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Impact of population structure on the apparent outcrossing rate of grain sorghum (*Sorghum bicolor*)

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Summary. Population structure, the physical arrangement of related and unrelated individuals, can have profound effects on the apparent outcrossing rate ($\hat{t}$) in a plant population. However, detailed experimental investigations of the impact of population structure on $\hat{t}$ are few. We compared the apparent outcrossing rates of experimental populations of grain sorghum with seed families spatially arranged in stratified and overdispersed treatments. Using alcohol dehydrogenase allozymes as genetic markers, $\hat{t}$ was calculated for each of the treatments at two locations over three years. For all six comparisons, the overdispersed treatment yielded significantly larger apparent outcrossing estimates than the stratified treatment. In one case, the difference was over five-fold. Site-specific and time-specific differences were small compared to treatment differences. Whether natural structuring plays a role in altering the effective outcrossing rates of natural populations has been addressed by only a few descriptive studies; structuring effects appear to have an impact in only about half of such studies. The sample is still too small to make any generalizations.

Population structuring can also be of significance in plant breeding programs. Controllable variation in $\hat{t}$ values of the magnitude reported herein may be useful in optimizing selection methods for quantitative characters in experimental plant breeding populations. Further work is under way to determine the effects of the variation in apparent outcrossing rates on genetic gains from selection.

Key words: *Sorghum bicolor* – Outcrossing rate – Planting design – Population substructuring – Breeding methods

Introduction

Apparent outcrossing rates are defined in terms of $\hat{t}$. For random mating, genotypes mate with each other in proportion to their frequency within a population $\hat{t}=1.0$. Values of $\hat{t}$ less than 1.0 occur when like genotypes mate among themselves more frequently than expected by random chance, as a result of selfing or other forms of assortative mating. On the other hand, dissassortative mating results in unlike genotypes mating at higher than expected frequencies; in this case $\hat{t}$ values greater than 1.0 may be observed (Clegg 1980).

Population structure, defined here as the physical arrangement of related and unrelated individuals within a population, can alone affect apparent outcrossing rates (discussed in detail by, e.g. Hamrick 1982; Shaw and Allard 1981; Shaw et al. 1981; Vasek 1968). For example, Ennos and Clegg (1982) demonstrated with computer simulation that if genotypes are clumped in space and pollen flow is limited, then apparent outcrossing is reduced relative to a randomly structured population. Such substructuring is probably responsible for the inverse relationship of population density to apparent outcrossing rates in populations of *Helianthus annuus* (Ellstrand et al. 1978). Whether substructuring is important in determining the effective outcrossing rates in natural populations has received little attention (reviewed by Clegg 1980) despite recently increased interest in measuring outcrossing rates in plant populations (e.g. Hamrick 1982; Levin 1978; Ritland and Jain 1981; Shaw and Brown 1982; Shaw et al. 1981). Furthermore, the problem has received very little experimental attention.

Ennos and Clegg (1982) manipulated the spatial arrangement of two experimental populations of the insect-pollinated *Ipomoea purpurea* so that in one population the two genotypes were randomly arranged; in the other they were arranged in two adjacent blocks. Progeny testing demonstrated that the randomly arranged population had a $\hat{t}$ value of 0.78, while the stratified population had a significantly lower value of 0.60. This experiment involved only one location and one season. Given the tremendous variation that has been reported in estimates of outcrossing rates from season-to-season and population-to-population (reviewed by Hamrick 1982; Schoen 1982), it is clear that further experimentation is necessary to establish whether this structuring effect is a general one.

To gain a more complete appreciation of how population structure affects apparent outcrossing rate, we structured populations of grain sorghum (*Sorghum bicolor* (L.) Moench.) in two different ways at two locations in each of three years. Specifically, we asked whether population structuring alters apparent outcrossing, and how the magnitude of such differences compare with those between sites and between years.

Materials and methods

The experimental organism

Grain sorghum is a predominantly selfed, wind-pollinated crop plant usually grown as an annual; its outcrossing rate has been estimated to range from 10% to 20% (Riccelli-Mattei 1968). Numerous diverse populations of grain sorghum have been synthesized, usually through the use of genetic male sterility employed to promote random mating. The population used herein was RP2B, a broad-based population synthesized from numerous U.S. and exotic lines and selected for temperate adaptation (Ross et al. 1977). This population has been random mated in isolation for a least four generations prior to initiation of this experiment. Following the last random mating, the population was grown again to produce (under bags) S_1 families from fertile plants. Approximately 100 S_1 families of five plants each were subsequently grown in the greenhouse. Two samples of 25 random male-fertile individuals (each from a different S_1 family) were drawn to constitute replicate experimental populations.

RP2B is polymorphic for codominant alcohol dehydrogenase allozymes (Ellstrand et al. 1983). This allozyme system served as the genetic marker for progeny analysis to determine apparent outcrossing rates.

Planting design

Test plots were planted at both the University of California Moreno Valley Field Station near Sunnymead and the University of California Experiment Station at Riverside. These sites are approximately 40 km apart and differ by 350 m in elevation. One replicate of 24 RP2B families was grown at each location. Seed was planted during the second week of June in 1980, 1981, and 1982. Each selection block was isolated from other sorghum by a minimum of 300 m. Seed were planted in rows spaced 76 cm rows having 10 cm between plants within each row. Two seeds per hill were planted, and soon after emergence plants were thinned to one per hill. After sowing, propazine was applied to control weeds. The fields were furrow-irrigated at weekly intervals following seedling emergence. Flowering began in mid-August; all plants had mature seed by October.

The research reported herein was part of an experiment designed to evaluate selection response. Individuals chosen to represent families for subsequent generations were the result of within-family selection for increased panicle length. Thus, populations grown in 1981 and 1982 resulted from selections made in 1980 and 1981, respectively.

Stratified treatment

The goal of this treatment was to place related individuals in proximity. Each of the 25 families was planted as a four-row block with 40 plants per row (Fig. 1). Each family block was assigned a position randomly within the 5×5 arrangement of families; this position was reassigned at each site every year.

Overdispersed treatment

The goal of this treatment was to surround each individual of a given family with members of different families. Every year each of the 25 families was assigned a random number from 1–25. Families numbered 1–13 were planted in sequence, one individual per family at a time, repeating the same sequence 15 times along every fourth row. Families 14–25 were planted in a similar manner in the row adjacent to the first set of families. Alternating pairs of rows were left unplanted to make the plots planted under both structuring treatments occupy the same field area (Fig. 2).

Progeny testing

Seeds for progeny testing were harvested from three randomly chosen plants per family, totaling 75 plants per population-treatment combination. In the stratified treatment, only plants from the inner two rows of each block were selected. Two seeds per plant were assayed electrophoretically for their alcohol dehydrogenase allozymes (details of electrophoretic procedures and interpretation of isozyme banding patterns are provided by Ellstrand et al. 1983). If one or both of the progeny were heterozygous, four additional progeny were

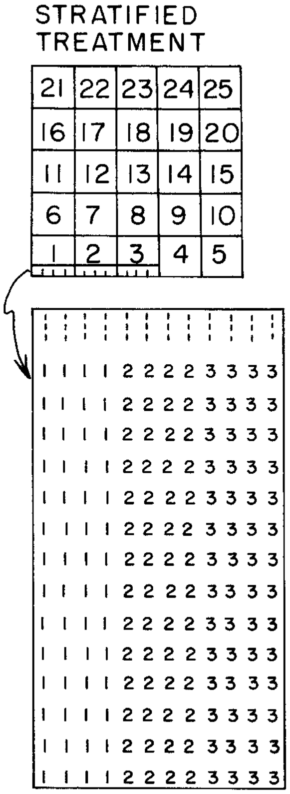

Fig. 1. Illustration of stratified planting design for 25 seed families. Inset shows detailed position of individual plants

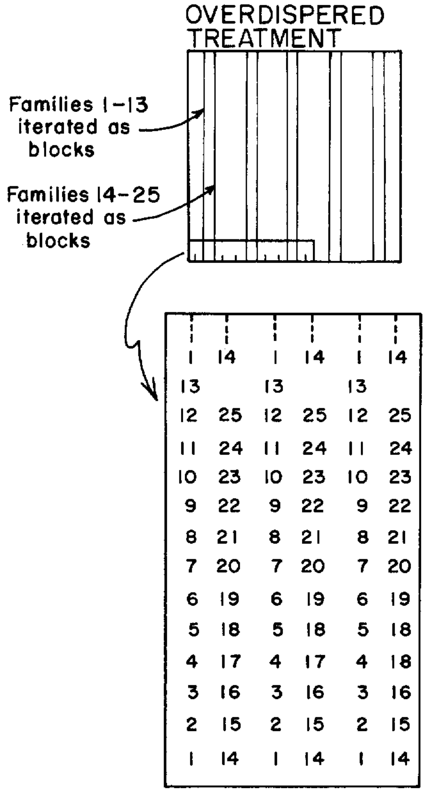

Fig. 2. Illustration of overdispersed planting design for 25 seed families. Inset shows detailed position of individual plants

sampled for that plant to ascertain the maternal genotype. This procedure is that of Brown's (1975) Design IV for efficient sampling for the estimation of population genetic parameters.

Calculation of apparent outcrossing

Approximations of $\hat{t}$ were computed following the method of Brown (1975), Design IV. This method first approximates $\hat{t}$ based on progeny and putative maternal genotypes and employs a joint maximum likelihood estimation. The new estimates of the genetic parameters are then used to attain a corrected value of $\hat{t}$. Iterations of these estimations are performed until the value of $\hat{t}$ varies by less than 0.01 in consecutive iterations.

Results

Estimates of $\hat{t}$ are given in Table 1. Values of $\hat{t}$ ranged from 0.09 (Riverside – 1980, stratified) to 0.68 (Riverside – 1982, overdispersed). The differences between the treatments were strong and systematic. Estimates of $\hat{t}$ were always significantly higher for the overdispersed treatment in each of the six paired comparisons. Overall, the trend is significant as well ($P < 0.01$; Wilcoxon's signed ranks test, one-tailed). The smallest difference was only an 8% difference (Moreno – 1981). But often the differences were profound; in one case (Riverside – 1982), the difference was more than five-fold. The difference between treatment means was more than two-fold (0.22 for stratified vs. 0.46 for overdispersed).

Table 1. Impact of population structuring treatments on apparent outcrossing rate estimates

Year	Location	Apparent outcrossing rate $\hat{t} \pm$ SE[a]		Year means
		Stratified treatment	Overdispersed treatment	
1980	Moreno	0.23 ± 0.01^{b}	0.37 ± 0.02^{de}	0.28
	Riverside	0.09 ± 0.01^{a}	0.43 ± 0.02^{fg}	
1981	Moreno	0.36 ± 0.02^{d}	0.44 ± 0.01^{g}	0.37
	Riverside	0.27 ± 0.02^{c}	0.40 ± 0.01^{ef}	
1982	Moreno	0.20 ± 0.02^{b}	0.46 ± 0.01^{g}	0.36
	Riverside	0.12 ± 0.02^{a}	0.68 ± 0.02^{h}	
Treatment means	Moreno	0.26	0.42	
	Riverside	0.16	0.50	
	Combined	0.22	0.46	
Location means	Moreno	0.34		
	Riverside	0.33		

[a] Nonsignificant differences are denoted by the same superscript

On the other hand, differences among years and sites were far less prominent. Although the two locations were about 40 km apart, the mean $\hat{t}$ values for each location were nearly identical (0.34 at Moreno vs. 0.33 at Riverside) ($P < 0.05$; Wilcoxon's signed ranks test, one-tailed). Likewise, the differences of mean $\hat{t}$ among years is relatively small compared to the treatment differences. 1980 was a relatively low year (mean $\hat{t} = 0.28$), but 1981 and 1982 had about the same, somewhat larger, value, 0.37 and 0.36, respectively.

Effects of structuring appeared to be more pronounced at Riverside than at Moreno (Table 1). Every year, the absolute difference in $\hat{t}$ at Riverside was more than one and a half times that at Moreno. Furthermore, the stratified treatment at Moreno was more highly outcrossed than its Riverside counterpart, and two of the three years the overdispersed Moreno population was less outcrossed than the analogous Riverside population. This apparent response difference cannot be attributed solely to location, however, as different samples of RP2B were used to generate the two base populations.

Discussion

Different kinds of population structuring have a profound effect on apparent outcrossing rates in *Sorghum* *bicolor*. The stratified treatment consistently produced lower outcrossing estimates relative to the overdispersed treatment. In fact, although other studies have demonstrated remarkable variation in outcrossing estimates among natural populations of the same species or over years within the same population (Hamrick 1982; Schoen 1982), the treatment differences reported here were far stronger than those previously reported differences between locations or between years. The differences in apparent outcrossing between treatments that we report here are to be expected. Estimates of apparent outcrossing are based on the expectations of a randomly mating population (Clegg 1980). But given the restricted pollen flow of a wind-pollinated plant like sorghum (Levin and Kerster 1974), deviations from random arrangement of related individuals should yield deviations from actual outcrossing rate (cf. Shaw and Allard 1981). Thus, stratified structuring of related individuals should result in a comparatively higher frequency of consanguineous matings and thus, estimate of apparent outcrossing lower than the actual rate of outcrossing. Conversely, an overdispersed structuring should result in a lower frequency of matings among related individuals compared to random expectation and therefore, an estimate of apparent outcrossing lower than the actual outcrossing rate.

The overall difference in apparent outcrossing rate due to our treatments is over two-fold. This disparity is considerably larger than that reported by Ennos and Clegg (1982) for differently structured experimental populations of *Ipomoea purpurea* (.60 vs. .78). Two reasons can account for this disparity. First, *Sorghum bicolor* is wind-pollinated, while *Ipomoea purpurea* is insect-pollinated. Ennos and Clegg (1982) discovered a considerable amount of pollen carry-over by insects which tended to homogenize the pollen pool of their stratified treatment. Obviously, pollen carry-over cannot occur in anemophilous plants like sorghum. Secondly, the experiments reported here involve a comparison of stratified versus overdispersed treatments; the *Ipomoea* experiment involved a stratified and a randomly structured treatment. The overdispersed treatment we designed should yield higher relative apparent outcrossing rates than a random treatment because, given limited pollen flow, the opportunities for consanguineous matings are reduced to the minimum.

If population structuring can influence apparent outcrossing rates, what role does it play in affecting inbreeding and effective outcrossing rates in natural populations? Since both seed and pollen dispersal are highly limited (Levin and Kerster 1974), it is likely that related individuals are spatially clumped in natural plant populations and that consanguineous matings of related neighbors should be common. However, only a few of the descriptive studies of apparent outcrossing rates in natural populations have examined the influence of population substructuring on plant mating systems.

The effect of such structuring can be readily tested by an examination of the distributions of outcrosses among the progenies of homozygous plants (Clegg 1980). If such distributions are significantly heterogenous among maternal plants, then either the population consists of local breeding sub-units or maternal outcrossing rates are highly variable from plant-to-plant. Such heterogeneity tests have proven significant for *Eucalyptus obliqua* (Brown et al. 1975), *Clarkia exilis* (Vasek 1967), and *Elymus canadensis* (Sanders and Hamrick 1980). However, population substructuring was ruled out as a reason for interfamily differences in the latter two studies because in the first, heterogeneity occurred among adjacent plants, and in the second, those loci with the large deviations from uniform outcrossing were those with the higher, rather than lower, effective outcrossing rates. No inter-family heterogeneity was detected in the outcrossing studies on *Eucalyptus pauciflora* (Phillips and Brown 1977), *Gilia achilleifolia* (Schoen 1982), or *Borrichia frutescens* (Antlfinger 1982).

Another way to test the effects of population structure is to compare outcrossing estimates derived from multilocus models with those from single-locus models; if structuring affects inbreeding, the single-locus estimates will be generally smaller than the multilocus estimate (Shaw et al. 1981). In studies of natural populations of *Pseudotsuga menziesii* (Shaw and Allard 1981, 1982), population substructuring appeared to play a role in affecting the apparent outcrossing at one locus while other loci did not show any decrease of apparent outcrossing relative to the multilocus estimate. No substructuring effect was detected in a randomly planted orchard of the same species as anticipated (Shaw and Allard 1982). Another such comparison involved two populations of *Limnanthes alba* (Ritland and Jain 1982); although the multilocus estimate exceeded the mean of the single-locus estimates in both populations, it did so significantly in only one. In summary, of the few studies that have examined the possibility of local breeding sub-units influencing outcrossing rate, only three have evidence that such may be the case. The sample is still too small to make any generalization.

Recurrent selection schemes for crop improvement programs emphasize the importance of maximizing recombination in order to maximize genetic gains. In fact, most such schemes include, either implicitly or explicitly, a generation largely, if not completely, devoted to achieving recombination (Empig et al. 1972). Considerable effort has been expended to develop random mating populations of predominantly self-pollinated species such as sorghum and soybean (*Glycine max*) (Ross et al. 1977; Brim and Stuber 1973). Quantitative genetic analysis within such sorghum populations has demonstrated the presence of substantial genetic variability which should be responsive to directional selection (Jan-Orn et al. 1976). Conversely, that low rates of recombination can provide significant selectable variation within a population has been shown by Foster et al. (1980), who found continued response to 10 cycles of mass selection for three traits within a predominantly self-pollinating population of grain sorghum. This finding suggests that optimum recombination for selection may not be synonymous with maximized outcrossing.

The population structuring approach described herein provides a relatively straightforward method with which to examine the effects of a range of apparent outcrossing rates on gain from directional selection in a wide range of crop plants. The method does not rely on manual pollination or other disruptive techniques to alter breeding systems, nor does it require a genetic alteration of mating systems to form populations with distinct outcrossing rates. Investigation of selection response as affected by the treatments described herein is under way and the results will be reported elsewhere.

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