Effect of Sexual and Asexual Reproduction on Race Abundance in Cereal Rust Fungus Populations

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ABSTRACT

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Several populations of cereal rust fungi that differed in the amount of sexual (= Type 1 where sexual reproduction occurred annually in a portion of the population) and asexual (= Type 2 where reproduction is primarily or wholly asexual) reproduction were compared for number of races and evenness of race frequency distribution. In all but one of eight cases, Type 1 populations had more races in a (visually) more even distribution than did comparable Type 2 populations. Simpson's and Shannon-Wiener indexes of diversity were used at an intraspecific level to estimate these differences. For all populations, the mean Simpson's indexes were 0.934 and 0.798 for the Type 1 vs Type 2 populations, respectively, and the mean Shannon-Wiener indexes were 2.76 and 2.10, respectively, which is a significant difference according to a paired *t*-test. Valid comparisons of two types of populations require that the same differential set be used on both since both virulence frequency and virulence associations, which determine the ability of differential cultivars to detect races, will affect the absolute size of diversity indexes.

Additional key words: Simpson's index of diversity, Shannon-Wiener index, gene-for-gene, *Puccinia graminis* f. sp. *tritici*, *Puccinia coronata*, *Puccinia recondita*, genetic variation.

Race surveys have been important in the effort to breed cereals resistant to rust diseases. Because of a need to detect potentially hazardous races before they constitute a major part of the population, extensive data from such surveys have been collected and published. However, few fundamental investigations of pathogen diversity and population structure have been made from these data. Our interest is in the study of diversity of races of pathogen populations that differ in the frequency of sexual and asexual reproduction occurring annually. Race abundance is analogous to the ecological concept of species abundance whereby the distribution of frequencies of species describes a particular community of organisms (8). Race abundance distribution is the distribution of frequencies of virulence phenotypes. These phenotypes are determined on the basis of their virulence or avirulence on a set of host differential cultivars. There are similarities and differences in the forces underlying distributions of species as opposed to races. Species are separate entities that do not usually exchange genetic material, while races, being intraspecific units, do frequently through sexual recombination and rarely through the parasexual cycle (3,17). The rate of such exchange should be expected to influence race distribution in a pathogen population. Habitat or niche preference and apportionment is a determinant of abundance of species and races. For races of plant pathogens, habitat includes the physical as well as the host environment. Races can be adapted to local regions (1;2,page 94;10;11;13) or to specific cultivars. The major determinant of species abundance is the manner in which environmental resources are apportioned among species in a community. Assumptions about resource apportionment form the logical basis of mathematical models that describe species abundance (8). These models may apply to race abundance, but the effects of genetic exchange and more direct (as opposed to indirect interspecific) competition are lacking in them.

Typically, species in a community occur in a range of frequencies—a few being abundant and well represented in samples, several occurring in intermediate frequencies, and many being rare, often represented only once in a sample if they are represented at all (8). Race abundance might follow this pattern due to unequal utilization of the host and physical resources because some races are widely adapted and relatively successful at reproducing and others are narrowly adapted and less successful. Assuming that fitness is not determined only by specific virulence genes, the effect of sexual recombination should be to minimize fitness differences by, more-or-less, randomly distributing many genes that affect fitness and occur at intermediate frequencies. This should manifest itself as many races occurring in equal frequency. Theoretically pathogen populations that are entirely asexually propagated should contain one or a few abundant races, depending on the uniformity of the environment. This pattern results from selection operating for longer periods of time, on the relatively stable phenotypes that compose the population and eliminating, for all practical purposes of sampling, all but the most successful. This pattern is not likely to be found, however, even in the complete absence of any means of genetic recombination by the pathogen. A few less abundant races will occur because of abiotic and biotic environmental heterogeneity. The objective of this study was to examine the pattern of race abundance in two *Puccinia graminis* Pers. f. sp. *tritici* populations, one of which reproduces sexually annually and the other normally only asexually, in order to see whether the patterns fit the expectations suggested above. Corroborative evidence was obtained from publishing race survey data of other rust fungus populations where direct comparisons of more- vs less-sexually reproducing populations had been made.

MATERIALS AND METHODS

Published race survey data were available for both sexual and asexual populations of *P. graminis* f. sp. *tritici* in 1975 (12). These data were used to obtain individual population race-abundance curves that show the frequency of races represented by *r* individuals where *r* is 1, 2, 3, etc. Such curves have been used for populations where the number of distinct units (species mainly) and the size of the sample collection were relatively large (9). Race frequency curves from the most to the least abundant races were plotted from earlier data on leaf rust of wheat (19) and crown rust of oats (4–6), as had been done by Stakman et al for stem rust of wheat (16).

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Direct comparisons of the number of different phenotypes obtained from different populations, using the same set of differential host cultivars are desirable. Unfortunately, sample sizes for populations compared were not always similar (4,12,16). The number of distinct phenotypes detected in the population with the smaller sample size should not be extrapolated to that number which would have been detected had a larger sample been taken, without knowing the actual distribution of phenotypes, because even assuming the best fitting theoretical abundance curve, extrapolated numbers of phenotypes do not "usually inspire confidence" (8, page 16). A more reasonable approach is to reduce the larger sample to the size of the smaller. If the smaller sample is not extremely small, the probability of obtaining the rarer phenotypes can be assigned according to Poisson expectations. This will not reveal which races will occur but does express the probability with which a race found in a large sample will occur in smaller samples.

Existing uredial populations of the cereal rust fungi contain progenies of sexual, parasexual, and asexual reproduction. In these fungi no natural population can be assumed to be reproducing completely either asexually or sexually (or parasexually). It is, however, convenient to group populations into types (ie, Type 1) where sexual reproduction occurs annually in all or a portion of the population, and Type 2, where the asexual cycle is the primary source of reproduction and little or no sexual reproduction occurs. Previous work (12) suggests that parasexual processes have not strongly influenced population structure in a Type 2 population of stem rust of wheat whose alternate host has been eliminated.

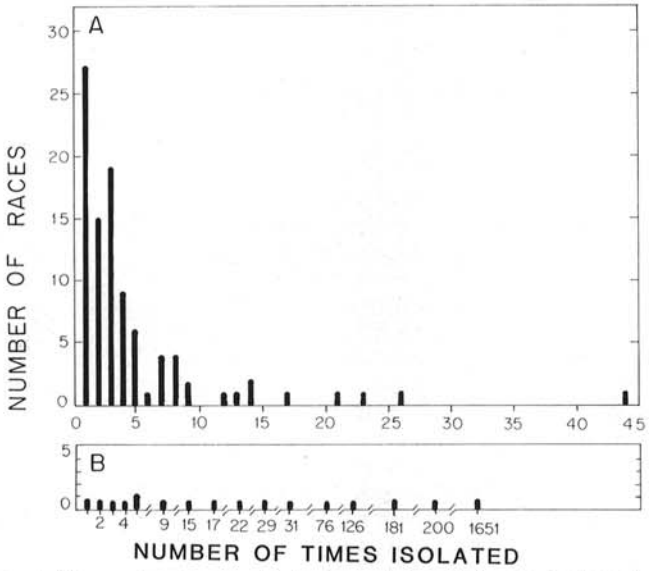

Fig. 1. The number of races obtained once, twice, etc. in samples from two populations of *Puccinia graminis* f. sp. *tritici*. **A,** A sexually reproducing population from Washington-Idaho and **B,** an asexually reproducing population from the United States east of the Rocky Mountains, where the alternate (sexual) host has been effectively eradicated.

Two indexes of diversity were calculated for each of the populations. Simpson's Index (9,15) was used because it has been mentioned in conjunction with host diversity (20, page 69 but the formula shown is not the original index) and pathogen diversity (12):

$$D = 1 - \sum_j \frac{N_j(N_j - 1)}{N\,(N - 1)}$$

in which N_j = the number collected of the jth phenotype (race) and N = the sample size.

The Shannon-Wiener index (14), also called the information function, was included because it has been adapted to include degree of difference between entities (species or races) in a discrete manner (8), a characteristic that could prove useful in future studies, and because it is considered less sensitive to changes in sample size than is Simpson's index (14). We chose to use the simplest form of this function because it provided similar results to those forms that are more suitable for small samples:

$$D' = -\sum_j p_j \ln p_j$$

in which p_j is the frequency of the jth phenotype.

RESULTS

Figure 1 shows the race abundance curves for 1975 U.S. *P. graminis* f. sp. *tritici* populations one of which was Type 1 (Fig. 1A) and the other of which was Type 2 (Fig. 1B). Since there were 100 different races identified in the Type 1 population, a race frequency plot in which each race is represented by a vertical bar was deemed impractical, and a race abundance curve was plotted instead (9, page 270). The two populations differed conspicuously in number of races and in their distribution. The difference in number of races can be adjusted for difference in sample size. If the asexual population was sampled only 426 times, it is likely that both of the races that were isolated once would be missed. This was calculated as follows: The best estimate we have of the frequency of a singleton race collected in the Type 2 population is $1/2{,}377 = 0.0004$. The likelihood of missing this race in a single trial (a sample of one) is the zero term of the Poisson distribution, $e^{0.0004}$ or 0.9996. In the 426 trials of the sample of the Type 1 population, this likelihood is the product of individual trial probabilities or 0.836, which means that

TABLE 1. Measurements of diversity for virulence for selected populations of *Puccinia* spp. Type 1 populations are defined as those having some sexual reproduction annually and Type 2 where reproduction is primarily asexual

Population of *Puccinia* spp.	Source	Type 1 No. of Races	Type 1 Sample Size	Type 1 Diversity indexes Simpson's	Type 1 Diversity indexes Shannon-Wiener	Type 2 No. of Races	Type 2 Sample Size	Type 2 Diversity indexes Simpson's	Type 2 Diversity indexes Shannon-Wiener
P. graminis tritici	(12)	100	426	0.974	1.78	17(12)[b]	2377	0.501	0.53
P. graminis tritici	(16)	24	238	0.836	2.30	19(10)	1622	0.678	1.33
P. recondita tritici NA65[a]	(19)	25	500±	0.931	2.90	17	500±	0.841	2.37
P. recondita tritici UN[a]	(19)	24	500±	0.917	2.76	8	500±	0.696	1.52
P. coronata	(5)	31	57	0.962	3.35	21	62	0.936	2.83
P. coronata	(4)	21	38	0.957	3.07	20(13)	110	0.905	2.58
P. coronata	(4)	17	29	0.941	2.50	25(13)	95	0.924	2.79
P. coronata[c]	(6)	43	760	0.951	3.42	32	565	0.900	2.84
Mean Index				0.934	2.76*[d]			0.798	2.10*

[a] NA65 and UN are different sets of differential hosts.
[b] Probable number of races that would have been detected (by Poisson expectations) if the sample size had equalled that of the corresponding Type 1 population.
[c] Populations of *P. coronata* are from eastern (where buckthorn is more abundant) and western Canada.
[d] Asterisks (*) indicates significant difference between comparable indexes for Type 1 vs Type 2 populations; $\alpha = 0.05$, according to a paired-t test.

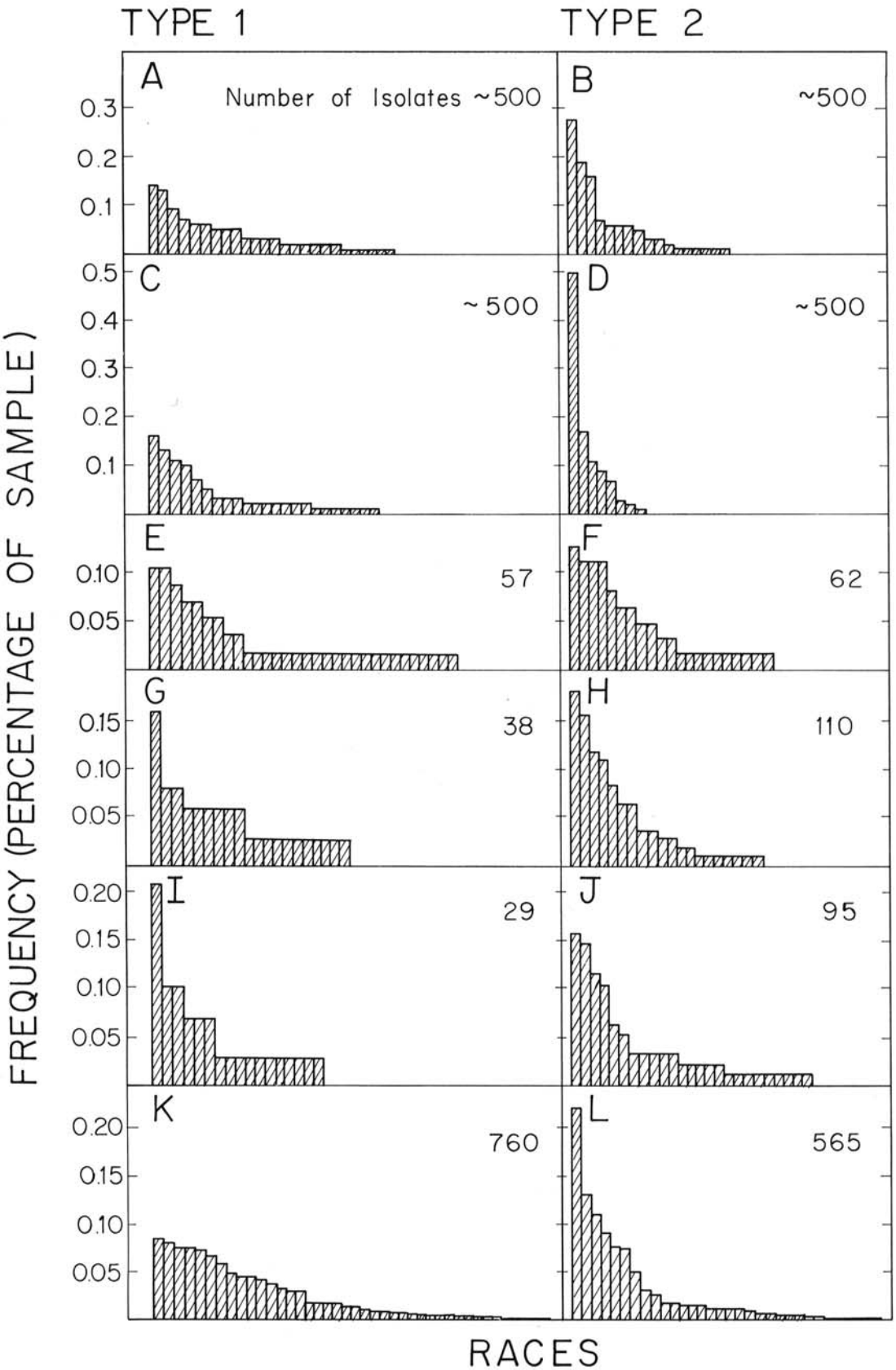

Fig. 2. Race frequency curves of six comparisons of Type 1 (partly sexually-reproducing) with Type 2 (primarily asexually reproducing) North American populations of cereal rust fungi whose races in each pairing have been characterized by using the same differential set of host lines and cultivars. In populations E-L, the most infrequently sampled races (on the right side) were singletons. Pairs AB and CD are *Puccinia recondita* f. sp. *tritici* characterized using the NA-65 and UN sets of differentials, respectively (19); E and F show *P. coronata* aecial and uredial collections, respectively (5), G and H are *P. coronata* aecial and uredial collections, respectively from western Canada, and I and J similarly from eastern Canada (4), and K and L are uredial collections from eastern and western Canada, respectively (6).

it is likely that this race would go undetected in the smaller sample, as would the races that were isolated two, three, or four times. A race that was isolated five times would be missed with a probability of 0.408, meaning that one of the two races detected is likely to be missed. All other races found in the large sample are likely to be obtained in a sample of 426. Thus, only 12 of the 17 races were sufficiently common in the Type 2 population to permit their detection if the sample size were as small as that taken of the Type 1 population. Along with the extreme differences between the two populations in number of races isolated, the patterns of race abundance were also distinct. The Type 1 population contained a continuous range of frequencies, from a few infrequent (5–10%) races to rather large number of very infrequent (<1%) races. The Type 2 population contained one extremely frequent race ($p = 0.69$) and many infrequent to very infrequent races, the most common of which was present as 80% of the population. The Shannon-Wiener index of diversity reflects the difference in number and distribution of races in these two populations, being 1.78 for the Type 1 population and 0.53 for the Type 2 population. The Simpson's index, which gives a similar comparison, and which has been published previously for these populations (11), was 0.974 and 0.501, respectively, for Type 1 and Type 2.

Because generalizations about these patterns are not possible with only one representative of a Type 1 and Type 2 population, we searched the literature for other examples of race frequency data which fit those categories. Table 1 presents Simpson's and Shannon-Wiener diversity indexes for these populations. Vertical comparisons of the diversity indexes of the rust populations are not meaningful since the number and quality (race differentiating capacity) of the differential cultivars varied among studies.

Figure 2 represents race frequency curves for these populations except those of Stakman et al (16), which were plotted in a similar manner already. The visual trends in nearly all comparisons were toward fewer races in the Type 2 populations in a pattern less even than with the Type 1 population. These trends are not evident in the 1964 eastern Canadian data of Fleischmann (4) (Fig. 2, I and J). In the other comparisons, the degree of difference in these trends varied, being most striking in the leaf rust populations using the UN set of differential cultivars (Fig. 2, C and D).

The diversity indexes of these populations which measure evenness and number also show differences between Type 1 and Type 2 populations (Table 1). In all but one case, the Type 1 populations had larger indexes of diversity than did the Type 2 populations. The single case in which this was not true was that of the 1964 Survey of eastern Canadian Crown Rust. A paired *t*-test of the eight pairs of indexes showed a significantly higher mean Shannon-Wiener index for Type 1 populations. This significance remained, even when the stem rust data for 1975 were excluded. Simpson's index was not similarly tested because variances about the two means were not homogeneous, by Bartlett's test for homogeneity.

DISCUSSION

Of all the data examined, those for 1975 on stem rust of wheat showed the largest difference between Type 1 and Type 2 populations. This is probably because these populations showed the greatest contrast in the amount of sexual recombination included in their life cycles. The other comparisons were of populations that differ only in degree of sexual recombination. Uredial collections, especially, can include many isolates that originated from aeciospores at the beginning of the season. This may explain why the 1963 eastern Canadian uredial collections of *P. coronata* (Fig. 2) exhibited a race abundance pattern that is more like a Type 1 population; ie, relatively low frequencies for the most common races and numerous infrequent races. The alternate host, buckthorn, is common and widespread in Ontario (5). Because the sample size was small, little can be said about the aecial population of *P. coronata* from eastern Canada. The aecial and uredial populations from western Canada in 1963 fit better the expected pattern of Type 1 and Type 2 populations, respectively, probably because buckthorns are less common there. The small sample of the aecial population prevents firm conclusions about the shape of the race frequency curve. The difference in abundance of buckthorn between eastern and western Canada is the basis for designating uredial populations (combined 1952-1961) as Type 1 and Type 2, respectively (6).

The two diversity indexes used in this study appear to provide similar information, so that either of them would normally provide an adequate measure of diversity. The one instance in which indexes were different is due probably to either the small size of the aecial sample which is known to more strongly influence Simpson's Index (upward) (14), or the presence of a small number of "dominant" (very prevalent) races, which is known to more strongly affect Simpson's Index than the Shannon-Wiener Index (18). The aecial collections contained one such dominant race while the uredial collections did not. Because of either or both of these effects, we believe that the Type 2 population is indeed more diverse, as measured by the Shannon-Wiener Index and shape of the race frequency curves.

For both sets of differential cultivars used, the data of Young and Prescott (19) show the Type 1 vs Type 2 population patterns. The UN differential host set, however, more stongly contrasts the two populations of *P. recondita*, as shown by the curves and by the larger differences between the two populations in either index of diversity using this set as opposed to the NA 65 differential host set. Their study clearly indicated that comparisons of sexual with asexual populations are influenced by the host differential set chosen. Absolute levels of diversity therefore have little meaning. Comparisons should not be attempted between pathogen populations of the same or different species unless similar criteria of diversity (differential host sets) are used. To minimize differences in criteria of diversity where the same differential sets of cultivars cannot be used, a uniform number of single-gene differential hosts should be selected for which the corresponding virulence genes occur at intermediate frequencies and are not associated in either population. Such selection will only be possible with the more intensively studied diseases.

In all cases but one, Type 1 populations had more races with a more uniform frequency distribution than Type 2 populations. Indexes of diversity provide the advantage of expressing both ideas as a single number. Plots of race abundance seem to be more revealing, but are often too voluminous to publish and are difficult to compare.

Diversity indexes are controversial. Hurlbert (7) thinks that they have been indiscriminantly applied, and that they are too elementary to adequately describe ecological communities. Pielou (8) states that they have too often been made the focus of research rather than being used as a tool. Many of Hurlbert's objections do not apply when the indexes are used to describe similar organisms at the intraspecific level. Diversity indexes have filled a need in ecological research, where they are being complemented by more specific and sophisticated measures of community or population structure. This process will also occur in describing populations of plant pathogens and their hosts, but probably at an accelerated rate, since an extensive literature on them already exists.

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