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# Mating System in *Artocarpus heterophyllus* Lam.

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***ABSTRACT.*** *The mating system of Artocarpus heterophyllus Lam. was investigated in a naturalised population at the University of Peradeniya Experimental Station, Dodangolla, Sri Lanka. Embryos of ungerminated seeds were used for starch gel electrophoresis and three polymorphic loci from two enzyme systems were scored. The data revealed that the species is highly outcrossing ($t > 1$). An outcrossing rate greater than unity, and an excess of heterozygotes indicates departure from the mixed-mating model. This departure from the model was due to violation of the assumption that there is no selection between pollination and the time of seed sampling.*

## INTRODUCTION

The mating system of a species characterises how gametes unite to form the next generation of a population. The patterns of mating in a particular species play an important role in determining the level and pattern of genetic variation and structure of future generations (Clegg, 1980; Hamrick and Godt, 1989). Knowledge of the mating system may provide important information for sampling of parental trees to establish and manage breeding populations and in the design of genetic conservation strategies (Adams and Birkes, 1991; Hamrick *et al.*, 1991). Information on mating system is also of practical relevance to the use of open-pollinated seeds for reforestation, since selfing or related mating in many species may result in inbreeding depression (Griffin, 1990).

Our knowledge of the mating systems of tropical trees has been based largely on inference from observations of floral morphology and sexuality, pollinators and their foraging behaviour, and controlled pollination

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experiments. Such studies have shown a predominance of mechanisms that favour outcrossing in tropical trees (Bawa *et al.*, 1985). However, they provide little or no information on the relative amounts of selfing and outcrossing within an individual species. The development of genetic markers and genetic models of how mating occurs within a population have made possible detailed quantitative studies of plant mating systems (Brown *et al.*, 1985; Ritland, 1990; Ritland and Jain, 1981). Estimates of outcrossing rates from such studies indicate that most tropical trees have high levels of outcrossing, associated in some cases with small, but significant, levels of inbreeding (Boshier *et al.*, 1995; Murawski and Hamrick, 1991; O'Malley and Bawa, 1987).

*Artocarpus heterophyllus* Lam. (jackfruit) in the family Moraceae is an important fruit and timber tree in south and south-east Asia. This tetraploid species is used in homegardens, plantations and enrichment planting in natural forests (Soepadmo, 1991). Despite the importance of *A. heterophyllus*, little is known about its basic biology, and nothing is known about the mating system. In this paper isozyme analysis of progeny arrays was used to estimate outcrossing rates of *A. heterophyllus*.

## MATERIALS AND METHODS

### Seed collection and electrophoresis

Open-pollinated syncarps were collected from 30 trees in a naturalised population of *A. heterophyllus* at the University of Peradeniya Experimental Station, Dodangolla, Sri Lanka during the period April to July 1996. Fifty randomly selected seeds from each syncarp were transported to Oxford, and 30 seeds were randomly selected from each syncarp, to provide a total of 60 progeny per individual tree. The whole embryo was dissected from the ungerminated fresh seed, and enzymes were extracted by grinding the embryo with extraction buffer, and subjected to electrophoresis on 11% horizontal starch gels. Sixteen enzyme systems were assessed for activity (Appendix 1). However, only two enzyme systems, aspartate aminotransferase (AAT) and 6-phosphogluconate dehydrogenase (6-PGD), were used for the mating system analysis due to either monomorphic or inconsistent banding patterns in the other enzyme systems. Detailed recipes of the staining solutions and buffer systems used are given in Pushpakumara (1997).

Genetic interpretation of the isozyme bands was based on the known sub-unit structure of the specific enzyme, expected banding patterns for heterozygotes, and the reported ploidy level of the species (Weeden and Wendel, 1990; Wendel and Weeden, 1990). The enzyme loci were numbered from the anode, with the fastest migrating locus designated as 1, whilst the alleles inferred at each locus were identified by letters.

**Mating system analysis**

Data were analyzed using the MLTR (for disomic loci) and TETRAT (for tetrasomic loci; Ritland, 1990) computer programmes, which are based on the mixed-mating model (Brown *et al.*, 1985; Ritland and Jain, 1981). The mixed-mating model is based upon four assumptions: (1) each viable offspring is the result of a random outcross (with a probability of t) or self-fertilisation (probability 1-t); (2) allele frequencies in the pollen pool are homogeneous over space and time; (3) alleles at different loci segregate independently; (4) the genetic markers are not affected by selection or mutation between the time of pollination and that when the seeds or seedlings are sampled.

Population estimates of single locus ($t_s$) and multilocus ($t_m$) outcrossing rates, fixation index for maternal trees ($F_m$), pollen (p) and ovule (o) allele frequencies were all estimated, without pollen and ovule allele frequencies set as equal. Standard errors of the outcrossing rates and allelic frequencies in the pollen and ovule pools of each locus were estimated using 100 bootstraps (Ritland and Jain, 1981). With the limited number of polymorphic loci, the initial single and multilocus estimates for the disomic loci were close to the constrained value of t = 2.00. Therefore, data analysis of both disomic and tetrasomic loci was carried out on a variety of levels to try to obtain more reliable estimates: (a) only for individual trees where the maternal genotype could be predicted at both disomic loci by examination of the progeny array data; (b) for individual trees where the maternal genotype could be predicted from the progeny array data for at least one diploid locus; (c) for individual trees for which the individual tree multilocus outcrossing rate was less than 1.90; and (d) for all trees. Individual tree outcrossing rates ($t_{mf}$) were separately estimated with and without pollen and ovule frequencies set as equal, and holding p or $t_m$ constant. The fixation index of progeny ($F_p$) was estimated separately for individual loci using the following formula: $F_p = (H_e - H_o) / H_e$; where $H_e$ and $H_o$ represent the expected number of heterozygous progeny under Hardy-Weinberg equilibrium, and the number of all genotypes observed to be heterozygous in the progeny, respectively. From progeny arrays for which the

maternal genotype could be determined from the progeny genotypes (only for 12 individual trees), a minimum detectable outcrossing rate ($t_{df}$) was estimated.

Statistical comparisons of outcrossing estimates and pollen and ovule allele frequencies were based on 95% confidence intervals. Population substructuring was studied by visually comparing the distribution of both maternal genotypes and the comparatively rare allele, *Aat-1c* in the study population. Deviations of progeny genotype frequencies from Hardy-Weinberg expectations for each polymorphic locus in the population were tested using a chi-square test, whilst independent segregation of the three loci was tested using a chi-square contingency table analysis. Where cells had too few observations, the genotypes were grouped, based on frequency and heterozygosity. For the *Aat-1* locus, two groups were made by combining the ac, bc and cd, and bb and dd genotypes, respectively, whilst for the *6Pgd-3* locus the aa and bb genotypes were grouped together. Since there were few observations of aaaa and aaab genotypes at the *6Pgd-1* locus, these data were excluded from the analysis.

## RESULTS

### Interpretation of isozyme bands

Two enzyme systems studied showed a variety of banding patterns, distinguished by both the position and relative staining intensity of the bands. AAT (dimeric enzyme) had two loci, *Aat-1* and *Aat-2* with overlapping mobilities. *Aat-1* was polymorphic with four allozymes, *Aat-1a, Aat-1b, Aat-1c* and *Aat-1d* (Figure 1). Variability was detected at *Aat-2*, but it was impossible to score, and thus was not included. *Aat-1* locus showed disomic inheritance.

Four loci, *6Pgd-1, 6Pgd-2, 6Pgd-3* and *6Pgd-4* were inferred for 6PGD (dimeric enzyme; Figure 2). *6Pgd-1* was polymorphic for two alleles and the genotypes at this locus were characterised by dosage effects showing both balanced and unbalanced heterozygotes (Figure 2). *6Pgd-2* was monomorphic for one allele, and completely overlapped with the *6Pgd-1a/6Pgd-1b* heterodimer. *6Pgd-3* was polymorphic for two alleles, whilst *6Pgd-4* was monomorphic for one allele that overlapped completely with the *6Pgd-3a/6Pgd-3b* heterodimer (Figure 2). Consequently, both *6Pgd-2* and *6Pgd-4* were excluded from the mating system analysis. Based on observation of the relative staining intensities of bands, it was postulated that *6Pgd-1* showed tetrasomic inheritance, whilst the *6Pgd-3* locus displayed disomic inheritance.

**Figure 1.** **Schematic representation of the isozyme banding patterns at AAT with their inferred genotypes in *A. heterophyllus.***
[Note: Genotypes aa, ab and cc at *Aat-1* locus were not found in the progeny arrays. N = 1530 seeds from 27 trees.]

## Outcrossing rates

Single ($t_s$) and multilocus ($t_m$) estimates of outcrossing rates for the population are presented in Table 1. *A. heterophyllus* was found to be highly outcrossing with $t_m$>1 at disomic loci, although the multilocus outcrossing rates varied with the level of analysis (Table 1). For each level of analysis, the mean single locus estimate was not significantly different (P≥0.05) from the multilocus estimates. The $t_s$ for the tetrasomic (*6Pgd-1*) locus was significantly lower (P<0.05) than the multilocus and mean single locus estimates for the disomic loci, but not significantly different from t = 1, except at analysis level (a) for which the estimate was significantly less than unity (Table 1). Apart from the maternal fixation index of the tetrasomic locus, the estimated fixation indices for both the adult population and the progeny arrays were negative, but generally constrained at F = -1.0 (Table 2).

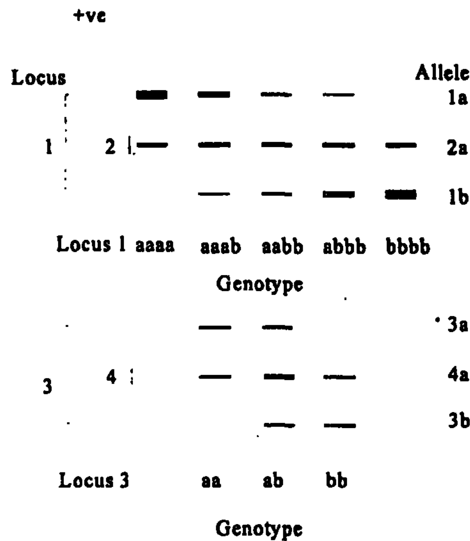


**Figure 2.** **Schematic representation of the isozyme banding patterns at 6PGD with their inferred genotypes in *A. heterophyllus*.** **[Note: Genotype aaaa at *6Pgd-1* locus was not found in the progeny arrays. N = 1268 seeds from 26 trees.]**

When individual tree multilocus outcrossing rates (ITMOR; $t_{mf}$) were estimated without constraining ovule and pollen allele frequencies to be equal, the ITMOR varied from 0.71 to 2.0. They were significantly heterogeneous ($P<0.05$), and none of the outcrossing rates was significantly below $t = 1$ ($P\geq0.05$). When the pollen allele frequency was held constant, the ITMOR changed significantly compared to previous values. Holding $t_{mf} = 1$ resulted in considerable variation in allele frequencies of the pollen sampled by individual trees. The mean individual tree minimum detectable outcrossing rate ($t_{df} = 0.42\pm0.03$) was significantly lower than $t = 1$ ($P<0.05$). The spatial distribution of maternal genotypes and the *Aat-1c* allele in the study population showed no evidence of genetic substructuring within the population. Allele frequencies in the pollen and ovule pool were significantly heterogeneous at all three loci (Table 3).

No evidence of association was found between any of the loci under study (Appendix 2), whilst a comparison of the genotypic frequencies in the progeny showed significant departures ($P<0.001$) from Hardy-Weinberg

equilibrium at all three loci (Appendix 3). For all three loci, an excess of heterozygote genotypes was evident; moreover, several expected genotypic classes were either absent or had comparatively few individuals (Appendix 2).

**Table 1. Estimates of single and multilocus outcrossing rates for *A. heterophyllus*.**

| Locus | Outcrossing rate (t) ± standard error<br>Level of analysis | | | |
|---|---|---|---|---|
| | (a) N = 4 | (b) N = 12 | (c) N = 13 | (d) N = 27 |
| **Disomic loci** | | | | |
| *Aat-1* | 1.14 ± 0.06 | 1.37 ± 0.16 | 1.44 ± 0.15 | 1.68 ± 0.12 |
| *6Pgd-3* | 1.99 ± 0.31 | 1.99 ± 0.22 | 1.99 ± 0.17 | 1.99 ± 0.19 |
| Mean single locus ($t_s$) * | 1.15 ± 0.30 | 1.39 ± 0.15 | 1.49 ± 0.16 | 1.83 ± 0.17 |
| Multilocus ($t_m$) * | 1.14 ± 0.32 | 1.29 ± 0.16 | 1.39 ± 0.18 | 1.85 ± 0.18 |
| **Tetrasomic locus** | | | | |
| *6Pgd-1* | 0.64 ± 0.11 | 0.83 ± 0.09 | 0.82 ± 0.15 | 1.17 ± 0.19 |

N represents the number of individual trees sampled, 60 seeds from two syncarps per tree, reduced in some trees by missing data. * Estimated from disomic loci.

## DISCUSSION

The results from this study are ultimately constrained by the small number of polymorphic loci available for use. Despite this, the results give some insight into the possible patterns of mating in the population of *A. heterophyllus* under study and clear indications of where future research should focus. All the multilocus outcrossing estimates were significantly greater than t = 1, although outcrossing rates theoretically range from 0 to 1. Such a problem has been reported by several authors (Brown *et al.*, 1985; Mandal and Ennos, 1995). The major causes suggested for these "biologically unreasonable" values (t>1) were sampling effects, non-assortative mating and the failure of the mixed-mating model to deal with multiple heterozygous maternal genotypes (Brown *et al.*, 1985; Clegg, 1980).

**Table 2. Estimates of fixation index for maternal and progeny genotypes of *A. heterophyllus*.**

| Locus | Fixation index (F) ± standard error | |
|---|---|---|
| | Maternal ($F_m$) | Progeny ($F_p$) |
| **Disomic loci** | | |
| *Aat-1* | -0.99 ± 0.01 ($N_1$ = 27) | -0.64 ($N_2$ = 1530) |
| *6Pgd-3* | -0.99 ± 0.01 ($N_1$ = 27) | -0.99 ($N_2$ = 1476) |
| Mean single locus ($t_s$) | -0.99 ± 0.01 ($N_1$ = 27) | - |
| Multilocus($t_m$) | -0.99 ± 0.01 ($N_1$ = 27) | - |
| **Tetrasomic locus** | | |
| *6Pgd-1* | 0.24 ± 0.03 ($N_1$ = 26) | -0.17 ($N_2$ = 1268) |

$N_1$ and $N_2$ represent the sample size of individual trees and seeds, respectively.

The mixed-mating model used for this study subdivides all mating events into those resulting from inbreeding and those produced by outcrossing to a random sample of the population (Brown *et al.*, 1985). Whilst estimates are susceptible to departures from the model's assumptions, the nature of the departures can provide an indication of how mating occurs in the population. There are a number of possible causes for departures from the assumptions of the mixed-mating model in *A. heterophyllus*. Firstly, correlated mating, owing to fewer actual pollen donors than the total set of potential fathers, and biased allelic frequencies in the pollen pool due to non-random sampling of pollen donors, may have violated assumptions (1) and (2), respectively, of the model. Correlated mating may occur when a relatively low number of individuals release pollen during the receptive period of female inflorescence. The syncarps from the population were collected from April to July 1996, and are likely to have been produced during a flowering season from February to May 1996. No flowering phenology data were available for this period; however, the 1993/4 and 1994/5 data showed high levels of flowering synchrony during the corresponding period of February to May (Pushpakumara, 1997), suggesting that phenology is unlikely to have been a constraint to random mating. Correlated mating may also occur when individuals in the population are genetically substructured (Boshier *et al.*, 1995), although there was no

evidence of such genetic substructuring in this study. The lack of a significant difference between the multi- and single locus outcrossing estimates here also provides no evidence of biparental inbreeding in the population. Thus, for this population of *A. heterophyllus* violations of assumptions (1) and (2) seems unlikely.

Secondly, disassortative mating which leads to an excess of heterozygosity as observed here, may be another cause of departures from assumption (1) in the mixed-mating model. There are no other data on this process from this study, and no reason to suggest that mating is biased in this way. Thirdly, segregation of alleles at a locus may be linked to other loci under study, violating assumption (3) in the mixed-mating model. This also seems unlikely in this study since chi-square contingency tables showed no association between genotypes of the three loci.

**Table 3. Ovule and pollen allele frequency estimates and associated standard errors (s.e.) for *A. heterophyllus*.**

| Locus | Allele | Sample size | Ovule (o± s.e.) | Pollen (p ± s.e.) |
|---|---|---|---|---|
| **Disomic loci** | | | | |
| *Aat-1* | a | 27 (1530) | 0.13 ± 0.04 | 0.18 ± 0.03 |
| | b | | 0.36 ± 0.04 | 0.30 ± 0.05 |
| | c | | 0.02 ± 0.00 | 0.01 ± 0.01 |
| | d | | 0.49 ± 0.00 | 0.51 ± 0.06 |
| *6Pgd-3* | a | 27 (1476) | 0.50 ± 0.00 | 0.37 ± 0.11 |
| | b | | 0.50 ± 0.00 | 0.63 ± 0.11 |
| **Tetrasomic locus** | | | | |
| *6Pgd-1* | a | 26 (1268) | 0.54 ± 0.05 | 0.17 ± 0.08 |
| | b | | 0.46 ± 0.05 | 0.83 ± 0.08 |

Sample sizes given as the number of individual maternal trees followed by the total number of progeny scored in parenthesis.

The evidence for the abortion of apparently fertilised ovules during syncarp development (Pushpakumara, 1997), with the potential for selection of certain genotypes prior to the time of seed sampling, is a clear indication of a failure in the assumption (4) of the absence of selection. If genotype frequencies of an adult population are determined solely by the mating system of the species, progeny and adult arrays should have the same fixation index.

Fixation index estimates of the disomic loci were constrained in this study, and not particularly informative. However, the tetrasomic maternal fixation index is higher than the progeny estimate due to an increase in heterozygosity in the progeny, and suggesting that factors other than mating system, such as selection or inbreeding depression, act during the development of ovules to seeds. It seems unlikely that the excess heterozygosity observed in this study was due solely to inbreeding depression, because selfed inflorescences produced similar levels of seed-set, seed weight and germination rate as those which were outcrossed (Pushpakumara, 1997). Thus, abortion of ovules may be a function of competition among embryos for resources rather than an expression of inbreeding depression.

The observation of variation in staining intensities with both balanced and unbalanced heterozygotes at the *6Pgd-1* locus may provide evidence of tetrasomic inheritance in *A. heterophyllus*, since they are considered as evidence of polyploidy in electrophoretic studies (Weeden and Wendel, 1990). The presence of both balanced and unbalanced heterozygotes, increased heterozygosity, and polysomic inheritance are associated with autopolyploidy (Soltis and Soltis, 1990; Weeden and Wendel, 1990). Thus, the results of this study agree with the suggestion that *A. heterophyllus* is an autotetraploid species (Soepadmo, 1991), although identification of the nature of polyploidy is not simple. In this study, however, disomic inheritance adequately explained the banding patterns at the *Aat-1* and *6Pgd-3* loci, consistent with the suggestion that polyploids tend to revert to a diploid level of gene expression. This phenomenon is common in ancient polyploids, where they undergo diploidisation by silencing of redundant genes, leading to loss of the polyploid expression of enzymes (Soltis and Soltis, 1990).

The results of this study suggest that *A. heterophyllus* has a high outcrossing rate. Although mating system analysis was constrained for the reasons given above, and the single locus outcrossing estimates are not precise, other than for the analysis level (a) the single locus outcrossing estimates for the tetrasomic locus were not statistically different from $t = 1$. It is, however, important to note that the analysis level (a) is not particularly useful, as too few families were used. The outcrossing nature of the species is also supported by the estimate of the minimum detectable outcrossing rate from this study. Mating system parameters have not previously been reported for *A. heterophyllus*. Whilst acknowledging the limitations and difficulties of this study, it provides strong evidence of outcrossing in *A. heterophyllus*, consistent with the results of other studies of the mating system of tropical trees (Murawski *et al.*, 1994; Murawski and Hamrick, 1991). However, it is

important to note that individual female flowers of *A. heterophyllus* have a single stigma for pollination (Pushpakumara, 1997) and this is the first report describing the mating system of an angiosperm species with multi-ovulated inflorescences where each ovary has its own stigma, providing opportunity to study resource competition within a syncarp among individual flowers.

## CONCLUSIONS

The evidence from the characterisation of the mating system in *A. heterophyllus* suggests that high levels of outcrossing can be maintained through post-zygotic selection. However, the question why some genotypes are favoured remains unresolved, although the reported results here may imply that both genotype and resource (space) availability are relevant to the successful development of ovules in *A. heterophyllus*.

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## APPENDICES

**Appendix 1. Enzyme systems screened in the mating system study of *A. heterophyllus.***

6-phosphogluconate dehydrogenase (E.C. 1.1.1.44); acid phosphatase (E.C. 3.1.3.2); aconitase (E.C. 4.2.1.3); alcohol dehydrogenase (E.C. 1.1.1.1); aldolase (E.C. 4.1.2.13); aspartate aminotransferase (E.C. 2.6.1.1); αβ esterase (E.C. 3.1.1.-); glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49); glutamate dehydrogenase (E.C. 1.4.1.2); glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.1.1.12); isocitric dehydrogenase (E.C. 1.1.1.42); malate dehydrogenase (E.C. 1.1.1.37); peroxidase (E.C. 1.11.1.7); phosphoglucose isomerase (E.C. 5.3.1.9); phosphoglucose mutase (E.C. 2.7.5.1); shikimate dehydrogenase (E.C. 1.1.1.25).

**Appendix 2. Contingency table analysis of independence for *Aat-1*, *6Pgd-1* and *6Pgd-3* genotype frequencies in *Artocarpus heterophyllus.***

| Locus and genotypes | | *Aat-1* | | | | |
|---|---|---|---|---|---|---|
| *6Pgd-1* | *6Pgd-3* | ac/bc/cd | aa/bb/dd | ad | bd | Total |
| aabb | aa/bb | 2 | 3 | 38 | 83 | 126 |
| | ab | 6 | 13 | 130 | 244 | 393 |
| abbb | aa/bb | 1 | 2 | 45 | 60 | 108 |
| | ab | 12 | 14 | 134 | 268 | 428 |
| bbbb | aa/bb | 0 | 2 | 6 | 8 | 16 |
| | ab | 0 | 2 | 14 | 24 | 40 |
| Total | | 21 | 36 | 367 | 687 | 1111 |

See materials and methods for grouping genotypes. $\chi^2 = 3.8$; $P = 0.15$; degrees of freedom = 2 for *6Pgd-1* and *6Pgd-3* loci; $\chi^2 = 1.8$; $P = 0.63$; degrees of freedom = 6 for *Aat-1* and *6Pgd-1* loci; $\chi^2 = 5.6$; $P = 0.48$; degrees of freedom = 3 for *Aat-1* and *6Pgd-3* loci.

**Appendix 3. Observed and expected genotype frequencies and chi-square test for departure from Hardy-Weinberg equilibrium for offspring at the *Aat-1*, *6Pgd-1* and *6Pgd-3* loci in *A. heterophyllus*.**

***Aat-1* locus**

| Geno | aa | ab | ac | ad | bb | bc | bd | cc | cd | dd | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Obs. | 0 | 0 | 13 | 503 | 10 | 4 | 952 | 0 | 14 | 49 | 1545 |
| Exp. | 43.1 | 163.1 | 5.2 | 261.6 | 154 | 9.8 | 495.1 | 0.3 | 15.7 | 379.1 | 1545 |

Geno. = genotypes; Obs. = number observed; Exp. = Number expected; $\chi^2 = 1306$; $P < 0.001$; degrees of freedom = 9.

***6Pgd-1* locus**

| Geno. | aaaa | aaab | aabb | abbb | bbbb | Total |
|---|---|---|---|---|---|---|
| Obs. | 0 | 2 | 587 | 612 | 67 | 1268 |
| Exp. | 19.8 | 144.6 | 396.5 | 484.5 | 222.6 | 1268 |

$\chi^2 = 394$; $P < 0.001$; degrees of freedom = 1.

***6Pgd-3* Locus**

| Geno. | aa | ab | bb | Total |
|---|---|---|---|---|
| Obs. | 22 | 1173 | 277 | 1472 |
| Exp. | 251 | 714 | 507 | 1472 |

$\chi^2 = 608$; $P < 0.001$; degrees of freedom = 1.