SELFING RATES IN TWO WILD POLYPLOID SOLANUMS[1]

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Abstract

The rate of selfing in the wild, tuber-bearing species *S. gourlayi* Hawkes (2n = 4x = 48) and *S. oplocense* Hawkes (2n = 6x = 72) was estimated from progeny of plants nulliplex for the "parallel spindles (*ps*) allele. The estimated values for plants grown in experimental plots were 22% for *S. gourlayi* and 1% for *S. oplocense*. The quantitative method used appears to be a simple and efficient way to calculate selfing rates in polyploids. Knowledge of selfing rates is useful for basic population genetic studies and for plant breeding and seed production of economic crops. There is a need for genetic markers in *Solanum* species if selfing rates are to be taken into consideration for the selection of an appropriate breeding method for the production of true potato seed.

Resumen

La proporción de autofecundación en las especies tuberiferas silvestres *S. gourlayi* Hawkes (2n = 4x = 48) y *S. oplocense* Hawkes (2 = 6x = 72) fue calculada aproximadamente por prueba de progenie de plantas nuliplexas para el alelo "husos paraleslos" (*ps*). Los valores estimados para plantas cultivadas en parcelas experimentales fueron 22% para *S. gourlayi* y 1% para *S. oplocense*. El método cuantitativo usado parece ser simple y eficiente para calcular la proporción de autofecundación en poliploides. El conocimiento de la proporción de autofecundación es útil para estudios básicos de genética de poblaciones y para mejoramiento de plantas y producción de semillas en cultivos económicos. Es necesario encontrar marcadores genéticos en especies de *Solanum* si la proporción de autofecundación va a ser tendia en consideración en la elección de un método apropiado para la producción de semilla botánica de papa.

Introduction

The genotypic structure of any population depends on the breeding behavior of the individuals that form it. It is, therefore, of interest to know

the rate of outcrossing not only for basic population genetic studies of wild species, but also for applied breeding and seed production of economic crops.

Inferences on the rate of cross-pollination can be made from the frequency of genetically marked individuals in the progeny of individual flowers, single plants, certain genotypes within a population, or the whole interbreeding population (4). The rate of cross-pollination will differ depending on the type of progeny used for estimation purposes. Furthermore, different results might be obtained from identical populations grown in different environments, since pollination ecology and dynamics can modify the breeding behavior of a species.

Methods to estimate the rate of cross-pollination have been proposed for diploid flowering plants (4) and for tetrasomic tetraploids (1, 5). The percentage of selfing in tetraploid Neo-Tuberosum material has been estimated (5), however, there are no reports on this value in wild polyploid Solanums.

S. gourlayi Hawkes (grl, $2n = 4x = 48$) and S. oplocense Hawkes (opl, $2n = 6x = 72$) are self-compatible species that, like wild diploids, are dependent upon bumble-bees (Bombus hortorum) for pollination. The rate of cross-pollination in these polyploids is not known either in nature or in the experimental field. It can, however, be estimated from progeny testing of plants nulliplex for the "parallel spindles" (ps) allele, which is the only genetic marker that has been reported for these species (2, 3). The ps allele controls the parallel orientation of the AII spindles during microsporogenesis. This mechanism results in First Division Restitution (FDR) 2n pollen.

The present report is mainly concerned with a quantitative approach used in the estimation of the rate of selfing in the two polyploid species under experimental field conditions, and its applicability in the production of true potato seed.

Materials and Methods

Twelve introductions of 4x grl and twelve of 6x opl obtained from the Inter-Regional Potato Introduction Project, Sturgeon Bay, Wisconsin, were grown in the field. Each introduction consisted of 14 plants in a row, at 45 cm spacing. The twelve rows of 4x grl were adjacent to the 12 of 6x opl, and rows of both species were surrounded by wild diploid and polyploid Solanum species. The introductions of 4x grl and 6x opl were screened for 2n pollen. The mechanism of 2n pollen formation was determined in a sample of plants; nine plants of 4x grl and five of 6x opl nulliplex for ps were identified. An average of ten open-pollinated fruits was harvested from each of those plants and seeds from individual plants were bulked. Progenies from each nulliplex plant were grown in a greenhouse, after

breaking seed dormancy with a 1500 ppm solution of GA. These progenies were screened for 2n pollen and the mechanism of 2n pollen formation was studied in a sample of them to confirm the·presence of *ps*. The open-pollinated progenies from each plant were classified into two phenotypic classes; "nulliplex" and "nonnulliplex."

Estimation of Rate of Selfing

In order to estimate the rate of selfing from progeny testing of nulliplex plants, a number of assumptions had to be made, as follows: 1) The gene frequency in the pollen pool is the same as in the zygotes that produced the pollen; 2) outcrossing estimates apply equally to all genotypes in the population; and 3) penetrance of the recessive allele is complete.

The rate of selfing (or outcrossing) in a tetraploid can be estimated by solving either of the following equations 1):

1) Observed nulliplex $= s + (1\text{-}s)$ $p_{(ps\ ps)}$

2) Observed nonnulliplex $= \lambda$ $(p_{(ps\ ps)} + p_{(ps\ ps)})$

where $s =$ percentage of selfing

  $\lambda = (1\text{-}s) =$ percentage of outcrossing

  $p\ (\underline{\quad}) =$ gametic frequency

The expected frequencies of segregating progeny in a hexaploid is conceptually the same as in a tetraploid, though the gametic frequencies vary.

To calculate gametic frequencies in the original populations it is necessary to determine zygotic proportions, which is a considerable task. A likely alternative to this would be to identify nulliplex and nonnulliplex plants in the experimental field (it could be one or a few of each, if our estimates are to be used on single plants), cross them to determine the genotype of the nonnulliplex plant(s), and harvest from them open-pollinated fruits and fruits resulting from control crosses, all in the first season. In the next season, test for nulliplex vs. nonnulliplex progenies from nulliplex and nonnulliplex parents of known genotype. By equating observed and expected segregation frequencies in progenies of either nulliplex and simplex, duplex or triplex, a system of simultaneous equations can be set and the values of *s* and *p* can be estimated.

A rough estimate of the frequency of the recessive allele, nevertheless, can be obtained from the frequency of nulliplex zygotes in the original population. The rate of cross-pollination obtained in this way would be an approximation of the real value.

Results and Discussion

Plants, 117, of 4x grl and 102 of 6x opl were screened for 2n pollen; 50% and 35% of them, respectively, were found to be nulliplex for *ps* (Table 1). Rough estimates of the frequency of *ps* in each polyploid species were obtained from the frequency of nulliplex plants in the original popula-

TABLE 1. - *Nulliplex and nonnulliplex plants in progenies of nulliplex plants of 4x grl and 6x opl.*

Species	No. plants tested	No. nulliplex plants	No. nonnulli- plex plants	Segregation frequencies
4x grl	117	58	59	0.50 : 0.50
6x opl	102	36	60	0.35 : 0.65

tions screened for 2n pollen. By assuming Hardy-Weinberg equilibrium in these populations, the frequency of *ps* was estimated to be approximately 0.60 in 4x grl and 0.70 in 6x opl (2).

Equations 1) and 2) were solved for *s* (rate of selfing) and λ (rate of outcrossing) as follows:

1) $s_{4x\ grl}$ = Obs. nulliplex - P$_{(ps\ ps)}$/1-P$_{(ps\ ps)}$
       = 0.50 - 0.36/1-0.36 = 0.218 ≅ 22%

   $s_{6x\ opl}$ = Obs. nulliplex - P$_{(ps\ ps\ ps)}$/1-P$_{(ps\ ps\ ps)}$
       = 0.35 - 0.343/1.0.343 = 0.010 ≅ 1%

2) $λ_{4x\ grl}$ = obs. nonnulliplex/P$_{(ps\ ps)}$ + P$_{(ps\ ps)}$
       = 0.50/0.16 + 0.48 = 0.781 ≅ 78%

   $λ_{6x\ opl}$ = Obs. nonnulliplex/P$_{(Ps\ Ps\ Ps)}$ + P$_{(Ps\ Ps\ ps)}$ + P$_{(Ps\ ps\ ps)}$
       = 0.65/0.27 + 0.189 + 0.441 = 0.989 ≅ 99%

If 6x opl has disomic instead of hexasomic inheritance, then the equations for *s* and λ are as follows:

1) $s_{6x\ opl}$ = Obs. nulliplex - P$_{(ps)}$/1-P$_{(ps)}$
       = 0.35-0.349/1-0.349 = 0.0015 ≅ 0.2%

2) $λ_{6x\ olp}$ = Obs. nonnulliplex/P$_{(ps)}$
       = 0.65/0.651 = 0.998 ≅ 99.8%

The values of λ and *s* estimated for each polyploid species are approximations of the real ones, because Hardy-Weinberg equilibrium in the original populations had to be assumed in order to obtain rough estimates of P$_{(ps)}$. In addition, full penetrance of the marker gene was assumed. If *ps* has incomplete penetrance in these materials then the proportion of observed nulliplex plants might have been underestimated.

The relation between gene frequency (*p*) and rate of selfing (*s*) when the frequency of observed nulliplex plants in the progeny of nulliplex mothers is 0.50, as in 4x grl, and 0.35, as in 6x opl, is shown in Table 2. It can be seen that either under or overestimation of *p* would lead to very different estimates of *s*. Therefore, *p* has to be more precisely estimated either from zygotic proportions or from expected and observed frequencies in any two of the four basic estimators; nulliplex, simplex, duplex or triplex, solved simultaneously for *p* and *s*, in order to minimize errors.

Large differences in rate of selfing were observed between progenies derived from different nulliplex plants of both specifics. Frankel and Galun

TABLE 2. — *Expected values of selfing (s) and gene frequency (p) for disomic, tetrasomic and hexasomic loci, with α = 0, if the percentage of nulliplex plants among progenies of nulliplex mothers is 50% and 35%, respectively.*

4x grl		6x opl		
Observed nulliplex = 0.50		Observed nulliplex = 0.35		
	Tetrasomic inheritance		Disomic inheritance	Hexasomic inheritance
s	p	s	p	p
0	0.71	0	0.35	0.71
0.1	0.67	0.1	0.28	0.65
0.2	0.61	0.2	0.19	0.57
0.3	0.53	0.3	0.07	0.41
0.4	0.41	0.35.	0	0
0.5	0			

(4) have pointed out that results like this can be expected with normally inbreeding plants and plants with a high seed set per flower (as in *Solanum*), where low frequency cross-pollination events may produce differentials in the progeny of fruit. Thus a majority of fruits may have a high rate of cross-pollination, whereas the average of the whole population may be exceedingly low. In this respect, Haldane (6) has pointed out that, in general, the proportion of recessives is little affected by a small amount of random mating in a self-fertilizing population, but a good deal by a small amount of selfing in a random mating population.

Nevertheless, the highest possible values for *s* in 4x grl and 6x opl, under the conditions of this experiment, are 0.50 and 0.35, respectively. Glendinning's (5) estimates of the rate of selfing in Neo-Tuberosum material, in contrast, ranged from 70% to 86%, with a mean of 80%. These estimates were based on a comparison of the percentage of pigmented seedlings obtained from; 1) a control cross between a donor clone (carrying the *P* allele, which confers purple pigmentation) and a receiver clone (carrying the *p* allele), and 2) open-pollinated fruits harvested on plants of the receiver that were grown in a plot of the donor.

It is not clear in his paper how isolated from each other the plants of each receiver were within a plot. In his calculations he did not take into consideration the unpigmented seedlings that could have come from outcrossing. Thus, if crossing between receivers occurred, his values would be overestimations of the real ones. The rate of selfing estimated on the basis of gametic and/or zygotic frequencies would avoid that problem, even though this method has its limitations and necessary assumptions. The frequencies of recessives and of heterozygotes in the progeny of recessives, which are observed directly, appear to be the best estimators of outcrossing (11).

Rate of Selfing

In population genetic studies zygotic frequencies at equilibrium in a tetraploid population with mixed self and cross-fertilization can be calculated once the rate of selfing or outcrossing and allele frequencies have been estimated (1, 6). Deviations from equilibrium can then be tested.

The use of more than one genetic marker in estimating the rate of selfing (or outcrossing) in diploid and tetraploid populations has been suggested (1, 4). Assumptions can be minimized if two or more genetic markers are used. Bennett (1) has suggested that it could be of interest to compare estimates of the amount of selfing obtained in different ways and for different marker loci, in order to test the various assumptions of equilibrium, unvarying frequencies of self-fertilization, and non-selective differences between genotypes.

The estimation of s or λ as done here can be carried out as efficiently in nature as in the experimental field. It would be of interest to obtain estimates of s in both environments in order to know how the breeding behavior of the species is altered when they are taken out of their natural habitats and grown under experimental conditions. This could give an idea of whether extrapolations of results could be, at least in certain conditions, valid.

In plant breeding and seed production cross-pollination must be controlled. Effective isolation of seed crops is required to avoid interpopulation crossing, which would result in contamination of cultivars. On the other hand, the facilitation of interpopulation crossing (or promotion of cross-pollination) is often necessary to produce hybrid seed or to secure wide crosses in a breeding program.

The use of true potato seed for the production of consumer potatoes in tropical areas of developing countries is being investigated for both economic and sanitary reasons. This is a new approach to potato production in many of these countries, therefore much research is needed in many disciplines, including breeding. The breeding method to be used for the production of true seed should capitalize on the type of gene action involved in tuber yield. Nonadditive genetic effects have been found to be preponderant in the determination of tuber yields in Phureja-haploid Tuberosum hybrids grown from tubers, at both the diploid and the tetraploid levels (7) as would be expected in uniparental organisms. Thompson (9) has reported genetic variances of mainly additive type for seed production and of mostly nonadditive type for tuber yield in a population of true seedlings derived from Neo-Tuberosum material.

The feasibility of F_1 hybrid seed production in India is being investigated (10). Controlled 4x-4x, 4x-2x (FDR), 2x (FDR)-2x (FDR), 4x-[4x-2x (FDR)] and 4x Andigena-4x Tuberosum crosses have been proposed by Peloquin (8) on the basis of the percentage of heterozygosity transmitted by the parents and the uniformity of the resulting progeny. This

same investigator has considered the use of open pollinated seed as an alternate approach when the hybrid seed is difficult to obtain in very large quantities, or when the added cost of uncontrolled pollinations exceeds the benefits of the expected yield increase. It is extremely important if open-pollinated seed is to be used in the production of true potato seedlings that clones be identified in which a large proportion of the open-pollinated seed is hybrid.

It, thus, appears of interest to arrive at a discriminate estimate of the natural cross-pollination rate for different materials, under set environmental conditions, if cross-pollination is to be either restricted or promoted artificially for seed production.

The quantitative approach used in the estimation of the rate of selfing (or outcrossing) in 4x grl and 6x opl can be used to obtain estimates of these parameters in cultivated material grown in the experimental field. Any recessive allele with full penetrance could be used as a genetic marker. Two generations, both of which could be grown in one year if facilities are adequate, would be required to obtain a good estimate of s (or λ). The breeding method to be used in the production of true seed should, then, be chosen in conformity with the results.

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