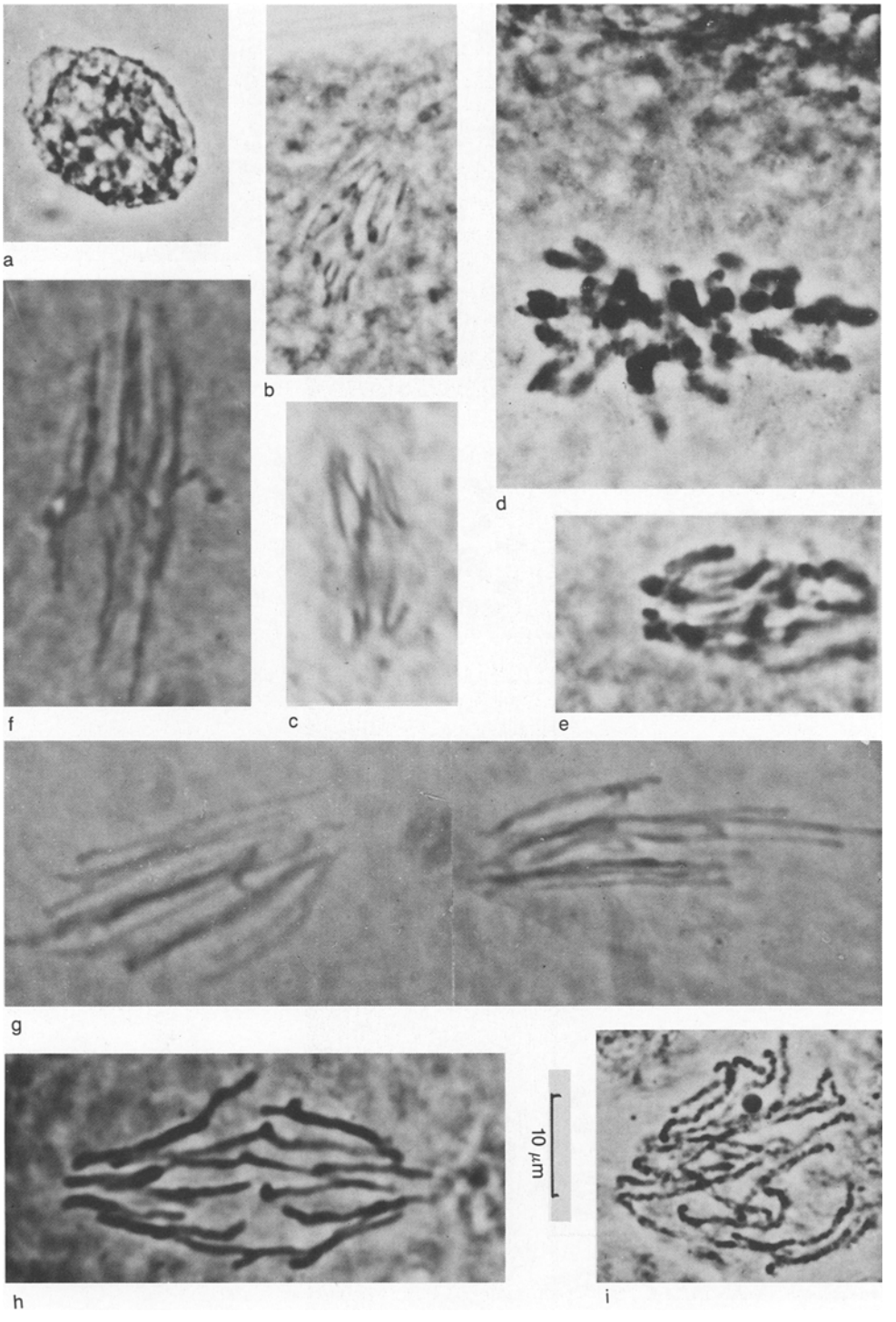


*Fig. 12.* Maturation and cleavage in *Neuroterus lenticularis:* (a) prophase of post-abortive division in unlaid egg of emerged female; – (b–c) first spindle in eggs laid 1–2 h, near margin; – (d) single anaphase, an exceptionally large spindle with diploid(?) chromosome number in an extruded egg; – (e) large spindle in anaphase, in an extruded egg; – (f) anaphase in single nucleus in egg laid 2–3 h, haploid and probably a cleavage spindle; – (g) two groups parallel to margin in egg laid after 1 to 1.5 h – haploid (?) derivatives of maturation or first cleavage division; – (h) late cleavage in egg giving two haploid groups; – (i) prophase in cleavage nucleus with diploid number of chromosomes.

source, three were definitely diploid in cleavage and five doubtfully so. In segmenting eggs of mixed origin, 35 were haploid (Fig. 12h), 21 were diploid (Fig. 12i) and many more were possibly diploid.

Only a few eggs succeed in reaching the embryonic stage under experimental conditions because degenertion usually sets in after the second day. Polyploid nuclei occasionally occur throughout the yolk in both healthy and degenerating eggs or may form a continuous layer in the serosa. In some apparently healthy eggs during late cleavage and blastoderm formation, pycnotic nuclei may be seen in the centre of the yolk. Their origin and possible fate is discussed later.

b. *N. baccarum:* Eggs of mated females are deposited singly on oak leaves. Among eggs fixed after 24 h, only one showed a single diploid nucleus. Another had several diploid nuclei and one had 20 cleavage nuclei which appeared to be diploid.

2a. *Biorhiza aptera:* Isolated females were set up to lay on growing saplings kept indoors. Some buds with eggs were removed for chromosome counts while others in which the same female had laid were allowed to develop through to maturity. Thus it was hoped any decisions taken on chromosome counts could be checked against the sex of the emerging unisexual brood. In all, over 2300 eggs were examined – 873 from four females in 1979 and 1476 from three females in 1980 (Table 3).

After oviposition the fusion nucleus becomes vesicular (Fig. 9b) then forms a spindle on which about 10 bivalents can be seen (Fig. 9c). In an egg showing only one nucleus, some rather massive anaphase chromosomes appeared to be still bivalent (Fig. 9e). No completed division nor any polar nucleus has been found. In cleavage nuclei the chromosomes are fairly discrete. The results of examination of eggs from seven females (Table 3) show that all the eggs from female 1 failed to develop beyond the fusiform nucleus; those from females 2, 5 and 7 gave haploid nuclei (Fig. 9d, f), only those from females 3 and 4 appeared to be diploid (Fig. 9e). Those from female 6 were thought to be developing as haploids. As it transpired, this interpretation in the case of the eggs from female 6 was correct, since eggs from the same female gave rise to 139 males which emerged from three separate galls on a young 30 cm sapling. Verification of the uncertain results from the other females was impossible because no other eggs produced mature galls. The quality of the photo-

*Table 3.* Results of experiments to relate chromosome number to sex in 2349 developing eggs of seven reared females of *Biorhiza aptera**.

| Female | Emergence date (survival period in days) | No. of eggs examined | Stage of development of selected eggs in 4 to 72 hours | Assessment of chromosome number and probable sex | | Sex of progeny |
|---|---|---|---|---|---|---|
| 1 | 31.X.79 (7) | 268 | fusion spindle only | – | – | – |
| 2 | 31.X.79 (10) | 168 | 1st spindle to cleavage; degeneration | 10 | male | |
| 3 | 21.XI.79 (9) | 170 | 1st cleavage to blocked mitoses | 20? | female? | |
| 4 | 19.XI.79 (24) | 267 | cleavage to blastoderm; degeneration | 20 | female | |
| 5 | 17.X.80 (35) | 323 | cleavage | 10 | male | |
| 6 | 17.XI.80 (28) | 580 | cleavage – blastoderm | 10 | male? | male confirmed |
| 7 | 23.XI.80 (21) | 573 | 1st spindle to advanced cleavage: degeneration | 10 | male | male |

* Females 1 and 2 were from same small sapling tree and may have been sisters: females 5 and 6 were from same root gall and were almost certainly sisters.

graphs in Figure 9 illustrates the difficulty met with in determining chromosome numbers in eggs. From the study of maturation and cleavage nuclei it is apparent that reduction must occur to yield haploid males, and if reduction occurs in all eggs, then in female-producing eggs diploidy must be restored by some type of regulation.

b. *B. pallida:* The female will lay, whether mated or not, and if given a sprouted acorn will insert the ovipositor into the rootlet, or even into the cotyledon or plumule. The eggs are rounder than those of *B. aptera* and have shorter pedicels which terminate in a small bulbous swelling. Eggs laid at one insertion of the ovipositor (about 40 in number) begin development more quickly and show less degeneration than eggs of *B. aptera.* In eggs deposited on an acorn rootlet, development proceeds as far as the embryo stage in about eleven days but never reaches the point of eclosion.

Most eggs were fixed at 0.5 h intervals up to 4 h and some were left for 9 h. As in the parthenogenetic egg, the maturation spindle is not very distinct but 10 dumb-bell shaped chromosomes directed towards the ends of the spindle indicate reduction. In a few eggs more than 4 h old the close apposition of one plate with another set of chromosomes on a half spindle might suggest a possible union of two sets of chromosomes (Fig. 9i). It is unlikely that a sperm nucleus is involved as a male pronucleus is usually vesicular in syngamy. Early cleavage nuclei appear to be haploid but in one egg less than three hours old at least one diploid cleavage nucleus was found.

Unmated females deposit eggs as readily as mated females but early development is much slower than in eggs from inseminated females. In unfertilised eggs 3 h old, cleavage nuclei with 20 chromosomes may be found (Fig. 9j), but later abnormal development with polyploid and pycnotic nuclei ensues. In eggs removed from dying females the fusiform nucleus does not begin development. It thus seems that in this species the act of oviposition is essential to initiate development.

3. *Diplolepis rosae:* Two days after emergence, females oviposit on young terminal shoots of dog rose. The insertion of the ovipositor is a lengthy process and for more than 1 h, clusters of eggs are laid in the base of the leaf bud or even in the flower bud. Over a thousand eggs were fixed at 0.5 h intervals so that all stages of maturation could be obtained even if the exact ages of the eggs were not known. In squash preparations eggs tend to burst posteriorly so the nucleus, lying in the pedunculated end of the egg, is often obscured by the chorion.

After oviposition the fusiform nucleus begins to swell into a vesicle within which chromatin masses are visible (Fig. 10 l). Later a spindle forms in which the chromosomes appear to be bivalent (Fig. 10m), but too tightly packed to give anaphase counts. The age of the egg, the form of the chromosomes and the number of nuclei in the egg give some indication of developmental progress but when only two nuclei are present and the age is uncertain it is difficult to decide whether these are maturation products (Fig. 10n) or cleavage nuclei, especially in orcein preparations in which asters typifying cleavages are not always rendered visible. From observations on the state of development in twelve batches of eggs fixed at timed intervals, it was calculated that each nuclear division lasts about two hours with formation of the first anaphase in 2 h, two cleavage nuclei in 5 h, 24 nuclei in 9 h and many blastoderm cells in 15 h. No haploid nuclei have been seen in the yolk of this species during later development and diploidy is established in second cleavage spindles (Fig. 10o). From the study of over 1400 eggs one can be fairly certain that maturation is meiotic, and that regulation occurs prior to or during the first cleavage division.

4. *Diplolepis eglanteriae:* In this triploid species, development proceeds with greater regularity than in diploid species. One hundred and sixty eggs laid in unopened rose leaves were fixed at intervals to give stages up to blastoderm. The earliest maturation stage, a large premetaphase plate with over twenty chromosomes, was found in several eggs (Fig. 11i), and anaphase spindles were found in older eggs (Fig. 11j–l). The spindles vary greatly in size and, even though larger than those found in other species, give only approximate triploid counts. No vesicular interphase forms and cleavage must follow on rapidly giving rise to healthy triploid blastoderm cells in

about 24 h (Fig.11l), with no sign of any pycnotic nuclei in the yolk. Several presumptive serosal cells were estimated to be polyploid and a hexaploid cell (follicle) is shown in Figure 4g. Degeneration does occur as in other species but is probably due to the adverse conditions within the severed rose shoots.

## Discussion

### *The chromosomes*

This survey reveals a type number of ten in seven species on oaks and nine in three species on roses. These numbers agree with numbers in *Neuroterus-baccarum-lenticularis, Andricus kollari* and *Diplolepis rosae* recorded by Doncaster (1910), Hogben (1920) and Dodds (1938, 1939), all of whom worked with sectioned material only. The species *Diplolepis eglanteriae,* first examined some 40 years ago (A. R. Sanderson, unpublished) and found to have more than the type number of 18 chromosomes, now has been shown to be a triploid with 27 chromosomes. It is interesting that *Cynips divisa* and *C. quercusfolii* were earlier placed in the genus *Diplolepis* – a taxonomic adjustment now justified on cytological grounds in *C. divisa* in which the chromosome number is 10. *C. quercusfolii* has not yet been examined cytologically. As the position of the centromere and details of chromosome structure are not clear, cynipid chromosomes do not lend themselves to detailed morphological study nor allow any discussion on the evolution of the karyotype.

Within a genus there is some degree of similarity in the karyotypes, the chromosomes in three species of *Neuroterus* and two species of *Andricus* being mainly acrocentric while in *Diplolepis* the chromosomes are mostly metacentric. A tendency towards precocious divergence of chromatids in somatic equatorial plates was noticed by Doncaster (1910) and although Dodds (1938, 1939) did not remark on it, his illustrations show the split clearly. The similarity of many acrocentric chromosomes in mitotic plates makes identification difficult and where, as in *A. kollari* (Fig 2j), adjacent chromosomes appear to be homologous their pairing may be accidental. The evidence for somatic pairing advanced by Dodds (1938) in support of a current belief in a diploid-tetraploid condition in Hymenoptera is not convincing. I inclined to this view at one time but cytological investigation of the honeybee *Apis mellifica* (Sanderson & Hall, 1948) and of sawflies (Sanderson, 1970) proved it untenable especially in the sawfly *Pteronidea ribesii* Scop. where, by use of squash technique, the haploid number was shown to be nine and not eight as earlier reported by Sanderson (1932) when using only sectioned material.

Polyploid somatic cells are common in insects, especially in parthenogenetic types such as the gynogenetic spider beetle *Ptinus clavipes* var. *mobilis* Moore, the triploid weevil *Listroderes costirostris* Schon. and sawflies (Sanderson, 1960, 1970 and 1973), all of which tolerate a high degree of chromosomal irregularity. Diploid nuclei are common in somatic cells of haploid gall wasp males as in other Hymenoptera and, where less than the haploid number of chromosomes appear as in loose spermatocyte cells, the loss can usually be attributed to breakage of the nuclear membrane. Serosal and ovariole epithelial cells (Figs. 4g, 10p) are prone to be polyploid. Nurse cells in developing ovarioles exhibit a fair degree of polyteny. As one of their functions is to increase the amount of RNA, the chromosomes replicate many times but the cells do not undergo any wasteful mitotic division. Each chromosome has several densely stained beaded strands which separate like chromatids, but never condense. In the triploid *Diplolepis eglanteriae,* the nurse cell chromosomes appear to have fewer strands than those of diploid species, probably because the increased number of chromosomes limits the space within the nuclear membrane.

In oak gall-wasps the diakinetic stage in the oocyte is usually so exceptionally clear that confirmation of chromosome number from haploid cells is not necessary. In thelytokous species where the form of diakinetic chromosomes is less clear no male haploid counts are available. I have reared over 200 adults of *Diplolepis rosae* and dissected over 130 individuals at different instars but have never found a male. Callan (1940) reported finding six males among 6000 females from galls collected in England, but unfortunately no cytological investigation was made.

*Meiotic prophase*

The prophase is meiotic in both gamic and agamic generations of all species examined, with the exception of the triploid *Diplolepis eglanteriae*. It is not clear what happens during the first diffuse stage which follows pachytene, but the appearance of distantly paired spiralised threads probably represents diplotene. This stage has not been mentioned by previous investigators who did not have the benefit of phase contrast optics. In the early stages of diakinesis bivalents resemble colchicine-treated metaphase chromosomes where sister chromatids are held together at the centromere. Although a few cross- and ring-bivalents have been seen, one gets the impression that meiosis is achiasmatic. In other groups of insects, lack of chiasmata and crossing over is usually confined to the heterogametic sex, e.g. male Diptera and female Lepidoptera (Suomalainen, Cook & Turner, 1973). I am not aware of reports of any genetical experiments in Cynipidae which would help to clarify meiotic behaviour during the prophase of oogenesis.

The side-by-side pairing occasionally found at a more condensed stage of diakinesis shown in *Neuroterus* in Figure 7e was also observed by Dodds (1939). He considered this 'parasynapsis' to be typical but it is probably no more than a brief stage of condensation which precedes the end-to-end position assumed later.

Early in congression, discordance between the movement of the chromosomes towards the equator of the spindle and the action of the centromeres results in a kind of premetaphase stretch. A similar movement is also apparent in the ichneumon *Nemerites* in which the spindle is also abortive (Speicher, 1937). In cynipids the position of the centromere is never clear but if it lies in the long arm then the homologues may be held together on the equator by a true chiasma. If they are held together by the centromeres only then the prolonged equatorial position of the latter may account for the failure of disjunction and spindle abortion.

*The abortive spindle*

In all Hymemoptera an abortive division is characteristic of spermatogenesis but an abortive spindle in oogenesis occurs only in the Cynipidae and the parasitic Apocrita. In the oocyte the spindle is at first wide and incompletely polarised possibly through lack of tension caused by the persistence of the nuclear membrane. As the still relatively long chromosomes begin to lose their identity and fuse laterally on the contracting spindle fibres it is not clear whether the nuclear membrane bursts or merely shrinks. The shape of the spindle varies from species to species but in all, contraction continues and the chromatin loses its stainability. The pale homogeneous chromatin mass in the fusiform nucleus is surrounded by a gel-like nucleoplasm bound by a firm membrane, whose refractive outline is readily detected under low powers of the microscope. After the egg is laid the nucleus again becomes vesicular and this return to an interphase distinctly separates the subsequent maturation division from the abortive division, which therefore must be considered as a blocked rather than a suspended first maturation division. In summer generations the interphase is of short duration but in long-living agamic females such as *Biorhiza aptera* and *Andricus kollari* it may persist for some weeks or even months under natural conditions before giving rise to another maturation spindle after the egg is laid.

*Maturation*

Of all the investigations undertaken those of maturation in over 4000 eggs have been the most unrewarding. Eggs laid singly in leaf buds are difficult to find and to handle and in only two species was it possible to utilise unlaid eggs from the egg masses extruded by expiring females. In the few species where eggs are laid in clusters, (*Biorhiza pallida-aptera* and *Diplolepis rosae*), eggs are easily extracted but their development cannot be timed accurately. In the laid eggs of both gamic and agamic females the compact chromosomes on the spindle appear to be bivalent and though never clearly countable during their poleward progression, it is believed that there are two haploid groups in mid-anaphase. The full telophase stage has not been seen and no polocyte has been identified with certainty.

In *Neuroterus lenticularis* Doncaster (1910, 1911)

thought that two divisions occurred in eggs destined to give males, and Dodds (1939) thought that only one spindle formed. Both authors recorded polar nuclei which by virtue of their marginal position would be more likely to be observed in their haematoxylin-stained serial sections than in dispersed squash material. However, although the orcein used here in squash preparations is a more selective stain for chromatin than haematoxylin, perhaps more reliable results could be obtained by using Feulgen technique, provided the problem of shrinkage and loss of material could be overcome. Today, the presence of one or more polar bodies is not acceptable as evidence of reduction. Doncaster and Dodds both believed that no maturation division occurred in eggs of *N. lenticularis* destined to give females. In the present study no difference was observed in the maturation of over 500 eggs from females developed in lentil galls collected over a wide area and almost certainly comprising both male- and female-producers. Reduction must occur in eggs of male-producing androphores as blastoderms clearly show hundreds of haploid nuclei. Eggs of some gynaephores showing a mosaic of haploid and diploid nuclei in the yolk would give rise, if viable, to females. The high degree of mortality in the parthenogenetic eggs could account for the relatively small numbers of currant galls produced in the spring compared to the enormous numbers of lentil galls produced by the sexual generation in the autumn. As for maturation in eggs of the bisexual generation, I would accept the statements of both Doncaster and Dodds that reduction occurs, although it has not been possible to demonstrate polar nucleus formation nor syngamy. In the acyclic *Diplolepis rosae* cleavage is diploid from an early age and in *D. eglanteriae* triploidy is established from the outset of cleavage.

*Regulatory mechanisms*

In a reduced egg, restoration of the diploid number is possible by (1) fertilisation or (2) automixis (Fig. 13).

1. *Fertilisation:* Although fertilisation by fusion of male and female pronuclei has not been observed in gall-wasps, it should be stressed that syngamy is difficult to demonstrate in any insect egg. Aster-like bodies found in the yolk of eggs of the gamic generation of *Biorhiza* could originate from sperms but similar centres are to be seen also in unfertilised eggs of *Neuroterus lenticularis* and other parthenogenetic insects e.g. sawflies. Rudimentary parthenogenetic development found in eggs of virgin females of *B. pallida* resembles the abnormal nuclear behaviour found in eggs of the unmated gynogenetic spider beetle *Ptinus clavipes* f. *mobilis* (Sanderson, 1960). It is possible therefore that in gall-wasps the sperm may only activate. As pseudogamy is not associated with any particular type of maturation or degree of ploidy and is to be found in ameiotic, meiotic, diploid and polyploid forms the possibility of its occurrence in gall-wasps could be an interesting additional area of study.

2. *Automixis:* This has been discussed by Suomalainen (1950), Peacock & Weidmann (1961) and Narbel-Hoffstetter (1964) in a wide range of parthenogenetic animals and more recently by Crozier (1975) in Hymenoptera. In gall wasp maturation, chromosomes appear to be bivalent and reduction is possible in all eggs, although it has not been observed. Disjunction must occur in eggs of male-producing females (androphores) (Fig. 13b) and probably also in eggs of females of the bisexual generation (Fig. 13a) If it occurs in eggs of female-producers (gynaephores) then diploidy must be restored by one or other of the methods set out in Figure 13 (c, d) either during or soon after maturation II.

In a few post-pachytene germinal vesicles the bivalents seem to show chromatids which in a normal maturation division would separate during the second division. In eggs of gall wasp gynaephores, duplication of chromosomes during maturation may be facilitated by a tendency for early separation of chromatids in somatic metaphase plates (Fig. 13c). In the acyclic species of *Diplolepis rosae* somatic chromatid separation is not noticeable and, since diploidy is here well established in early cleavage, it is possible that restitution is not by early chromatid separation but by recombination of anaphase plates, with the spindle sinking into the yolk and acting as the first cleavage spindle (Fig. 13e).

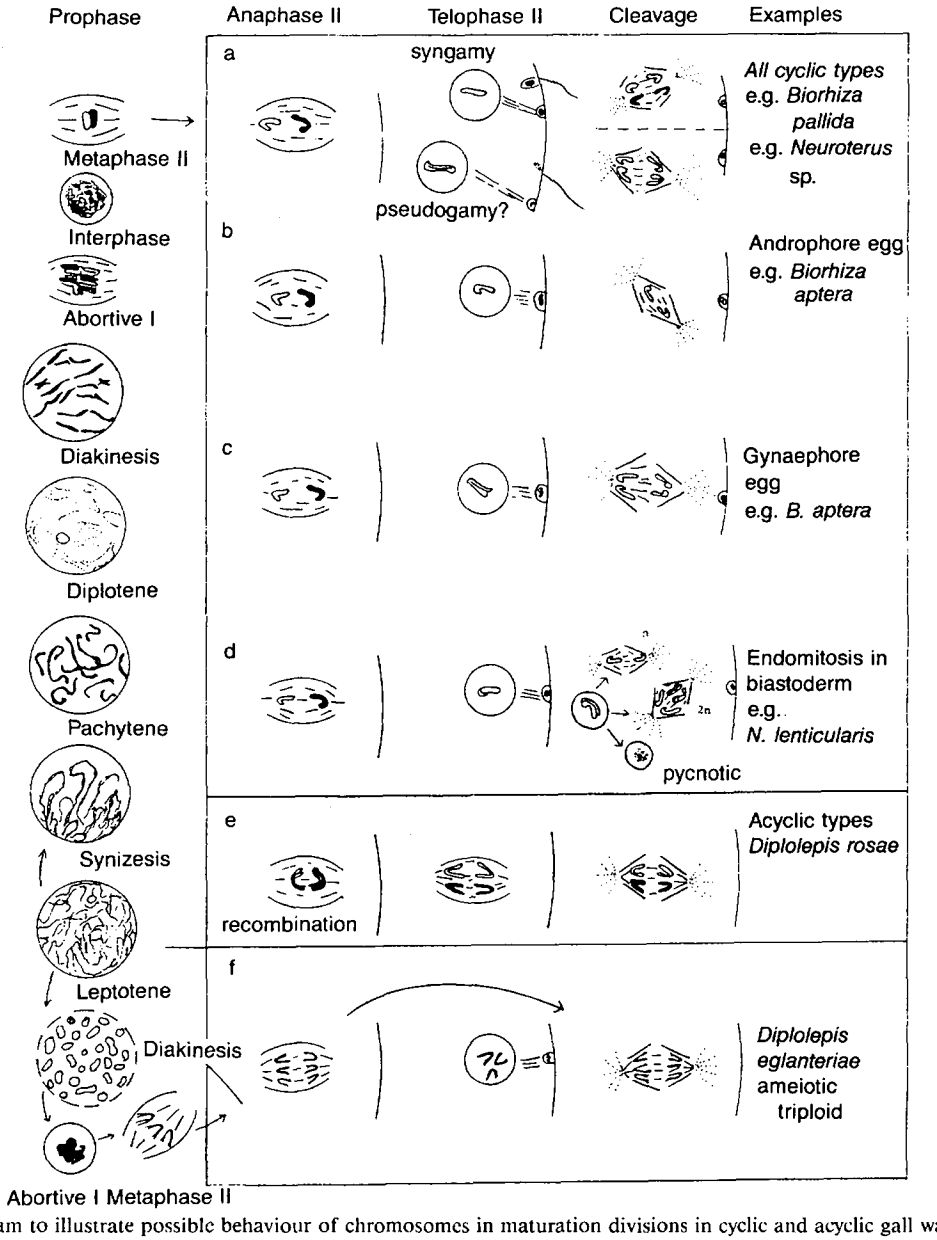


*Fig. 13.* Diagram to illustrate possible behaviour of chromosomes in maturation divisions in cyclic and acyclic gall wasps with special reference to regulation of chromosome number. Prophase stages from leptotene (below) to the second maturation division are shown in the left hand column. The second maturation division may involve reductional or pseudoreductional disjunction and a polar body may not be formed. Only one pair of chromosomes is shown in the diploid species. In the triploid *Diplolepis eglanteriae* synapsis does not occur but an abortive first spindle forms; the second spindle is non-reductional.

*Cyclic:* (a) Bisexual: gamic (Disjunction of homologues with reduction in eggs of bisexual generation female, followed by syngamy (or, if pseudogamous, by endomitotic restitution); e.g. eggs of *Biorhiza pallida* and *Neuroterus* sp.); – (b) Parthenogenetic: agamic (Disjunction of homologues in eggs of male-producing androphores, e.g. *Biorhiza aptera, Neuroterus lenticularis*); – (c) Parthenogenetic: agamic (Disjunction with endomitosis in anaphase in eggs of gynaephore, e.g. *Biorhiza aptera*); – (d) Parthenogenetic: agamic (Disjunction to form a haploid pronucleus; early cleavage with duplication in blastoderm stage giving a mosaic of haploid, diploid and pycnotic nuclei in the yolk e.g. *Neuroterus lenticularis*).

*Acyclic:* (e) Parthenogenetic (Disjunction and later recombination of anaphase plates; diploid in early cleavage, the maturation spindle becoming the first cleavage spindle, e.g. *Diplolepis rosae*); – (f) Parthenogenetic (No disjunction – ameiotic; triploidy maintained e.g. *Diplolepis eglanteriae*).

In the acyclic triploid *Diplolepis eglanteriae* there is no synapsis nor any meiotic phenomena during prophase, no reduction and therefore no regulation (Fig. 13f).

A method of regulation by fusion of the pronucleus with a polocyte nucleus occurs in the psychid moth *Luffia hemisphaericum* (Suomalainen, 1940) and in the sawfly *Diprion polytomum* (Smith, 1941). This method is quite feasible in insects because polar nuclei are retained within the yolk but in the absence of polar nuclei it cannot operate in gall wasps. Restoration by fusion of two cleavage nuclei was found in the whitefly *Trialeuroides vaporariorum* (Thomsen, 1927) and in the scale insect *Gueriniella serratulae* (Hughes-Schrader & Tremblay, 1966) but no fusion of any vesicular nuclei has been seen in any gall wasp. The apparent association of two chromosome groups in *Biorhiza* (Fig. 9i) was considered to be unusual and perhaps accidental. Finally, a less well known form of regulation by endomitosis in the blastoderm stage is worthy of consideration (Fig. 13d). This is a form of regulation found in phasmids. Bergerard (1958) described how virgin-laid eggs of the parthenogenetic *Clitumnis extradentatus* may develop to eclosion if kept at suitable temperatures. The reduced eggs begin development with the haploid number of chromosomes but many nuclei may become diploid and eventually form a blastoderm. The chromosomes undergo an extra replication whereby large 'diplochromosomes' are formed. In anaphase the two chromatids remain attached at the centromere until the nuclear membrane forms. Their subsequent separation restores the diploid number in some nuclei which then enter the peripheral blastoderm layer, while nuclei which fail to diploidise remain deep in the yolk and form pycnotic nuclei. A similar method of regulation may operate in *Neuroterus lenticularis* where in some blastoderms, many nuclei lying deep in the yolk have very tightly packed plates of chromatin (Fig. 13d). These 'tight' plates are common in eggs which have many diploid nuclei and may indicate a breakdown process in haploid nuclei which have failed to diploidize and enter the peripheral layer. Doncaster (1910) referred to similar pycnotic nuclei in blastoderms of both generations of *Neuroterus*. The very large spindles shown in Figure 12e were in extruded eggs and may have arisen by early diploidisation under irregular conditions.

### *Endomeiosis*

The term *ameiosis* applies where no effective pairing of homologues occurs and the term *endomeiosis* where homologues pair and then disjoin before the end of prophase, as in aphids. The diversity of endomeiotic processes exhibited by parthenogenetic animals has been discussed by Narbel-Hoffstetter (1964) particularly as to when and where disjunction occurs. Pairing of a transitional nature may occur during prophase with despiralisation and terminalisation of chiasmata leading to formation of univalents *after diakinesis* but before growth of the oocyte.

In *D. rosae* Hogben (1920) considered that the diploid number reappeared for a time and was followed by a later second pairing. The prediffuse phases shown in Figure 10d and in Figure 8j could be interpreted in this way. The disjunction and later end-to-end pairing may well be brought about by the somewhat irregular behaviour of the centromeres. In the thelytokous cockroach *Pycnoscelus surinamensis* L. the centromere divides during prophase not only in mitotic divisions but in post-pachytene stages in the oocyte (Matthey, 1945). This brings about complete deconjugation of the homologues as evidenced by the diploid number of chromocentres visible in the following diffuse stage. In anaphase I the disjoined chromosomes are ring-like through early repulsion between the new centromeres and attraction between their distal ends. The chromatids separate at the second division and thus diploidy is maintained. Matthey (1945) applied the term 'centromeric anticipation' to this early division of the chromosomes, and because of this tendency for chromatids to behave like chromosomes considered *Pycnoscelus* to be a potential polyploid.

### *The prophase in triploids*

The karyotype has been studied in many triploid parthenogenetic forms particularly in Coleoptera

(Narbel-Hoffstetter, 1964) but the prophase has been examined in only one or two species. The triploid Australian vegetable weevil, *Listroderes costirostris* Schon. has an enigmatic bouquet stage comparable to pachytene with the thirty chromosomes forming a haploid number of loops apparently by tandem linkage in threes, but reappearing after the diffuse stage as thirty univalents (Sanderson, 1973) which undergo a non-reductional maturation division. The triploid gynogenetic *Ptinus clavipes* f. *mobilis* Moore has a bouquet stage with the triploid number of loops (Sanderson, 1960) and therefore may be classed as pseudomeiotic. In the triploid cynipid *Diplolepis eglanteriae* there are no detectable synaptic phenomena and here parthenogenesis is ameiotic.

The evidence presented here on maturation and development in the cynipid egg is incomplete with respect to meiotic prophase, completion of the second maturation division and liberation of a polar nucleus, and, in the bisexual generation, the fusion of male and female pronuclei. Although chromosome behaviour is difficult to follow through the diffuse phases of the prophase it is clear that the evanescent post-pachytene despiralisation does not lead to complete disjunction of homologues since bivalents can be followed from the diakinetic stage up to their alignment on the abortive spindle. Special investigation into the behaviour of the centromere may lead to a better understanding of not only its cytological role in automixis but also its genetical role in sex determination. The data obtained from breeding experiments will be presented elsewhere.

## Summary and conclusions

(1) The most reliable chromosome counts are obtained from oogonia, oocytes in diakinesis and first and second spermatocytes. In heterogonous cynipids the chromosomes are mainly acrocentric and the basic number is ten. In the acyclic types studied the chromosomes are metacentric and the basic number is nine. Tentative idiograms have been prepared from the data available although the position of the centromere is never clear.

(2) In all females the prophase in the oocyte is meiotic. Following synapsis and pachytene there are two diffuse periods in the germinal vesicle during growth of the oocyte, the second occurring between an evanescent form of diplotene and a very distinct diakinesis in which homologous chromosomes are elongated and joined end-to-end. Chiasma-like configurations are difficult to interpret.

(3) An abortive first maturation spindle forms within the nuclear membrane but no disjunction occurs, the bivalents adhere laterally on the equator then form a fused mass on a shrunken fusiform spindle. After the egg is laid a vesicular nucleus gives rise to a second maturation spindle in which the chromosomes still appear to be bivalent. No completed telophase stage nor any liberated polar nucleus has been seen. It is thought that in female-producing parthenogenetic eggs maturation is quasi-reductional and regulation in cyclic types is by an endomitotic split in anaphase chromosomes before cleavage and in acyclic types by recombination of homologous chromosomes during anaphase.

(4) In developing parthenogenetic eggs, male blastoderm cells have haploid nuclei; eggs destined to develop into females have both haploid and diploid nuclei in the yolk but, although the method of regulation to full diploidy is not clear, it may be effected by diplosis in blastoderm cells.

(5) All males are haploid and spermatogenesis is typically hymenopteran with abortive first and normal second spermatocyte divisions.

(6) Sperms have not been found in the yolk of eggs of sexual females.

(7) Eggs of unmated females of *Biorhiza pallida* undergo rudimentary parthenogenetic development but degenerate after a day or two.

(8) In the thelytokous genus *Diplolepis* the chromosomes are metacentric and the basic number is nine. In diploid species synapsis, pachytene and post-pachytene are followed by the equivalent of a diakinetic stage. In *D. rosae* bivalents appear as nine double spheres which are never stretched out lengthwise on the spindle and which later form a dense band on the equator

of the abortive spindle. The fusiform spindle persists until the egg is laid. Diploidy is restored during the second maturation division or during the first cleavage division.

(9) In the triploid *D. eglanteriae* (3n=27) there is no synapsis and the triploid number of univalent spheres is clearly seen in the 'diakinetic' stage. An irregular abortive first maturation spindle with irregular chromatin masses is followed by a fusion nucleus. A second maturation spindle forms but has not been seen to complete its division on the margin of the egg. Early cleavage nuclei are triploid and cleavage is more regular than in the diploid species *D. rosae*.

(10) Probable methods of automictic regulation of chromosome number are discussed.

*Postcript*

*Study of the Cynipid group is not recommended for those whose time is limited but there is scope for further investigation into the behaviour of the centromere in oogenesis and maturation, the role of the sperm and the possible existence of both diploid and triploid races in* Diplolepis eglanteriae.

*On retiral I had hoped to complete the work on Cynipidae much sooner and to resume investigations begun earlier on the parthenogenetic snail* Potomopyrgus jenkinsi *Smith. But deteriorating eyesight now enforces me to abandon all microscope work and to terminate a life-long series of studies in parthenogenesis.*

## Acknowledgements

I have to thank the following members of staff in the Department of Biological Sciences, University of Dundee for their assistance in the preparation of this paper: Professor W. D. P. Stewart FRS for his encouragement and helpful advice; his secretarial staff for much assistance and the technical staff for the supply of stains and reagents necessary for the preparation of microscope slides and photomicrographs. I also acknowledge with gratitude the very useful discussions with my former colleague Dr. D. W. Hall.

To the University of Dundee, I shall be ever grateful for allowing me the continued use of the research microscope in my home since 1971 when I retired from the University. My thanks are also due to many younger friends who by their keen eyesight and youthful agility enabled me to obtain much of the gall wasp material.

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