A Growth Chamber Comparison of Traits of Aggressiveness in Sexual and Asexual Populations of *Puccinia coronata*

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ABSTRACT

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Uredial isolates of *Puccinia coronata*, derived from aecia on *Rhamnus cathartica* in Minnesota, were compared for several traits of aggressiveness with uredial isolates from southern Texas where the role of the alternate host is reduced or nonexistent. Mean values of most traits differed significantly within each population. Uredial dimensions and urediospore production exhibited significantly higher mean values in the sexual population from Minnesota than in the asexual population from Texas. Uredial latent period and time to formation of telia were also significantly shorter in the sexual population. In contrast, there were no significant differences between the sexual and asexual population in the amount of genotypic or phenotypic variation, suggesting that sexual reproduction conferred no advantage to the sexual population in producing a greater range of variability for traits of aggressiveness. Confidence limits ($P=0.05$) of broad-sense heritability estimates for all traits ranged from 18 to 96% in the asexual population and from 26 to 98% in the sexual population, but no significant difference in heritability was detected for any trait between the two populations.

Additional key words: *Avena sativa*, crown rust, oats.

The origin and maintenance of variation in pathogen populations is of concern to plant pathologists. This concern is based upon the premise that sexual reproduction is more beneficial to a population than asexual reproduction by creating greater genetic variation, which by natural selection produces higher levels of fitness (7,17). The benefits of recombination in this hypothesis were proposed by Felsenstein (6) to apply only to traits that exhibit simple Mendelian inheritance and then only in small or finite populations. Whether or not gene recombination will confer an immediate advantage to a population when multiple loci, linkage, or large population sizes are involved is not clear (6,15,16). Indeed, sexual recombination is not required for high levels of variability, as evidenced by the level of genetic variation in parthenogenetic animals (14,20,26) and self-pollinating plants (1,13).

Only a limited number of experiments or surveys of plant pathogens have specifically compared the consequences of sexual reproduction with those of asexual reproduction. In a survey of *Puccinia coronata* Cda. populations in the USA, Simons et al (23) detected greater phenotypic variation at 24 loci for virulence on oats (*Avena sativa* L.) in a sexual than in an asexual population of the pathogen. Similar results were obtained by Roelfs and Groth

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(21) in sexual and asexual populations of *Puccinia graminis* Pers. from wheat in the USA. Groth and Roelfs (8) later combined data from different sexual and asexual *Puccinia* sp. populations that showed significantly greater phenotypic variation of virulence genes (by paired *t* test) in the sexual populations. Our examination of these data from the four populations of *P. coronata* alone, however, did not reveal significant differences (by paired *t* test) between sexual and asexual populations. The study reported here was carried out to measure the potential benefits of sexual reproduction for certain traits of aggressiveness in a Minnesota population of *P. coronata* where the sexual cycle occurs annually on the alternate host, *Rhamnus cathartica* L. Aggressiveness is defined in this study as the relative rate at which a virulent isolate produces a given amount of disease (27). The Minnesota population was compared in the growth chamber with an asexual Texas population in an attempt to determine: whether sexual reproduction produces a greater range of genotypic or phenotypic variation for traits of aggressiveness than does asexual reproduction and whether sexual reproduction provides an adaptive advantage to the population by giving rise to some offspring with higher levels of aggressiveness than does asexual reproduction.

MATERIALS AND METHODS

The sexual population consisted of 20 purified single uredial isolates each originating from separate aecia collected at random in 1979 from the Minnesota buckthorn nursery at St. Paul by P. G. Rothman (22). The original inoculum that infected the buckthorn came from a diverse group of cultivars and lines of several *Avena* spp., whose prevalence and longevity in the nursery were dependent upon their resistance or susceptibility to the pathogen population as determined by Rothman and co-workers (23). The asexual population consisted of 20 purified single uredial isolates from 11 cultivars or lines of *A. sativa* at seven nurseries in southern Texas. The uredia were collected by M. E. McDaniel in 1979. Adequate quantities of inocula were produced on the oat cultivar Markton to permit inoculation of 24 differential host cultivars and lines of oats (23). Each cultivar and line possessed a single known gene for crown rust resistance. Infection type of each isolate-differential combination was rated according to the guidelines of Murphy (18). Those isolates with susceptible or moderately susceptible infection types were classified virulent, while those with resistant or moderately resistant infection types were classified avirulent.

Growth chamber studies were performed to measure various components of aggressiveness. A randomized complete block design with three separate growth chambers serving as complete blocks was used. Each experimental unit consisted of 10 seeds of the susceptible cultivar, Markton, planted in a row in 10-cm-diameter clay pots with standard greenhouse fertilizer and autoclaved soil. When the second leaves of the seedlings were fully expanded (approximately 14 days after planting), the plants were inoculated by using an aliquot quantitative inoculator, at a concentration of 2 mg of spores per milliliter of Soltrol 170 mineral oil. Only fresh spores with a minimum of 80% germination on 2% water agar were used. After inoculation, the seedlings were held in a dew chamber for 18 hr at 25 C and then placed in the growth chambers maintained at 21 ± 1 C air temperature with a 14-hr day length at 10,000 lux illumination.

Eleven traits of aggressiveness were measured. Uredial latent period and telial latent period, defined as the number of days from inoculation to first uredial eruption of the host epidermis and first telia formation, respectively, were recorded. Uredium size was determined by measuring length and width of three randomly selected mature isolated uredia per leaf from three secondary leaves per replication (a total of nine uredia per replication). Detached leaves placed next to a metric ruler were photographed and slides produced from the photographs were used to project an image onto a screen for estimation of uredial dimensions. Uredium area was calculated as π (length × width)/4 (25). The numbers of uredia per square centimeter of leaf for the primary and secondary leaves were calculated separately and in combination to determine if age of host tissue affected uredial density.

In separate experiments, spores produced by each isolate were collected every 3 days by tapping spores from the leaves into preweighed glass vials. Data for total uredial number and total area of inoculated leaves were also collected. Analyses of variance were performed for each trait for examining variation between and within each fungal population. Because uredial and telial latent periods were recorded as discrete data, a "log-linear analysis for categorial data" (2) was used for the two traits.

Component analysis of variance (10) was used to estimate broad-sense heritability for each trait based on isolate means. The isolate (I) source of variation (d.f. = 19) with mean square M1 has an expected mean square composed of $\sigma_e^2 + 3\sigma_I^2$. The replicate within isolate source of variation (d.f. = 40) with mean square M2 has an expected mean square of σ_e^2. The genotypic component of variance σ_g^2 = (M1−M2)/3. Error component (σ_e^2) = M2. Phenotypic component $\sigma_p^2 = \sigma_g^2 + M2/3$. Heritability $= [\sigma_g^2/\sigma_p^2] \cdot 100$. Confidence limits ($P = 0.05$) were calculated for the genotypic component and for each heritability estimate (24).

RESULTS

The primary objective of the study was to compare the aggressiveness of sexual and asexual populations of *P. coronata*. Significant differences were detected in the mean values of several aggressiveness traits between the two populations (Table 1). Uredial length, uredial area, spore weight per uredium, and spore weight per total uredial area were all significantly higher in the sexual population than in the asexual population. The uredial latent period was significantly lower in the sexual population, although the absolute difference was not great. A shorter uredial latent period would presumably favor increased aggressiveness. The total time from inoculation to the appearance of telia as well as the time from the first uredium eruption until the appearance of telia was much longer for the asexual isolates than for the sexual isolates. Since the sexual population was dependent on the formation of telia for survival over the winter, early formation of telia would be expected to confer an advantage, and selection for this trait has apparently taken place. Within each population, the isolates showed significant ($P = 0.01$) differences in mean values for each of the traits of aggressiveness except uredial latent period, telial latent period, and the difference between these latent periods. Variability for these traits within each population could not be estimated due to the discrete data structure, several zero values in the different categories of the log linear analysis (2), and the relatively low number of replications. It must be noted that uredial length, uredial width, uredial density (primary leaf), uredial density

TABLE 1. Population means for traits of aggressiveness of sexual and asexual populations of *Puccinia coronata* measured in the growth chamber

Trait	Unit of measurement	Mean of asexual population	Mean of sexual population[a]
Uredial length	mm	1.86	1.94*
Uredial width	mm	0.60	0.76
Uredial area	mm^2	0.87	1.15*
Uredial density (primary leaf)	uredia/mm^2	2.95	3.23
Uredial density (secondary leaf)	uredia/mm^2	1.89	2.16
Uredial density (primary and secondary leaves)	uredia/mm^2	2.23	2.54
Spore weight/uredium	mg	0.73	1.19*
Spore weight/uredial area	mg/mm^2	112.45	156.33*
Spore weight/uredial size	mg/mm^2	560.19	814.64*
Uredial latent period	days	7.32	7.10*[b]
Telial latent period	days	28.72	20.68*[b]

[a] Asterisk (*) indicates significant difference as measured by an *F* test, $P = 0.05$.

[b] Means compared by log-linear analysis (2).

(secondary leaf), spore weight per uredium, and uredial latent period were truly independent variables. These were then used to calculate the remaining five traits whose values were dependent upon the independent variables.

The phenotypic variances of each of the traits were compared between populations with an F statistic (*unpublished*). The phenotypic variances for most of the traits in the sexual population were appreciably higher than the corresponding variances of the asexual population. The only exceptions to this trend were the variances for uredial density on the primary leaves and for spore weight per uredium. For these two cases the values for the asexual population were slightly higher than those for the sexual population. Even though there was a trend toward greater variance in the sexual population, comparisons of the variances failed to reach the $P = 0.05$ level of significance for any trait.

When the genetic variances within a population were considered, all traits exhibited values significantly greater than 0 ($P = 0.05$) (*unpublished*). When the genetic variances of traits of the asexual population were compared with corresponding variances of the sexual population, the trend toward a greater variability in the sexual population was less evident than with the phenotypic variances. For example, while the uredial dimension and uredial density traits exhibited a trend of greater variation in the sexual population, variability of spore weights was generally greater in the asexual population. As with the phenotypic variances, the genetic variances of traits were not significantly different in the two populations. In addition to the traits of aggressiveness, population differences for identified virulence loci were compared. Roughly equal numbers of virulence loci were detected between the two populations, but a greater number of distinct virulence phenotype combinations were observed in the sexual population (17 sexual vs 7 asexual), presumably a result of recombination of the virulence genes. This trend is consistent with a previous study of the two populations (23). Although valid tests for independence of virulence and aggressiveness genes could not be conducted, the number of identified virulence genes and the level of aggressiveness were poorly correlated, with r values ranging from -0.20 to 0.30 for the different traits.

Associations between the various traits would suggest that the components of aggressiveness are not independent. Phenotypic correlation coefficients between traits within populations (19) were calculated and showed that certain trait associations existed within each population. For example, uredial latent period was positively correlated with telial latent period ($r = 0.83$*) and with the difference between these latent periods ($r = 0.82$*) in the asexual population; no such correlations ($r = 0.09$, $r = 0.05$, respectively) were detected in the sexual population. Uredial density (primary and secondary leaves) exhibited a positive association with the difference between telial and uredial latent periods ($r = 0.72$**) in the asexual population, but the association ($r = 0.44$) did not hold in the sexual population. High levels of gene recombination that break up certain gene complexes may help explain the lack of associations in the sexual population, but appropriate gene markers were not available to test the hypothesis. In general, spore weight traits were negatively correlated with the other traits in both populations, probably due in part to the relatively high inoculum concentrations.

TABLE 2. Estimated heritability values from the component analyses of variance for traits of aggressiveness in sexual and asexual populations of *Puccinia coronata*

Trait	Heritability estimates (%)			
	Asexual population	C.L.[a]	Sexual population	C.L.[a]
Uredial length	65	18–69%	86	50–87
Uredial width	83	42–83%	80	59–81
Uredial area	79	35–80%	75	47–85
Uredial density (primary leaf)	96	82–96%	98	88–98
Uredial density (secondary leaf)	92	65–92%	90	62–91
Uredial density (pri. + sec. leaf)	95	77–95%	98	88–98
Spore weight/uredium	90	58–91%	81	39–82
Spore weight/total uredial area	86	50–87%	82	40–83
Spore weight/uredial size	93	70–93%	92	64–93

[a] Confidence limits ($P = 0.05$).

Heritability estimates and their 95% confidence limits for all traits except uredial latent period and those involving telia are shown in Table 2. The values of confidence limits for the various aggressiveness traits ranged from 18 to 96% in the asexual population and from 26 to 96% in the sexual population. There is a trend toward higher heritability values in the sexual than in the asexual population, but not all of the traits follow this trend and in any event the overlap of confidence intervals precludes the possibility of significant differences occurring between the two populations.

DISCUSSION

Gene recombination is thought to confer an immediate advantage by producing more variable progeny with some portion of the population exhibiting higher levels of fitness than would occur with asexual reproduction (16). A comparison between the two populations for phenotypic variances of traits of aggressiveness does reveal a general trend for higher levels of variation in favor of the sexual population. The differences in variability are not statistically significant, however, suggesting that sexual reproduction provides little advantage over asexual reproduction in producing a wider range of phenotypic variation for traits of aggressiveness. A comparison of genetic variances failed to detect any population differences and thus supports those conclusions obtained with phenotypic variation.

The lack of an advantage to sexual reproduction conforms most closely to the "Hill-Robertson Effect" (6,12,15). The "Hill-Robertson Effect" predicts that gene recombination is beneficial primarily in small populations (ie, populations smaller than the reciprocal of the mutation rate). In such populations chance events will generate linkage disequilibrium. Under these conditions change in gene frequency for a particular locus under a given selection pressure is reduced compared to the response observed when populations are large. This means that selection of individual genes in different genetic backgrounds will increase the variation in number of progeny produced and the amount of genetic drift. Gene recombination would have the effect of reestablishing a random association among the loci and result in an increase in the response to selection. Thus, the beneficial effects of recombination would be observed in small populations but not in large populations.

It is unlikely that the levels of phenotypic and genetic variation detected in the asexual population are due to the effects of migration or gene flow from the sexual population. If migration were extensive both populations would exhibit similar genetic make-up, under weak to moderate levels of selection (3, page 267). This is clearly not the case for those virulence loci that showed strong population differences for the number of distinct phenotypes.

Significantly higher mean values for several traits in the sexual population (Table 1) suggest that gene recombination confers an advantage by creating a greater range of variation through which natural selection acts to produce higher levels of aggressiveness on the cultivar Markton. If we assume, however, that the range of genotypic and phenotypic variation is similar for both populations, then it is likely that different selection pressures for the two populations would account for the differences in mean values. If this is true, the case for using the mean of all isolates to compare the variability between the two populations is weakened. Whether or not different selection pressures also affect the amount of genotypic and phenotypic variation is uncertain. Further studies with unselected markers could indicate the true level of genetic variation in the two populations.

Another important consideration, mentioned by Roelfs and

Groth (21), is whether the sample isolates are representative of the population. That is, could a different population structure be deduced from a larger number of isolates? Although the sample size was sufficient to detect significant mean population differences, additional studies are obviously needed to answer the question.

High heritability values for some traits suggest a potential for increased levels of fitness in both populations, but these estimates did not evaluate potential genotype × abiotic environment interactions, which could substantially reduce the level of heritability for these traits. Emara and Freake (4) have reported aggressiveness in *Ustilago hordei* to be sensitive to different environmental conditions, which resulted in relative low heritability estimates ($h^2 = 28\%$). Conclusions about the level of genetic control of aggressiveness in this and in previous studies (5,9,11) should therefore be extrapolated to other environments with caution.

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