their planning jurisdictions. These calculations can be updated periodically to account for shifts in labor costs, equipment costs, and patterns of utilization.

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7. In this analysis, it is assumed that the piece of equipment has already been acquired. Hence, the cost of acquisition, or "sunk" cost, is not considered as a fixed cost. Average costs are computed as the sum of the cost of continued possession of the equipment (fixed costs) and direct operating costs (variable costs). In the region of interest, regular and overtime variable costs are assumed to be linear functions of the patient load. Since the marginal costs of regular and overtime operation are computed as the derivatives of the respective variable costs, they are assumed to be constant in the region of interest.
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10. If a simplified "occupancy chart" is represented as a straight line with slope m and time period x then it can be shown that the proportionality constant $K = 1/(2x^2m)$. In reality, an occupancy chart can be described as a sequence of such straight lines. The computer simulations described in this report (see Figs. 3 and 4) are based directly on the "occupancy chart" shown in Fig. 2. No simplifying assumptions concerning the occupancy chart were made in the computations.
11. When considering the decision to acquire a second machine, costs of acquisition as well as costs of continued possession must be considered in the decision. Hence, in this analysis fixed costs (F) include both the cost of acquisition and the cost of continued possession of the machine.

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Homoeologous Chromosome Pairing: Frequency Differences in Inbred and Intraspecific Hybrid Polyploid Ferns

Abstract. *The homosporous fern* Ceratopteris thalictroides *(Parkeriaceae) has evolved a polyploid genetic system that serves to store and release genetic variability in spite of self-fertilization and Mendelian homozygosity. This is demonstrated by the segregation of a gametophyte mutant within two inbred tetraploid lines and in their intraspecific hybrid. The segregation behavior can be explained by a model involving a duplicated locus and regular homoeologous chromosome pairing. Homoeologous pairing occurs at low levels in the inbred lines and at a high level in the hybrid, indicating intraspecific differences in chromosome pairing affinities.*

Homosporous ferns are characterized by a life cycle that includes independent diploid and haploid phases. The haploid phase is represented by hermaphroditic gametophytes that produce both male and female gametes by mitotic divisions. Self-fertilization of an individual gametophyte can occur when both types of gametes are produced simultaneously. Because only mitotic divisions are involved, all gametes produced by an individual gametophyte have identical genotypes, and self-fertilization should result in the formation of completely homozygous diploid sporophytes in one generation of selfing. At the diploid level, this system appears maladaptive in that no variability, aside from mutation, can be stored or released by the genetic system of a sporophyte once selfing has occurred (*1*). It has been proposed, however, that homosporous ferns possess a polyploid genetic system in which genetic variability can be stored and released in spite of selfing (*2*). Within such a system heterozygosity is maintained within duplicated loci that are located on homoeologous (similar but not fully homologous) chromosomes. In addition, although meiotic pairing occurs preferentially between homologous chromosomes and does not result in segregation, the stored genetic variability is occasionally released when homoeologous pairing and subsequent segregation occur during meiosis (*2, 3*).

Evidence for this type of system in ferns has been primarily cytological (*4–6*). However, a study of the genus *Ceratopteris* has provided genetic evidence showing that a duplicated locus and segregation through homoeologous pairing occurs within a polyploid inbred homosporous fern (*3*). In this study, a model was formulated showing that the frequency of homoeologous pairing within a duplex heterozygote tetraploid sporophyte (for example, *AAaa*) could be determined by multiplying the frequency of double recessives (*aa*) segregating in the gametophyte generation by a factor of 4 (*7*). An additional and expanded study of this behavior, which demonstrates the presence of homoeologous pairing involving an identical duplicated locus in two inbred lines of the fern *Ceratopteris thalictroides*, is reported here. In addition, the study shows that in an intraspecific F_1 hybrid involving the two inbred lines the levels of homoeologous pairing and segregation are greatly increased.

Two inbred lines, 230Xn and 205I, of tetraploid *C. thalictroides* ($2n = 154$) were used (*8*). Both stocks were produced by selfing isolated gametophytes that were obtained from the original spore collections. Although the resulting sporophytes were complete homozygotes in a diploid sense, each inbred line segregated for pale green gametophyte mutants at low frequencies (Table 1 and Fig. 1). The pale mutant gametophytes were phenotypically identical in both lines. They grew very slowly, and frequently died under standard culture conditions on mineral nutrient medium. Pale gametophytes that reached sexual maturity on mineral nutrient medium produced pale sporophytes when selfed, but the pale sporophytes could not be grown to maturity.

The observed segregation in the gametophyte generation can be explained by assuming that the sporophytes were duplex heterozygotes (*AAaa*) for a locus affecting gametophyte pigmentation and that homoeologous pairing released the variability stored within the duplicated locus. Thus, according to the model (*3*), homozygous (*AA*) and heterozygous (*Aa*) green gametophytes and homozygous (*aa*) pale gametophytes were present in the gametophyte generations (Fig. 1). The segregation frequencies of the double recessive mutants, 1.57 percent for 230Xn and 0.67 percent for 205I, indicate levels of homoeologous pairing of 6.28 and 2.68 percent, respectively. Homozygous green gametophytes (*AA*) were presumably present at the same low frequencies, but they were phenotypically indistinguishable from the heterozygous green types.

In addition to segregating for the pale gametophyte mutant, line 230Xn also segregated for a previously reported gametophyte mutant that produced nonmotile spermatozoids (*4*). Gametophytes with nonmotile spermatozoids could not self, although they possessed functional archegonia with viable eggs. Intraspecific crosses between the two inbred lines were obtained by using swimming spermatozoids from green gametophytes of the 205I line to fertilize green, spermatozoid mutant gametophytes from the 230Xn line. The resulting intraspecific F_1 hybrids were morphologically indistinguishable from the parents, but, be-

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cause they were produced on the 230Xn mutant gametophytes in the presence of 205I spermatozoids, their hybrid origin was certain. The fertility of the hybrids was 32.1 percent compared to a combined average spore viability of 82.8 percent in the inbred lines. The reduced spore viability in the hybrids may have been associated with the frequent appearance of one or two univalent chromosomes during meiotic divisions. Similar behavior has also been observed within the 230Xn inbred line (*4*), and it is not apparently associated with segregation behavior described here. Otherwise, meiosis in the hybrids was generally normal with regular bivalent formation. The intraspecific hybrids segregated for the same mutants as the parents, but the pale gametophyte mutants were present at a much higher frequency of 13.2 percent (Table 1). If the hybrids are assumed to be duplex heterozygotes (*AAaa*) derived from crossing heterozygous green (*Aa*) 205I and 230Xn gametophytes (Fig. 1), the level of segregation indicates that homoeologous pairing in the hybrids occurred at a frequency of 52.8 percent.

The extreme difference in segregation behavior between the inbred parents and the intraspecific hybrid can be explained by a tendency toward more random pairing in the hybrid with respect to the set of four chromosomes with which the locus is associated. For instance, if it is assumed that the 230Xn parent has contributed chromosomes 1^A and 2^a and that the 205I parent has contributed chromosomes 3^A and 4^a, random pairing within the hybrid would result in the formation of *A/a* pairs two-thirds of the time. This would result in a 1 *AA*:4 *Aa*:1 *aa* (16.7 percent *aa*) segregation in the gametophyte generation (*3*). The observed segregation frequency of 13.2 percent *aa*, however, indicates that these pairs formed only 52.8 percent of the time. Thus, although it approached randomness, pairing occurred preferentially between the chromosomes containing identical alleles. In terms of homologies, the parents possessed fully homologous chromosomes as a result of inbreeding but homoeology within the set of four permitted a limited amount of pairing and segregation. In the hybrid, full homologies were not present within the set of homoeologous chromosomes although chromosome pairs 1^A3^A and 2^a4^a formed preferentially because of closer affinities.

A small F_2 population of five sporophytes was obtained by isolating and selfing green gametophytes that were generated from an F_1 hybrid sporophyte. One of the F_2 sporophytes did not segregate for pale gametophytes, and this was presumably derived from homozygous green (*AA*) gametophytes. The F_2 sporophytes that segregated for the mutant were duplex heterozygotes derived from selfing heterozygous green (*Aa*) gametophytes. These sporophytes segregated near the parental frequencies (Table 1), an indication that full homologies were restored within these sporophytes since they were the products of selfing. As a result, meiotic pairing was predominantly homologous while occasional homoeologous pairing resulted in the limited segregation of pale mutants.

Table 1. Segregation frequencies of pale green gametophytes and spore viabilities in *C. thalictroides* sporophytes.

Sporophyte generation	Genotype	Gametophytes scored (No.)	Frequency of pale (*aa*) gametophytes (%)	Spore viability (% germination)
Parent 205I	*AAaa*	1200	0.67	92.1
Parent 230Xn	*AAaa*	637	1.57	73.5
F_1 hybrid	*AAaa*	1000	13.20	32.1
F_2 generation*	*AAaa*	1200	1.16	95.0
Pale × green cross	*Aaaa*	400	47.00	30.5

*Four heterozygous sporophytes were tested for segregation.

Confirmation that the same pale mutant was segregating within both of the inbred lines and within the intraspecific hybrid was obtained by hybridizing pale, presumably homozygous, gametophytes (*aa*) from the hybrid with green, presumably heterozygous, gametophytes (*Aa*) from line 205I. Hybridizations were produced by growing pale gametophytes in axenic culture on the standard medium supplemented with 2 percent sucrose (*9*), conditions under which pale gametophytes became green and grew readily. Some pale gametophytes grown in this fashion also contained the spermatozoid mutant and could not self. These gametophytes were fertilized with spermatozoids from green gametophytes of line 205I. The resulting sporophytes were green and normal in appearance when grown on standard medium and, in accordance with the model, were presumed to be simplex heterozygotes (*Aaaa*). These sporophytes segregated for green and pale gametophytes at an observed ratio of 212 green : 188 pale. These results closely approximate the expected 1 *Aa*:1 *aa* segregation of a simplex heterozygote if the two inbred stocks both contained the same mutant ($\chi^2 = 1.44$; $P = 0.2$ to 0.3). Reciprocal crosses with heterozygous eggs from green gametophytes that contained the spermatozoid mutant and sperm from young pale mutants yielded similar results.

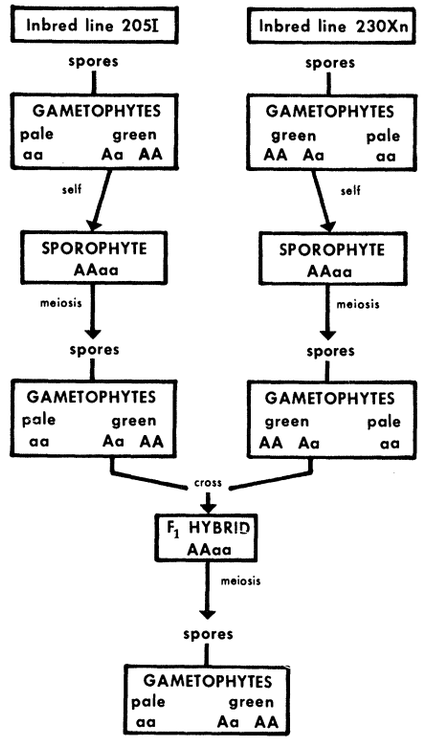

Fig. 1. Segregation of pale gametophytes within two inbred lines of *C. thalictroides* and their intraspecific hybrid. Heterozygosity is maintained within a duplicated locus and released through homoeologous pairing during meiosis.

This study provides genetic evidence that demonstrates homoeologous chromosome pairing and the restricted segregation of an identical marker within two inbred lines of *C. thalictroides*. Previous genetic demonstrations of homoeologous pairing have either not fully explained the manner of inheritance within the investigated system (*4*, *6*) or have inbred lines that were initally derived from synthesized interspecific hybrids (*3*, *6*). In my study, because both stocks had not been subject to any artificial hybridization, it is likely that the previously reported genetic behavior also occurs under natural conditions. It is also evident that intraspecific differences in chromosome pairing homologies exist within the species. Within each inbred line, full

homologies are present because of selfing and, as a result, homoeologous pairing and segregation occur only occasionally. In the intraspecific hybrid, however, full homologies are not apparent and pairing behavior approaches randomness within a set of four homoeologous chromosomes. Greater similarity is apparent between some of the homoeologous chromosomes, however, because the segregation ratios indicate a low level of preferential pairing between chromosomes carrying the same allele. The pairing behavior described above appears to depend primarily on the degrees of homology rather than on genetic factors. For example, when full homologies were restored in the F_2 generation by selfing gametophytes from the F_1 hybrid, normal pairing behavior was restored uniformly within the F_2. In that cytological analyses have not shown the presence of multivalents in either the F_1 hybrid or within the inbred lines, the limitation of pairing to bivalent formation is evident. Whether this condition is a result of genetic or physical restrictions on pairing behavior remains unknown.

Intraspecific hybridization can be a significant force in promoting the release of stored variability within the described polyploid genetic system. Such hybridizations not only have the potential to generate new genotypic combinations but they also serve to release the variability stored within the duplicated loci through high levels of homoeologous pairing. Given this, and the maintenance of moderate levels of spore viability and full sporophyte vitality in the F_2 generation, the possibilities of producing new adaptive combinations are greatly increased over the situation within inbred lines. Because the level of segregation within this system is dependent on the degree of homology shared by chromosomes contributed by separate parents, it may be expected that different intraspecific combinations will segregate for the same or similar characteristics at different rates. Thus, intraspecific differences involving chromosome pairing affinities may be detectable within such a system even though morphological differences between sporophytes are not evident. In that *C. thalictroides* has been described as a polymorphic species (*10*), the ability to differentiate between individuals on the basis of chromosome pairing affinities may be an important factor to be considered in future taxonomic treatments of the genus.

The previous demonstration of homoeologous pairing and restricted segregation in duplex heterozygote *Ceratopteris* sporophytes (*3*) involved a diploid form of the genus ($n = 39$). Obviously, although this is the lowest extant number in the genus, the diploid form is, in fact, of polyploid derivation and tetraploid for those loci that have been shown to be maintained in a duplicated state. The present example dealing with *C. thalictroides* involves the tetraploid form of the genus ($n = 78$). Evolutionarily, it must represent an ancient octaploid. However, the inheritance pattern is identical to the model developed from the extant diploid form. Therefore, in spite of a major difference in ploidy level, both forms contain at least one locus present in a tetrasomic state.

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7. The model is based on the observation that, if chromosome pairing within a bivalent forming duplex heterozygote is restricted to homologous chromosomes (A1/A1, a2/a2), no segregation can occur (0 percent *aa*); but if pairing is restricted to homoeologous pairs (A1/a2, A1/a2) segregation will be at a maximum of 25 percent *aa*. Thus, intermediate levels of segregation can be interpreted on the basis of intermediate levels of homologous and homoeologous pairing. Since one-quarter of the meiotic products are double recessives when pairing is 100 percent homoeologous, the level of homoeologous pairing can be calculated from the segregation frequency by multiplying by a factor of 4. Multivalent forming individuals must be treated somewhat differently because of the possibility of double reduction. A detailed description of the model is given by Hickok (*3*).
8. Stock 230Xn was derived from a spore collection obtained from a Malaysian plant growing in the Botanic Garden at the University of Malaya. Stock 205I was obtained from a spore collection taken from a plant growing at the Royal Botanic Gardens, Kew, England. The wild source of this plant is unknown. General culture methods utilized to establish homozygous sporophytes by selfing isolated gametophytes have been described by L. G. Hickok and E. J. Klekowski [*Am. J. Bot.* **60**, 153 (1973)].
9. Spore collections that had been surface-sterilized by treatment with dilute sodium hypochlorite [M. Schedlbauer and E. J. Klekowski, *Bot. J. Linn. Soc.* **65**, 399 (1972)] were sown on standard mineral nutrient medium solidified with 1 percent agar and contained within sterile disposable petri dishes (100 by 15 mm) (*8*). Subsequent pale gametophytes that developed were isolated into 250-ml flasks containing the same medium supplemented with 2 percent sucrose. All procedures were carried out in a laminar flow hood.
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Long Ascending Projections from Substantia Gelatinosa Rolandi and the Subjacent Dorsal Horn in the Rat

Abstract. *Small neurons of the substantia gelatinosa Rolandi and the subjacent dorsal horn of the spinal cord have been thought to exert a direct modulatory effect only on neurons located within a distance of a few spinal segments. By using the technique of retrograde transport of horseradish peroxidase, however, it has been found that in the rat a significant number of these cells, particularly those of the subjacent dorsal horn, ascend many spinal segments to the lateral cervical nucleus and to the lower brainstem. These data provide an anatomic basis for a role of substantia gelatinosa Rolandi and subjacent dorsal horn cells in modulating or contributing to sensory information transmission not only in nearby segments but in far distant structures.*

Cells of the substantia gelatinosa Rolandi [SGR; Rexed's lamina II in the cat (*1*)] and of the subjacent dorsal horn (SDH; corresponding approximately to Rexed's lamina III) make numerous synaptic contacts with primary afferent fibers and with the dendrites of larger neurons entering these areas from above and below (*2-7*). The axons of these larger neurons, in turn, make segmental and propriospinal connections (*8*), and many are now known to ascend for long distances before synapsing in the brainstem and thalamus (*9*). Although direct physiological evidence is lacking, the connections formed by the small SGR and SDH cells have long been thought to qualify them for an important role in the modulation of reflexes and of information transmission to the brain (*4, 6, 7, 10*). Szentágothai (*4*) reported that lesions within laminae II and III of the cat dorsal horn produced degeneration at a distance of not more than three spinal segments. Consequently, the area directly affected by the modulating influences of SGR and SDH cells has been thought to be quite restricted. However, in the course of studying the cells of origin of the spinocervical tract in the rat, we have seen and now report that in this species many SGR and SDH cells possess far longer projections.

Adult male Sprague-Dawley rats were deeply anesthetized with sodium pentobarbital, and a laminectomy was per-

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