Mixed first and second division restitution in male meiosis of *Hierochloë odorata* (L.) Beauv (Holy Grass)

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Hierochloë odorata is a rhizomatous perennial grass of northern latitudes with a highly restricted distribution in Britain and Ireland. Six clones sampled from Scotland are tetraploid ($2n = 4x = 28$) and most show irregular male meiosis, characterized by the exclusion of laggards as micronuclei, and are probably pseudogamous. In both these respects they resemble *H. odorata* from Swedish Lapland which is octaploid ($2n = 8x = 56$). Material from one Scottish locality shows meiotic restitution in the majority of its pollen mother cells (77 per cent). One in two first divisions leads to restitution (52 per cent) and one in seven of these (14 per cent) remains undivided even at telophase-II. Of those dyads formed from normal first division, almost half (46 per cent) show failure of one of the second divisions but only one in 20 shows double second division failure. This remarkable mixture of first and second division restitution is reflected in an extensive range of ploidy ($n = 2x, 4x, 8x$) and pollen grain size ($35–113 \ \mu m^3$). Seed-set in this material is low (2 per cent). Genetic variation in British material is revealed by isozyme electrophoresis while studies of total seed proteins in Swedish material indicate genetic uniformity. Implications for parthenogenesis are discussed.

Keywords: clonal reproduction, *Hierochloë*, isozymes, meiotic restitution, parthenogenesis, polyploidy.

Introduction

Restitution occurs when a cell fails to divide despite replication of the chromosomes, thus resulting in a doubling of the chromosome number usually present in that type of cell (Rosenberg, 1926). Such a mechanism is essential to parthenogenesis. Mitotic restitution may occur just prior to meiosis as in the grasshopper *Warramaba virgo* (White, 1970) or by post-meiotic doubling which leads to instant total homozygosity as seen in whiteflies, *Aleyrodidae* (John & Lewis, 1975). Restitution during meiosis leads to the production of unreduced or polyploid gametes. Conversely, unreduced gametes have been used as evidence of restitution in many species including red clover (Parrot & Smith, 1984), *Trillium* spp. (Stern, 1946) and potato (Fukuda, 1927). Parthenogenesis affects many genera as shown by reports of unreduced gamete formation (Harlan & De Wet, 1975; Cuellar, 1987).

First division failure has been reported in many species including potatoes (Ramanna, 1983) and meadow grasses (Müntzing, 1940). It may reflect non-disjunction at the first division (Wagenaar, 1968) or aberrant spindles during the second division causing the products of the first division to be reunited. Spindle aberrations may be caused by second division spindles lying next to each other (Mok & Peloquin, 1975a), by tripolar spindles (Veilleux *et al.*, 1982) or by the fusion of the second division spindles to form one large spindle (Ramanna, 1979). All three types have been found in potato (Veilleux *et al.*, 1982).

Second division restitution is less common than first but has been reported in several plants including species of *Datura* (Satina & Blakeslee, 1934) and some orchids (Teoh, 1984). It can arise when cytokinesis occurs prematurely such that the second meiotic division does not occur. This has been observed in potato (Mok & Peloquin, 1975a). Such failure may affect one or both of the second divisions and has been attributed to recessive genes such as *elongate* in maize

(Rhoades & Dempsey, 1966), *dyad* in *Datura* (Satina & Blakeslee, 1934) and the restitution pollen gene in alfalfa (McCoy, 1982).

Hierochloë odorata is a grass with an arctic/subarctic distribution and is highly restricted in Britain. The present study forms part of an investigation of genetic variation in a species of restricted distribution using cytological and electrophoretic techniques. In some Arctic/alpine grasses, such as *Poa alpina* (Müntzing, 1940; MacEwan, 1986) and *Hierochloë alpina* (Weimarck, 1976), parthenogenesis is accompanied by extensive aneuploidy. By contrast, *H. odorata* shows variation in ploidy but no sign of aneuploidy. While an understanding of the mechanisms of parthenogenesis requires the study of female meiosis, we can look at male meiosis for evidence of meiotic breakdown (Cuellar, 1987).

The material in this study is referred to as *Hierochloë odorata, sensu lato.* The tetraploid most closely resembles *H. odorata* spp. *odorata* while the octaploid best fits the description of *H. hirta* spp. *arctica* as given by Weimarck (1971).

Materials and methods

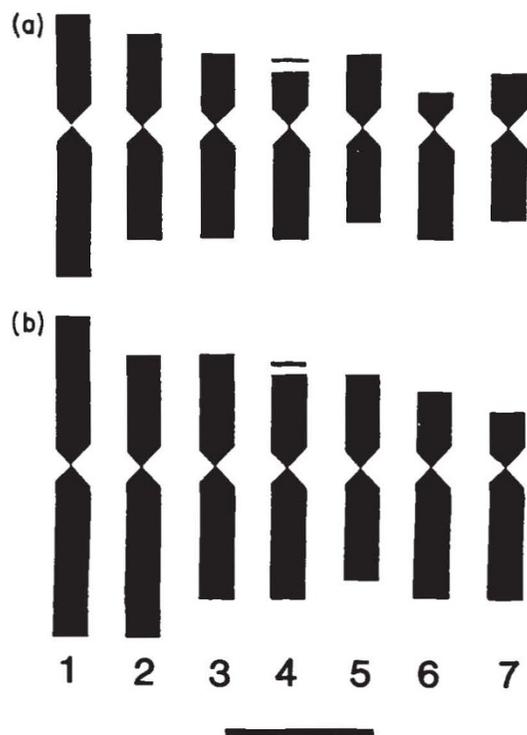Flowering tillers of *Hierochloë odorata* were collected from Scotland and Swedish Lapland. Root tips for

(a)

(b)

1 2 3 4 5 6 7

Fig. 1 Karyotype idiograms of *Hierochloë odorata* (a) octaploid $2n = 8x = 56$; (b) tetraploid $2n = 4x = 28$. Bar represents 2 μm.

mitotic preparations were pretreated in colchicine (0.05) per cent) for 5 h and fixed in ethanol: acetic acid (3:1) for at least 24 h at 5°C. Fixed material was hydrolysed in 1M HCl at 60°C for 6 min, stained in feulgen and tapped out in lacto-propionic orcein. Inflorescences were fixed as above for meiotic analysis of pollen mother cells (PMCs), single anthers being tapped out in lacto-propionic orcein (Dyer, 1963). Isoenzymes of phosphoglucose isomerase and esterase were analysed using horizontal starch gel electrophoresis. Leaf samples were crushed in a 0.05 M citrate buffer (pH 8.3) and applied to starch gels (12 per cent) on paper wicks. Gels were run in a 0.2 M borate buffer (pH 8.3) for 25 min at 35 mA, 140 V; the wicks were removed and the gels run for a further 4 h at 50 mA, 150 V. Sodium dodecyl sulphate polyacrylamide gel electrophoresis was used for seed protein analysis. Seeds were crushed in a 0.125 M borate extraction buffer (pH 8.9) containing mercaptoethanol and polyvinyl pyrrolidone, and were applied to wells on polyacrylamide gels (7.5 per cent). Gels were run in vertical bed apparatus in a 0·5 M HCl buffer (pH 6.8) at 50 mA, 150 V and stained in coomassie blue for 1.5 h.

Results

Mitotic complements

Karyotypes of *Hierochloë odorata* from six Scottish clones and from two Swedish populations were examined (Fig. 1). Scottish plants were all tetraploid with 28 chromosomes ranging in length from 2.5 to 4.3 μm. Up to four distal nucleolar organizing regions (NORs) were seen on the short arm of chromosome four (Fig. 2a). By contrast, 20 plants from each Swedish population were all octaploid with 56 chromosomes ranging in length from 2.0 to 3.5 μm (Fig. 2b). The NORs again were located distally on the short arm of chromosome four, although no more than three were seen in a single cell.

Male meiosis in tetraploids

Pachytene nuclei displayed a high degree of pairing, including occasional synaptic exchanges (Fig. 3a). At metaphase-I, the 28 chromosomes were organized into 14 bivalents (Fig. 3b) in 85 per cent of cells. Pairs of univalents occurred in 10 per cent of cells while the remaining 5 per cent contained one, rarely two, multivalents. Adjacent and alternate ring and chain quadrivalents were seen. Trivalents were all convergent except for one saucepan trivalent. Numbers of chiasmata ranged from 25 to at least 35 per cell but the possibility of differences between clones was not assessed.
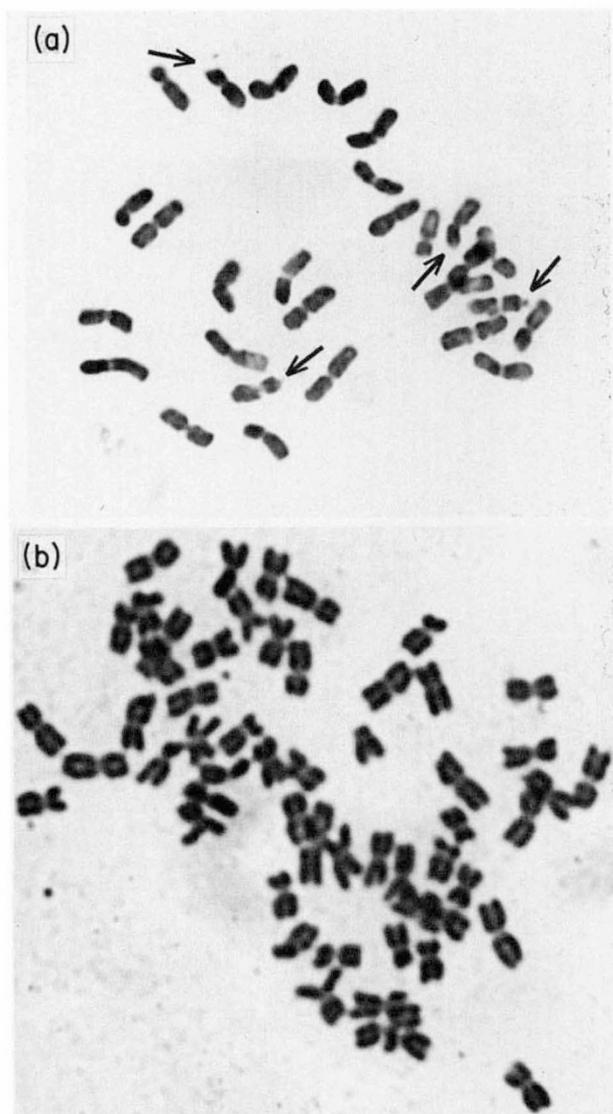
Fig. 2 Standard karyotypes of *Hierochloë odorata*.
(a) Scottish tetraploid complement $2n = 4x = 28$, with
nucleolar organizing chromosomes (arrowed); (b) Swedish
octaploid complement $2n = 8x = 56$. Bar represents 10 μm.

In all plants examined, laggards were seen in some ana-
phase-I PMCs (Fig. 3c). Bridges and acentric fragments
occurred in 2 per cent of anaphase-I PMCs from the
Ravenshall clone (Fig. 3d, e). In five of the clones
investigated telophase-I and telophase-II indicated
normal division with production of dyads and tetrads
respectively. In the Ravenshall clone, however, all
PMCs at telophase-I lacked the first division cell plate.
Of these PMCs 52 per cent were monads, resulting
from first division restitution (Fig. 3f). Telophase-II
PMCs all contained their second division cell plate but
lacked the first division plate. Five categories of PMC
were observed: intermingled within each anther (Fig. 4)
but displaying a range of frequencies (Fig. 5). Tetrads

represented 23.4 per cent of PMCs, while 22 per cent
were triads and 7.2 per cent monads (Fig. 3i–k). The
remaining PMCs were dyads but could be separated
into two classes. The cell plate separates the two nuclei
in 94.5 per cent of dyads (Fig. 3h) while in 5.5 per cent
both of the restitution nuclei were seen to interrupt the
cell plate (Fig. 3g). These two kinds of second division
dyad will be referred to as types I and II respectively.

Telophase-II in other clones revealed extensive pro-
duction of micronuclei in up to 10 per cent of PMCs
(Fig. 6a). A maximum of four micronuclei was seen in
any microspore (Fig. 6b). Within a single clone, the fre-
quency distribution of micronuclei per microspore did
not fit the Poisson distribution but rather was slightly
but significantly contagious ($\chi^2_{[1]} = 62.479$, $P < 0.001$)
(Table 1a). Such non-randomness seems to be largely
attributable to the non-random distribution of micro-
nucleate microspores amongst tetrads, cases of a single
micronucleate spore within a tetrad occurring at less
than half their expected frequency (Table 1b). Pollen
grain diameter exhibited a limited range (19–25 μm) in
five Scottish clones but was more variable in the
Ravenshall clone (19–30 μm) (Fig. 6c), due to the pre-
sence of a few giant grains (Fig. 7).

Male meiosis in octaploids

Although metaphase-I has been observed in octaploid
plants, it is not amenable to analysis (Fig. 6d). Univa-
lents appear to be frequent and some multivalents may
also be present. Bridges and fragments occurred spor-
adically at anaphase-I (Fig. 6e) but laggards were
common at this stage (Fig. 6f). Telophase-I and telo-
phase-II showed no evidence of restitution but
micronuclei were observed in about 80 per cent of
microspores (Fig. 6g, h) (Table 2). Within a single
clone, the incidence of micronuclei conformed to a
Poisson distribution at telophase-I but displayed evi-
dence of slight uniformity by telophase-II (Table 2).

Enzymic variation

Electrophoresis on esterase in seed progeny of Scottish
tetraploids revealed variation between half-sibs. Of six
half-sibs from Ravenshall, four were heterozygous for
esterase 2 and two were homozygous for the slow
allele. Four out of five half-sibs from Southwick were
homozygous for the slow allele while one plant was
heterozygous. Two alleles for PGI were found and
plants were either heterozygous or homozygous for the
fast allele. Four Mary's Knowe half-sibs were all heter-
ozygotes while two of three Ravenshall half-sibs and
one of four Southwick half-sibs were homozygotes for
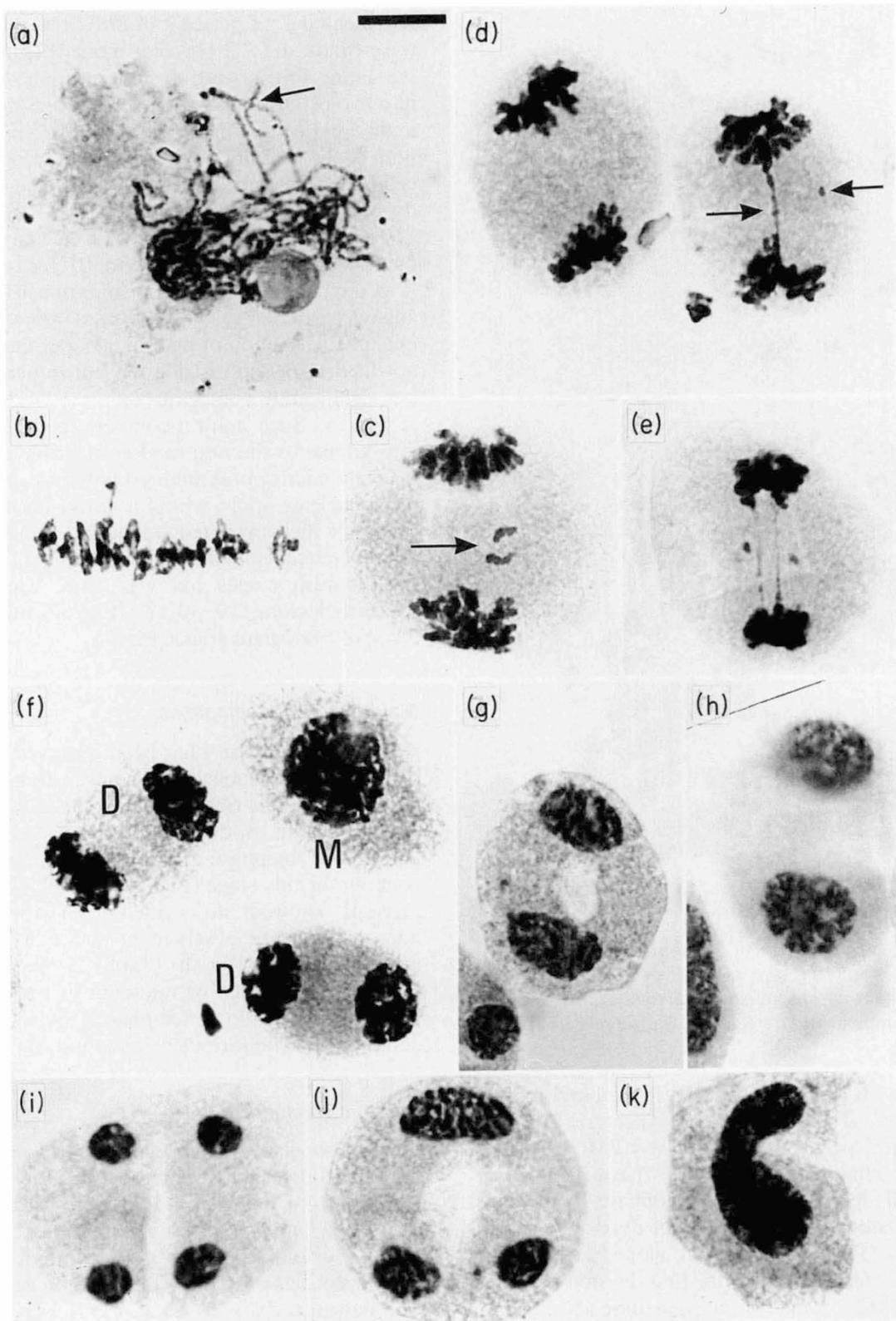the fast allele.

Fig. 3 Meiosis in tetraploid *Hierochloë odorata* from Ravenshall Point. (a) Pachytene with pairing partner exchange (arrowed); (b) metaphase-I; (c) anaphase-I with laggard univalents (arrowed); (d) anaphase-I showing formation of bridge and fragment (arrowed); (e) anaphase-I showing double bridge and fragment formation; (f) telophase-I with first division dyads (D) and restitution monads (M), (g–k) Telophase-II, (g) second division restitution (SDR) dyad; (h) first division restitution (FDR) dyad; (i) tetrad; (j) SDR triad; (k) monad from successive FDR and SDR. Bar represents 10 μm.
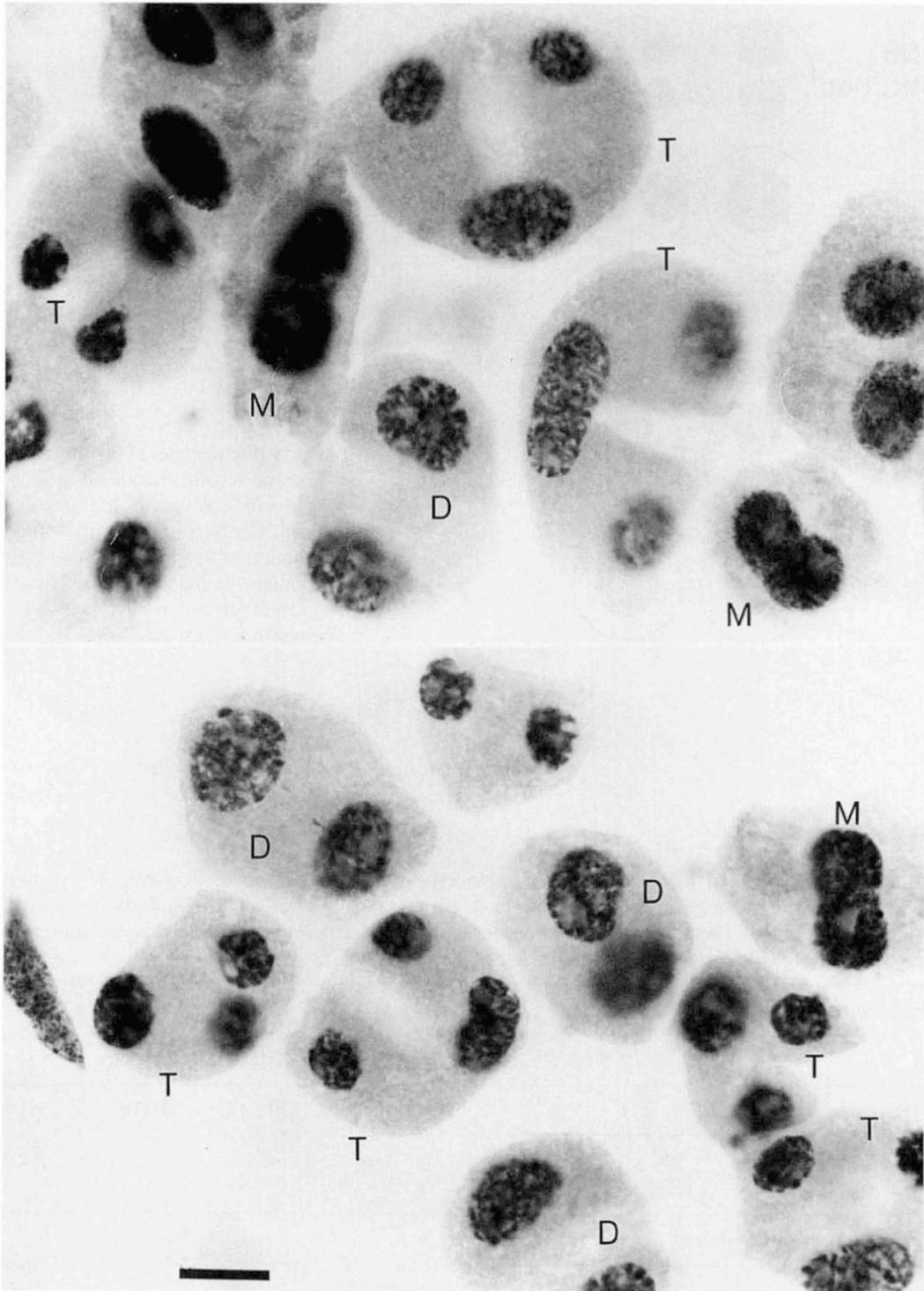
Fig. 4 Products at telophase-II of mixed first and second division restitution in *Hierochloë odorata* from Ravenshall Point. (M) monad, (D) dyad, (T) triad. Bar represents 10 μm.
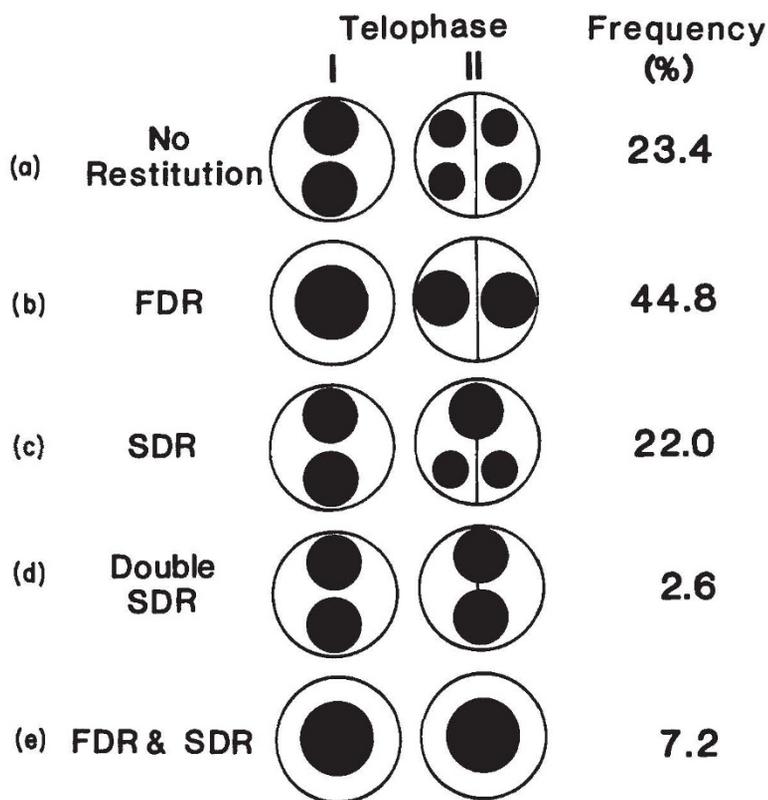
Fig. 5 Frequencies of restitution at the first and second meiotic divisions in *Hierochloë odorata* from Ravenshall Point. The frequencies of the different products of 928 pollen mother cells at telophase-II are given. (FDR) first division restitution (SDR) second division restitution.

Table 1 Incidence of micronuclei in microspores from a single clone of tetraploid *Hierochloë odorata*. The slight but very highly significant deviation from randomness amongst microspores (a) appears largely to reflect the distribution of micronucleate microspores amongst tetrads (b). The proportions of micronucleate (p) and amicronucleate microspores (q) were estimated from the data. Expected frequencies are given in parentheses.

(a) Micronuclei/microspore

0	1	2	3	4	>4	Total microspores	Mean	Variance	Goodness of fit to Poisson
4813	188	20	2	1	0	5024	0.047	0.056	$\chi^2_{[1]} = 62.479$
			23						$P < 0.001$
(4791.5)	(227.0)		(5.5)						

(b) Microspores with micronuclei/tetrad

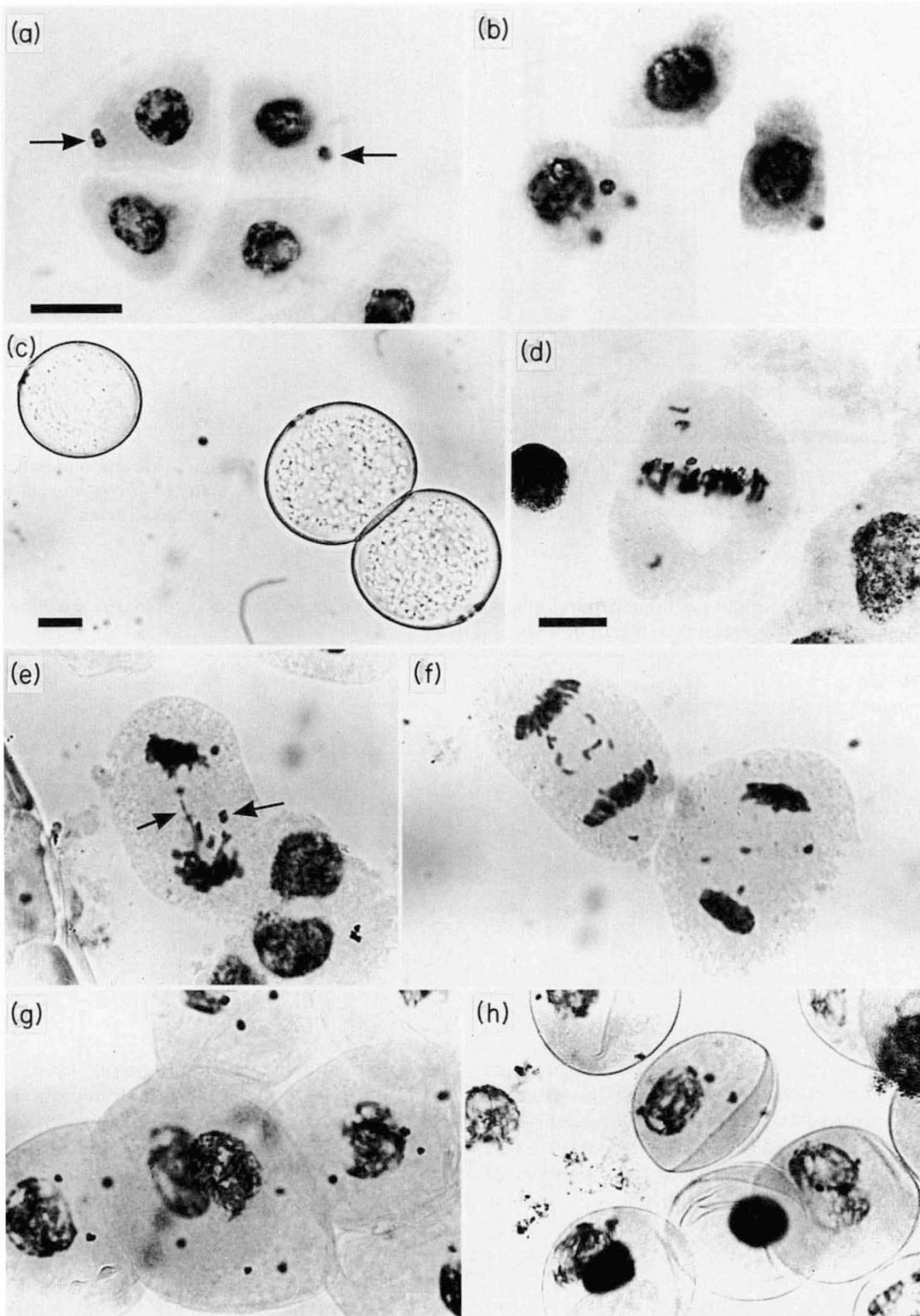| 0	1	2	3	4	Total tetrads	p	q	Goodness to fit to the binomial $(p+q)^4$
1119	82	38	15	2	1256	0.042	0.958	$\chi^2_{[1]} = 203.956$
			55					$P < 0.001$
(1057.9)	(185.5)		(12.6)					

Fig. 6 Meiosis in *Hierochloë odorata*, (a–c) Tetraploid $2n = 4x = 28$; (d–h) octaploid $2n = 8x = 56$. (a) Telophase-II tetrad showing micronuclei (arrowed); (b) telophase-II microspores with up to four micronuclei; (c) variation in pollen grain size in the Ravenshall clone; (d) metaphase-I showing univalents; (e) anaphase-I with bridge and fragment (arrowed); (f) anaphase-I with laggard univalents; (g) telophase-I with micronuclei; (h) telophase-II with micronuclei. Bar represents $10 \mu m$ (a = b, d = e – h).
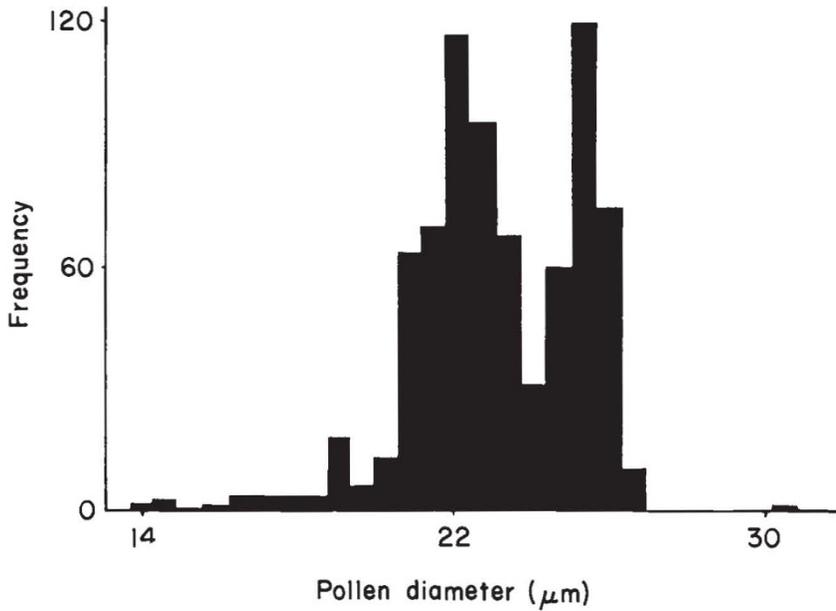
Fig. 7 Variation in pollen grain diameter in *Hierochloë odorata* from Ravenshall Point.

Table 2 Frequency distributions of micronuclei at telophase in PMCs from a single clone of octaploid *Hierochloë odorata*, compared with their Poisson expectations (in parentheses)

Micronuclei/daughter nucleus							Total nuclei	Mean	Variance	Goodness of fit to Poisson
0	1	2	3	4	5	> 5				
Telophase-I										
199	349	252	136	52	22	0	1010	1.563	1.481	$\chi^2_{[5]} = 9.358$
(211.6)	(330.7)	(258.5)	(134.7)	(52.6)	(16.4)	(5.5)				$P > 0.050$
Telophase-II										
295	402	351	130	51	14	0	1243	1.422	1.310	$\chi^2_{[4]} = 12.068$
					14					
(299.7)	(426.3)	(303.2)	(143.8)	(51.1)	(19.9)					$P < 0.050$

SDS PAGE showed variation in the seed proteins within families and between populations of Scottish *H. odorata*. In a sample of 16 half-sibs five different banding patterns were observed (Fig. 8a). No variation was observed in the banding patterns of four families of octaploid half-sibs from Sweden (Fig. 8b).

Discussion

Nuclear restitution is responsible for unreduced gametes and therefore essential to parthenogenesis in the strict sense (Gustaffson, 1946). Although the present study relates only to male meiosis, there is every likelihood that a similar spectrum of meiotic behaviour exists on the female side. In *Agropyron scabrum*, for

example, increases in the frequency of first division restitution in both male and female meiotic cells concord with a rise in the incidence of parthenogenesis (Hair, 1956). Similarly, in *Hierochloë* itself, male meiosis shows broad correlations with female meiosis across a spectrum of sexual and apomictic species and, a wide range of ploidy (Weimarck, 1973). First division restitution causes diplosporous parthenogenesis in some cases and can even result in the formation of embryo sac-like structures in anthers of some forms of *H. alpina* (Weimarck, 1970a).

The mixture of frequent first and second division restitution in the tetraploid clone of *Hierochloë odorata* from Ravenshall seems to be very unusual. A similar syndrome is known to be induced in the presence of
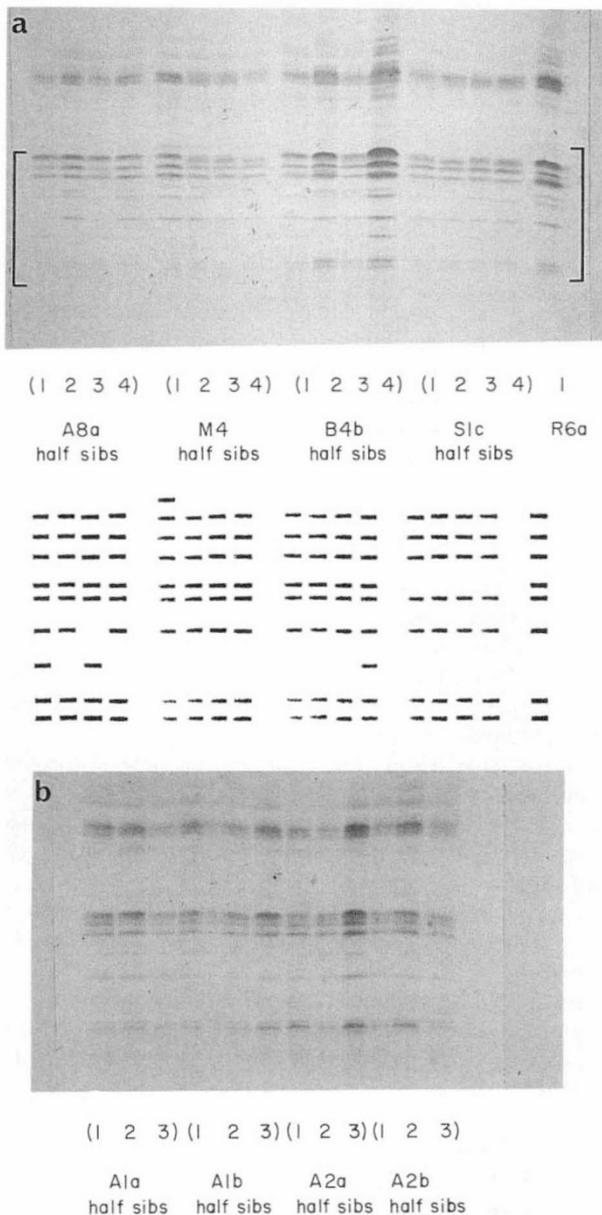
Fig. 8 SDS polyacrylamide gel electrophoresis of total seed proteins in *Hierochloë odorata*. (a) Variation within and between four families of Scottish tetraploid half-sibs, idiogram represents bracketed area A = Alewater, M = Mary's Knowe, B = Blackpool Moss, S = Southwick, R = Ravenshall Point; (b) uniformity in half-sib families from two octaploid populations at Abisko in Swedish Lapland. (A₁, A₂).

high numbers of B-chromosomes in diploid *Hypochoeris maculata* but the incidences of restitution are not as high as those reported here (Parker *et al.*, 1978). Although asynapsis and extensive non-disjunction are generally known to induce restitution, especially at the first meiotic division (Wagenaar, 1968), there is no evidence that these agents are active in the Ravenshall

clone of *Hierochloë odorata*. The apparent regularity of metaphase pairing in this material and the absence of micronuclei at telophase therefore attest to the failure of anaphase as the primary rather than secondary cause of nuclear doubling. These considerations make it unlikely that restitution has been precipitated by hybridization, as may have occurred in other plant species (Gustaffson, 1936; 1947; Richards, 1973; Cuellar, 1987). Hybridization is thought to be an unlikely cause of parthenogenesis in most invertebrates (Suomaleinen, 1950), with the notable exception of *Warramaba virgo* (Webb & White, 1975; Hewitt, 1975), but to have played an important role in the evolution of many parthenogenetic vertebrates (Bell, 1982), especially lizards (Cole *et al.*, 1983; Moritz, 1984).

With the exception of the Ravenshall clone, tetraploid *H. odorata* from Scotland appears to be sexual but of low fertility. Univalents and micronuclei are likely to account for the low pollen fertility, while the low seed set may reflect a combination of self-incompatibility and a limited availability of compatible mates. In these respects, Scottish material resembles its tetraploid relatives from Canada and Scandinavia (Weimarck, 1973). By contrast, octaploid plants from Swedish Lapland show much greater meiotic irregularity and are likely to be apomictic. Aposporous octaploids are already known from Sweden (Weimarck, 1967) and from North America (Norstog, 1963).

Because parthenogenetic organisms are not amenable to standard breeding experiments, particularly if obligately non-sexual, their patterns of variation have often been analysed in terms of isozyme mobility on electrophoretic gels (Cuellar, 1987). Such an approach allows clonal uniformity to be tested and levels of heterozygosity to be assessed. In weevils, for example, Saura *et al.* (1976) have used enzyme studies to support the idea that tetraploid apomicts are monophyletic, while Lokki *et al.* (1976) have shown that apomictic triploids maintain higher levels of heterozygosity than related sexual diploids. A detailed chromatographic study of species of *Hierochloë* has proved less diagnostic (Weimarck, 1970b). Although our own evidence does not permit levels of heterozygosity to be compared, it does provide powerful support for sexual reproduction at the tetraploid level but clonal reproduction in octaploids.

The timing of nuclear restitution is well known to influence levels of heterozygosity (John & Lewis, 1975). Premeiotic restitution, as occurs in *Warramaba virgo* (White & Webb, 1968) and many species of worm (Cuellar, 1987), conserves heterozygosity whereas post-meiotic restitution eliminates it. As far as meiotic restitution is concerned, the effects depend
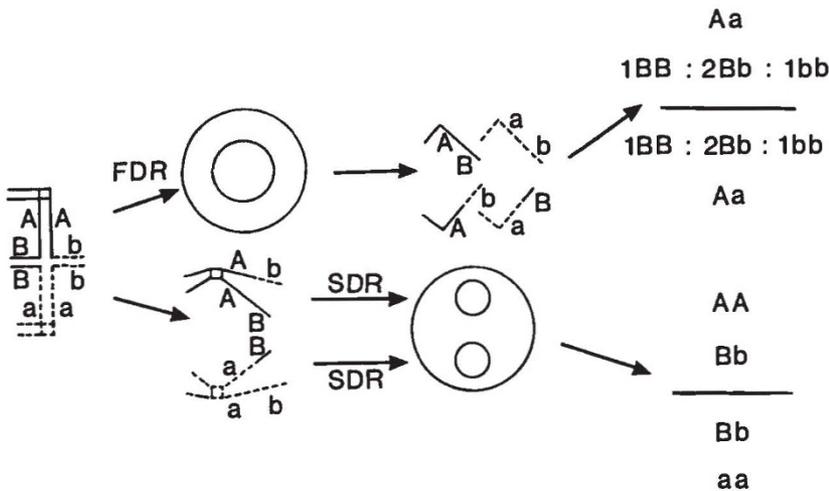
Fig. 9 Genetic effects of meiotic restitution. First division restitution (FDR) conserves heterozygosity in regions proximal to the chiasma while a 50 per cent reduction occurs in distal segments. Second division restitution (SDR) eliminates heterozygosity in regions proximal to the chiasma while heterozygosity in distal regions is conserved.

very much on the relative locations of genes and chiasmata (Fig. 9). First division restitution conserves heterozygosity of proximal segments but reduces that of distal segments by a half. This mechanism is by far the most common in flowering plants and is also common in ferns (Cuellar, 1987). It is the only form of parthenogenesis that gives rise to recombinants, however infrequent. Thus the system is deemed subsexual (Darlington, 1939; Hair, 1956). By contrast, consistent failure of the second meiotic division is rare, one of the few cases reported being that of the diploid brine shrimp *Artemia salina* (Barigozzi, 1944). Interestingly, the triploid inducer gene (*tri*) in diploid barley causes second division restitution in about 50 per cent of EMCs but has no effect on PMCs (Finch & Bennett, 1979). Second division restitution is known to produce less effective unreduced pollen in potato than that which results from failure of the first meiotic division (Mok & Peloquin, 1975b; Simon & Peloquin, 1976).

Our own study has shown that first and second division restitutions can occur within a single clone and may indeed even coincide. An experimental study should reveal to what extent the two types of restitution are sensitive to environmental disturbance, as well as their underlying mechanisms.

Acknowledgement

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