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Mutation-selection Equilibrium as a Possible Cause of an Interchange Chromosome Polymorphism in a Cultivar of Rye, *Secale cereale* L.

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Summary. A persistent chromosomal polymorphism exists in a population of cultivated rye, *Secale cereale* (Candela et al. 1979). In order to ascertain the possible causes that maintain it, we have estimated the fitness values of structurally homozygous and heterozygous plants and the mutation rate of spontaneous interchange. The estimates of the selection coefficient against heterozygotes ($s = 0.15 - 0.40$) and of the mutation rate $u = 6.12 \times 10^{-2}$ support a mutation-selection equilibrium as a likely cause of the interchange chromosome polymorphism.

Key words: Interchange – Chromosome polymorphism – Mutation – Selection – Equilibrium – Rye

Introduction

Candela et al. (1979) have found that the interchange chromosome polymorphism present in the 'Ailés' cultivar of rye, *Secale cereale* L., exhibits the following characteristics: (i) a rather constant frequency (20 percent) of structural heterozygotes for several generations; (ii) that the chromosomal polymorphism is due to many reciprocal translocations; among 11 heterozygotes analyzed, seven different interchanges were detected, involving five out of the seven chromosomes of the complement; and (iii) as a consequence, the frequency of homozygotes for any translocated arrangement must be very low (all the 26 homozygotes analyzed had the standard arrangement).

The maintenance of such polymorphism may be due to several causes. One is that structural heterozygotes have higher fitness than homozygotes; this has been reported in rye under particular circumstances of inbreeding or high sowing rates (Rees 1961; Bailey et al. 1976). A second possibility is that structural heterozygotes are selectively neutral, and thus the heterozygotes accumulate through the generations. Finally, it is possible that structural heterozygotes have a selective disadvantage but this is compensated by a sufficiently high rate of chromosome mutation, leading to a mutation-selection equilibrium.

In the present paper, we estimate the fitness of structural homozygotes and heterozygotes and the rate of chromosomal mutation in order to decide among these possible explanations.

Materials and Methods

(i) *Materials*

Fitness is estimated in two samples (I and II) of cultivated rye, *Secale cereale* L., taken from the locality of Ailés, (Zaragoza, Spain). The two samples belong to the same generation analyzed by Candela et al. (1979) but they have been grown in different fields and years. Sample I (which is A3 in Candela et al. 1979) was grown in 1975–76. Of the 62 plants analyzed, 13 were interchange heterozygotes (HT) on the basis of one or more multivalent associations at Metaphase I; the other 49 exhibited 7 bivalents (7^{II}) and were therefore classified as structural homozygotes (HM). Sample II was grown in 1978–79. Of the 195 plants screened for Metaphase I configurations, 30 were classified as heterozygotes. Fitness was analyzed in these interchange heterozygotes as well as in 30 homozygotes randomly chosen among the 165 homozygous plants in the sample.

The chromosomal mutation rate was estimated in homozygous plants from samples A3 and C1 described by Candela et al. (1979).

(ii) *Methods*

Fitness

Plants were sown 30 cm apart in rows separated by 60 cm. The fitness of each plant is measured by the number of viable progeny produced. This value is calculated by multiplying the number of seeds harvested from each plant under natural open pollination by their viability (measured by the number of germinating seeds on wet filter paper in a sample of one hundred seeds under standard conditions of humidity and temperature).

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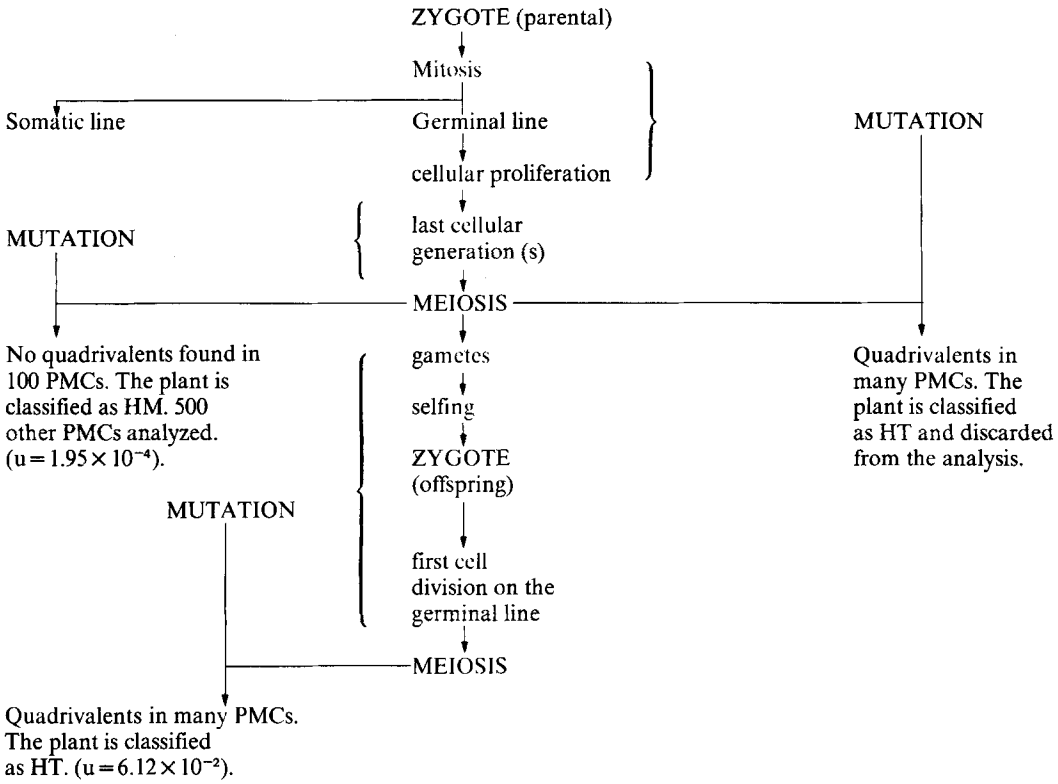
Fig. 1. Diagram showing the different stages in which the interchange spontaneous mutations can occur in relation to the methods followed in this investigation. HM = homozygote; HT = heterozygote; PMCs = pollen mother cells



The following components of fitness are also estimated: efficiency of tillering (number of tillers producing seed), mean number of seeds per ear, total number of flowers per plant, viability of male gametes, and fertility of female gametes. The viability of male gametes is measured by the percentage of pollen grains that become stained with a mixture of acetic carmin and glycerine. The fertility of the female gametes is estimated by the ratio of the number of seeds produced to the total number of egg cells (flowers).

The mean fitness values of the HM and HT plants are compared by *t* tests. All structural homozygotes (for the standard and any translocated arrangements) are included among HM plants, while all the interchange heterozygotes (irrespective of the number of translocations) are included in the HT class. Thus any differences found must be attributed to the chromosome condition per se, i.e., to the condition of homozygosity versus heterozygosity.

Chromosome Mutation Rate

The starting point are plants classified as homozygous by their meiotic configurations. All the pollen mother cells (PMCs) analyzed show 7^{II}. This method of classification seems appropriate because in the heterozygous plants multivalent associations are present in a high percentage of the PMCs. The HM plants are selfed and the Metaphase I configurations of their progeny examined. The presence of quadrivalents is taken to indicate that an interchange mutation has occurred in the last stages of parental gametogenesis or during early embryo development. 500 PMCs of one anther are also analyzed in some of the HM plants. The presence of a quadrivalent is interpreted as being due to an interchange during the first stages of the microsporogenesis (Fig. 1).

Results

Fitness

The estimates for fitness and fitness components are, in the two samples analyzed, either equal or higher in the HM than in the HT plants (Table 1). The *t* tests indicate that HM and HT plants are similar in flowers per ear and in viability of the offspring, whereas the HM plants have higher pollen viability and egg cell fertility than the HT plants. In addition, the HM plants show significantly higher numbers of tillers and total number of flowers per plant than the HT plants in sample I, while no significant differences exist in sample II for these two components.

Fitness, measured as the total viable offspring, is significantly higher in HM than in HT plants in sample I, but the difference is not significant in sample II.

Interchange Mutation Rate

Eight out of 113 plants in the first generation of selfed homozygous plants and 1 out of 34 in the second

Table 1. Mean and standard error for fitness and fitness components in homozygotes and in interchange heterozygotes of the 'Ailés' cultivar of rye. P represents the significance of the differences between homozygotes and heterozygotes on the basis of *t*-tests

	Sample I (1975 – 76)			Sample II (1978 – 79)		
	Homozygotes	Heterozygotes	P	Homozygotes	Heterozygotes	P
Tillering	39.9 ± 1.8	29.15 ± 3.5	0.01 – 0.02	22.4 ± 2.4	23.5 ± 2.0	> 0.5
Flowers/plant	2,931.4 ± 128.1	2,214.5 ± 281.7	0.02 – 0.05	1,446.1 ± 165.2	1,439.9 ± 128.8	> 0.5
Flowers/ear	74.23 ± 1.0	74.4 ± 1.9	> 0.5	64.2 ± 1.1	61.1 ± 1.7	0.1 – 0.2
Pollen viability	93.38 ± 0.7	84.4 ± 2.4	< 0.001	94.7 ± 0.8	89.2 ± 2.1	0.01
Eggcell fertility	61.64 ± 1.3	48.5 ± 1.8	< 0.001	65.8 ± 2.4	56.0 ± 2.3	0.001 – 0.01
Offspring (seeds per plant)	1,792.9 ± 81.0	1,087.6 ± 150.7	< 0.001	945.2 ± 120.4	805.5 ± 78.0	0.2 – 0.4
Offspring viability	99.0 ± 0.2	98.2 ± 0.4	0.1 – 0.2	99.2 ± 0.2	99.0 ± 0.2	0.2 – 0.4
Fitness (total viable offspring)	1,775.6 ± 80.8	1,070.4 ± 149.2	< 0.001	936.8 ± 118.9	797.7 ± 77.4	0.2 – 0.4
No. of plants analyzed	49	13		30	30	

generation exhibited 5 bivalents and 1 quadrivalent ($5^{II}+1^{IV}$) at Metaphase I. Hence, the interchange spontaneous mutation rate is estimated as $u=6.12\times10^{-2}$ per plant per generation, with an error of $\sqrt{u(1-u)/n}=2\times10^{-2}$. However, only 4 PMCs showed $5^{II}+1^{IV}$ among the 500 PMCs per anther examined in 41 homozygous plants. Hence, the interchange spontaneous mutation rate during the early stages of microsporogenesis is estimated as 1.95×10^{-4} per PMC (with an error of 0.97×10^{-6}).

Discussion

The somewhat different results obtained in the two samples may be due to the different environmental conditions in which the two samples were grown. Nevertheless, in both samples the homozygous plants show, on the whole, higher fitness than the heterozygotes. Needless to say, it remains possible that, among the many interchange heterozygotes examined, some might have higher fitness than the homozygotes.

The 40% selective advantage of the homozygous plants in sample I can be attributed to a greater number of flowers per plant (24%) and a higher egg cell fertility (21%). Obviously, the different viability of pollen grains has no effect on the fitness estimates because the experimental plants were open pollinated and thus fertilized by a mixture of pollen from the whole population. In sample II, the only character showing significant differences is egg cell fertility (15%). On this basis, it seems reasonable to conclude that $s=0.15$ in sample II in spite of the lack of significant difference in overall fitness. Thus, in the 'Ailés' cultivar, the selection coefficients against interchange heterozygotes are estimated as $s=0.4$ and $s=0.15$ for samples I and II, respectively. This conclusion eliminates the overdominance hypothesis as an acceptable explanation for the maintenance of a 15–20% interchange heterozygosity.

The spontaneous mutation rates for chromosomal interchanges, estimated by several authors in organisms as different as grasshoppers, mice, *Drosophila* and man, range from 10^{-3} to 10^{-4} (Lande 1979). For example, Jacobs (1977) estimates a rate of 1.3×10^{-4} per gamete per generation in a sample of children from parents free of translocations. Freire-Maia (1961) found only one new translocation in 1,377 polytene chromosomes from the offspring of females fertilized under natural conditions in *Drosophila ananassae* (rate 7.2×10^{-4}); while Plough (1941), using a segregation test, estimated in *D. melanogaster* an interchange mutation rate of 1.4×10^{-3} (one new translocation among a total of 677 individuals), and Alexander (1952) estimated a mutation rate of 2.5×10^{-3} in a sample of 15,000 gametes of *D. virilis*. White (1973) has reported that in *Moraba scurra* one among 1,000 individuals carries a recently arisen reciprocal translocation. In plants, Brandham (1976) analyzed diploid and tetraploid species of the genus *Haworthia* and observed new translocations in 3 out of 666 diploid gametes (4.5×10^{-3}), but none among 1,398 haploid gametes tested.

In contrast to the above data, the rate of 6.12×10^{-2} interchange per generation estimated in the 'Ailés' cultivar is at least one order of magnitude greater. This might reflect the existence of a causal factor increasing chromosome mutations in this particular population. Yamaguchi et al. (1976), for example, proposed the

presence of mutator genes in certain lines of *Drosophila melanogaster* in order to account for a rate of 1.12×10^{-2} chromosome aberrations (mostly inversions; the rate of reciprocal translocations was 3.6×10^{-4}). We should point out that in the 'Ailés' cultivar we have also observed Anaphase I bridges and fragments, which may reflect inversion heterozygosity (unpublished data), such as was observed by Yamaguchi et al. (1976).

The difference between the two interchange mutation rates estimated in our study (1.95×10^{-4} PMC/generation and 6.12×10^{-2} individual/generation) indicates that chromosome instability is greater in early embryo development than in the early stages of microsporogenesis (Fig. 1).

Since there is no overdominance in the population of 'Ailés' cultivar, the question arises whether the chromosomal polymorphism can be accounted for as the result of mutation-selection equilibrium. If this occurs, then $u = sq$, where $q = \Sigma\, q_i$ is estimated as $q = 0.1$, q_i being the frequency of each individual interchange (Candela et al. 1979; see Nagylaki 1977). If $s = 0.40$ (sample I), then $u = 4 \times 10^{-2}$; if $s = 0.15$ (sample II), then $u = 1.5 \times 10^{-2}$. Thus, a mutation rate of the order of 10^{-2} is sufficient to obtain equilibrium. This value of u is in good agreement with the value found in the 'Ailés' population.

In conclusion, the estimates obtained for the selection coefficient against interchange heterozygotes and for the mutation rate indicate that the chromosomal polymorphism for reciprocal translocations found in the 'Ailés' cultivar is maintained by a mutation-selection equilibrium.

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