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Allozyme Uniformity within and Variation between Races of the Colonizing Species *Xanthium strumarium* L. (Noogoora Burr)

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Abstract

The degree of polymorphism at 13 enzymic loci was tested in 12 populations of *Xanthium strumarium* representing all four naturalized races of this species in Australia. The races are *X. chinense*, *X. italicum*, *X. pennsylvanicum* and *X. cavanillesii*. Very little variation was found within races of *X. strumarium* at the loci studied. In marked contrast to this lack of intraracial variation there was considerable interracial genetic differentiation at several loci. It is concluded that genetic polymorphisms and hence populational buffering, at least at the enzyme level, has been of little significance in the adaptation of this species during its colonization of Australian environments. The observed outcrossing rate in *X. italicum* was zero (inestimable in the other races) with an upper confidence limit of 10–12%, confirming previous observations that *X. strumarium* is predominately self-pollinated.

Introduction

Colonizing plant species often occupy variable and fluctuating environments. Therefore, such species must have considerable flexibility if they are to maintain their adaptation to each of the environments they encounter. There are two obvious mechanisms by which a plant species population can achieve the flexibility necessary to adjust to the heterogeneity of natural environments (Thoday 1953; Lewontin 1957; Allard and Bradshaw 1964; Bradshaw 1965). The first is *individual buffering* whereby individuals within a population can adjust their phenotypic responses so that each member of the population can cope with a wide range of environments. An important component of individual buffering, particularly in colonizing plant species, is *phenotypic plasticity* or environment-induced variation in morphological and growth characteristics (Bradshaw 1965; Harper 1967; Marshall and Jain 1968). The second is *populational buffering* which refers to buffering above and beyond that attributable to individual buffering, and which results from the co-existence of different genotypes, each adapted to somewhat different environments, in the one population (Allard and Bradshaw 1964).

The present study is concerned with the ecogenetics of *Xanthium strumarium* L., a predominantly self-pollinated monoecious annual (Love and Dansereau 1959), in Australia. *X. strumarium* can be divided into eight complexes or races on the basis of burr morphology and four of these races (*X. chinense*, *X. italicum*, *X. cavanillesii* and *X. pennsylvanicum*) have been introduced into different parts of Australia from the New World. Since they were introduced at different points on the Australian mainland, since the species is predominantly self-fertilized, and since they have different photoperiodic requirements for flowering (McMillan 1975), the four races rarely hybridize under field conditions and have maintained their genetic integrity since their introduction.

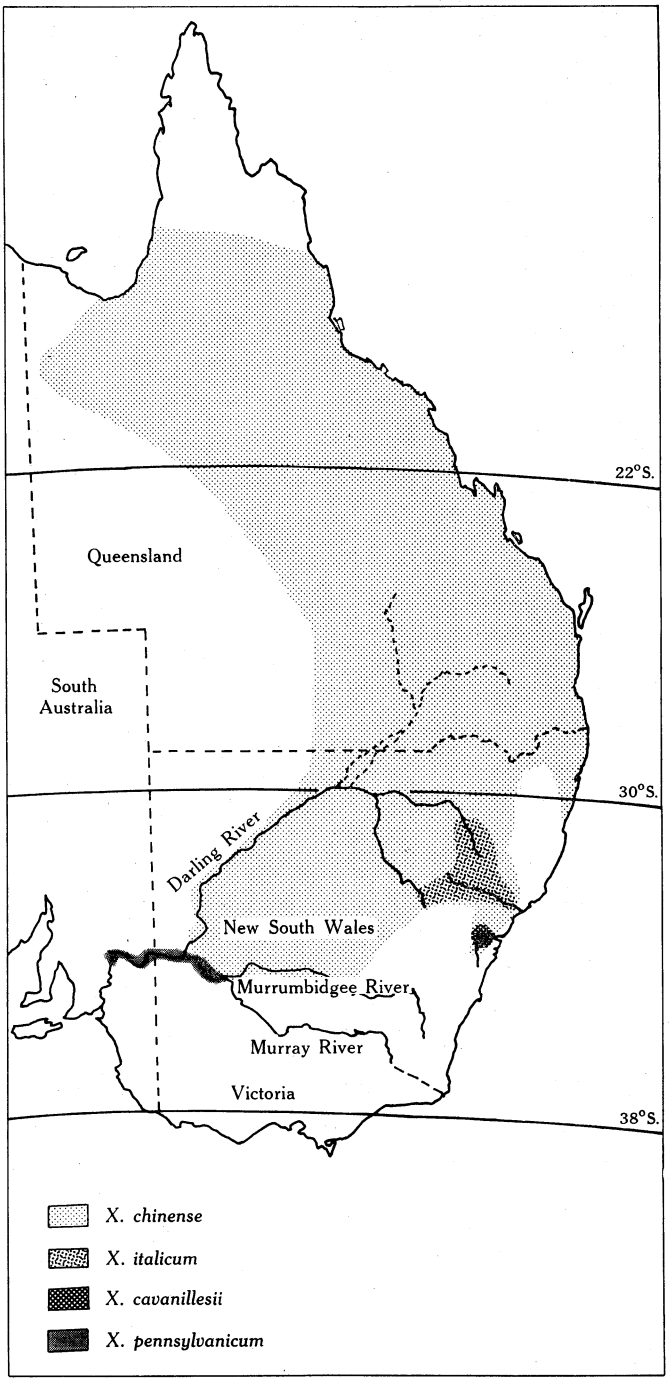

Fig. 1. Distribution of the four races of *X. strumarium* in Australia.

All races occupy similar, highly unstable and unpredictable habitats, namely river banks and associated flood plains and less often, man-disturbed habitats, and between them they have very successfully colonized large regions of Australia. However, from their current distribution in Australia (Fig. 1) it is clear that the races of *X. strumarium* differ dramatically in their colonizing ability. We have sought to determine whether these differences are due to differences in individual buffering (phenotypic plasticity) or populational buffering (genetic polymorphism) among the racial groups of *X. strumarium*. As a first step in this analysis we estimated the levels of allozyme variation within and between races of *X. strumarium* and we report these estimates here.

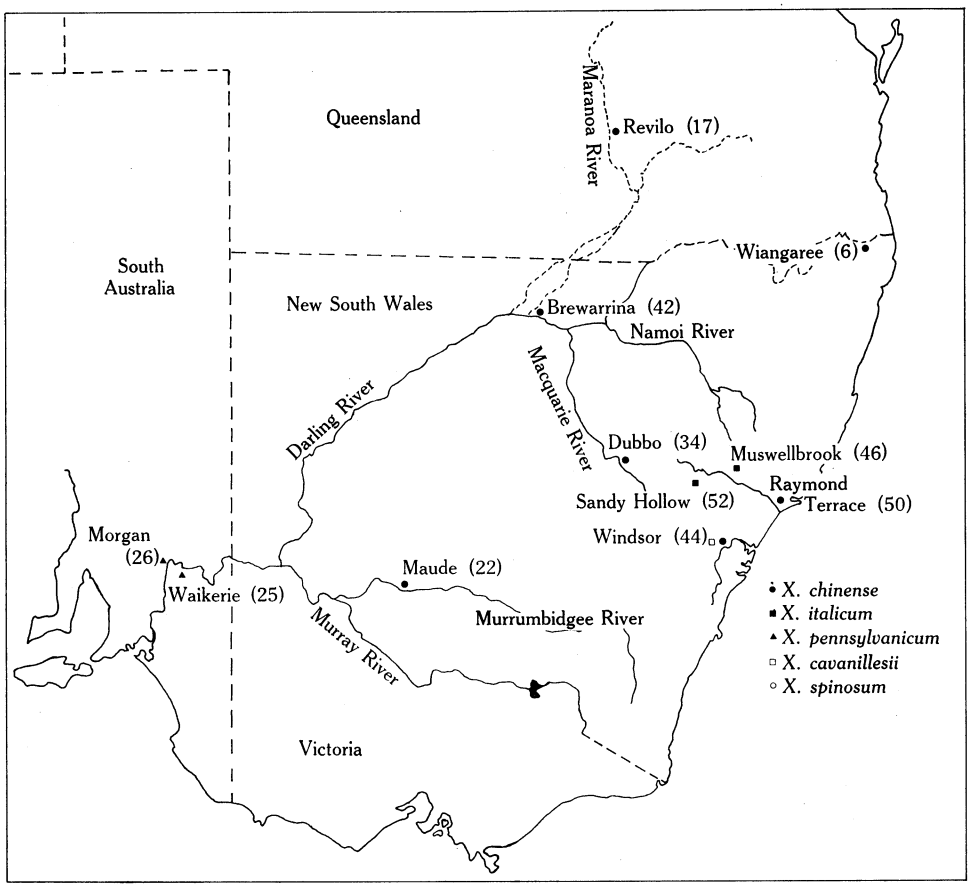

Fig. 2. Distribution of *X. strumarium* populations surveyed for allozyme variation in south-eastern Australia. The numbers given to the populations are shown in parentheses.

Materials and Methods

Samples were collected and analysed from a total of 12 populations of *X. strumarium* in south-eastern Australia. The distribution of the sites and races sampled is shown in Fig. 2. Where possible at least 10 fruits were collected from each plant and 50–150 randomly chosen plants were sampled from each population.

Allozymic variation in seven assay systems encoded by 13 genetic loci was analysed in over 1200 individual plants of the four races of *X. strumarium*. Extracts for electrophoresis were prepared by grinding single whole seeds in a mortar and pestle in 0·4 ml of 0·1 M phosphate buffer, pH 7·0,

containing dithiothreitol at 1 mg/ml. Three electrophoretic systems were used: (1) 13% starch gels in tris–citrate–lithium hydroxide–borate buffer (Brewbaker *et al.* 1968), (2) 13% starch gels in 5 mM histidine buffer, pH 8·0 (Brewer and Sing 1970), and (3) 7% acrylamide gels in tris–citrate buffer, pH 8·7 (Brewbaker *et al.* 1968).

Glutamate–oxalate transaminase (aspartate aminotransferase, EC 2.6.1.1) (*Got*), leucine aminopeptidase [aminopeptidase (cytosol), EC 3.4.11.1) (*Lap*), alcohol dehydrogenase (EC 1.1.1.1) (*Adh*) and glutamate dehydrogenase (EC 1.4.1.2) (*Gdh*) were detected on lithium hydroxide gels, malate dehydrogenase (EC 1.1.1.37) (*Mdh*) and acid phosphatase (EC 3.1.3.2) (*Aph*) were demonstrated on histidine gels, and esterases (EC 3.1.) (*Est*) were detected on the acrylamide gels. The staining procedures were identical to those described by Brewbaker *et al.* (1968) and Shaw and Prasad (1970). Genetic analyses of the allozyme variants showed simple codominant inheritance of alleles at all the variable loci (Moran 1975).

In surveying the populations for allozyme variants a two-stage sampling procedure was used. In the first round of sampling two progeny from each field plant were assayed. If one or both of these were heterozygous at any locus, a further sample of progeny was assayed to determine the genotype of the maternal parent at that locus to 95% certainty. This double sampling procedure is optimally efficient for the estimation of genetic parameters in predominantly inbreeding species (Brown *et al.* 1974; Brown 1975).

Results

Allelic frequencies at the 13 loci assayed in the 12 populations of *X. strumarium* are given in Table 1. In scoring the gels the locus specifying the allele with the fastest anodal migration was designated 1, the next 2, etc. Alleles at each locus were designated F (fast), S (slow) or N (normal or most common allele). In Table 1 dashes indicate that the allele or locus was not detectable in the population, whereas blank spaces indicate that the population was not scored for that locus.

Within-race Variation

The most striking feature of these data is the extremely low level of intraracial polymorphism in *X. strumarium*. *X. italicum* was polymorphic at the *Est*-4 locus in two populations surveyed and for *Got*-3 in one population. The other three races, namely *X. chinense*, *X. pennsylvanicum* and *X. cavanillesii*, were completely monomorphic—within a race all individuals were homozygous for the same electrophoretic allele at all loci. These races appear to be represented in Australia by a single genotype at these loci.

The genotypic frequencies at the polymorphic *Est*-4 and *Got*-3 loci in *X. italicum* are given in Table 2. The low levels of heterozygosity observed at these loci support the contention that *X. italicum*, despite its monoecious breeding system, is predominantly self-pollinated. All open-pollinated progenies which contained a heterozygote on the first screening of these populations proved on further analysis to be derived from heterozygous parental plants in the field. No true outcrosses were detected at either of the polymorphic loci in *X. italicum*. Therefore, a direct estimate of the outcrossing rate in *Xanthium* from these data is zero. We can assign an upper confidence limit to the outcrossing rate, t_m, by calculating how large the level of outcrossing would have to be to observe no outcrosses among the number of progeny sampled 5% of the time (Fisher and Yates 1957). The estimates of t_m calculated in this manner (Table 2) varied from 10 to 12% with a mean of 11%. Table 2 also gives estimates of Wright's fixation index, $\hat{F}$, where

$$\hat{F} = 1 - \hat{R}/2\hat{q}(1-\hat{q}),$$

where $\hat{R}$ is the observed heterozygosity and $\hat{q}$ is the observed frequency of the most

Table 1. Gene frequencies at 13 loci in *X. strumarium*

Site	Sample size	*Est*-1 N	*Est*-2 N	*Est*-4 F	*Est*-4 N	*Est*-4 S	*Aph*-1 N	*Gdh*-1 N	*Mdh*-1 N	*Mdh*-2 N	*Adh*-1 N	*Adh*-2 N	*Adh*-2 S	*Lap*-1 N	*Lap*-1 S	*Got*-1 N	*Got*-2 N	*Got*-3 N	*Got*-3 S
									(*a*) *X. pennsylvanicum*										
25 Waikerie	66	1·0	1·0	—	—	1·0	1·0	1·0	1·0	1·0	—	1·0	—	—	1·0	1·0	1·0	1·0	—
26 Morgan	128	1·0	1·0	—	—	1·0	1·0	1·0	1·0	1·0	—	1·0	—	—	1·0	1·0	1·0	1·0	—
									(*b*) *X. italicum*										
46 Muswellbrook	146	1·0	1·0	0·85	0·13	0·02		1·0	1·0	1·0	—	1·0	—	1·0	—	1·0	1·0	0·98	0·02
52 Sandy Hollow	78	1·0	1·0	0·79	0·21	—		1·0			—	1·0	—			1·0	1·0	1·0	—
									(*c*) *X. chinense*										
6 Wiangaree	59	1·0	1·0	1·0	—	—	1·0	1·0			1·0	—	1·0	1·0	—	1·0	1·0	1·0	—
11 Oxford	168	1·0	1·0	1·0	—	—	1·0	1·0			1·0	—	1·0	1·0	—	1·0	1·0	1·0	—
17 Revilo	124	1·0	1·0	1·0	—	—		1·0	1·0	1·0	1·0	—	1·0	1·0	—	1·0	1·0	1·0	—
22 Maude	126	1·0	1·0	1·0	—	—	1·0	1·0			1·0	—	1·0	1·0	—	1·0	1·0	1·0	—
34 Dubbo	177	1·0	1·0	1·0	—	—	1·0	1·0			1·0	—	1·0	1·0	—	1·0	1·0	1·0	—
42 Brewarrina	176	1·0	1·0	1·0	—	—	1·0	1·0			1·0	—	1·0	1·0	—	1·0	1·0	1·0	—
50 Raymond Terrace	115	1·0	1·0					1·0						1·0	—	1·0	1·0	1·0	—
									(*d*) *X. cavanillesii*										
44 Windsor	62	1·0	1·0	—	—	—[A]	1·0	1·0	1·0	1·0	1·0	1·0	—	1·0	—	1·0	1·0	—	1·0

[A] *Est*-4 could not be detected in *X. cavanillesii*.

common allele. Table 2 also gives the expected equilibrium values of F assuming no selection,

$$F_e = (1-t)/(1+t),$$

and assuming outcrossing occurred at the estimated maximum rate, i.e. $t = t_m$. Comparison of the observed and expected values of the fixation index (F) indicates that the observed levels of heterozygosity are close to the confidence limit considering the breeding system of *X. strumarium*.

Table 2. Estimates of genotypic frequencies, maximum outcrossing rates and fixation indices in polymorphic populations of *X. italicum*

Population	Locus	FF	FN	NN	NS	SS	$\hat{t}_m$ [A]	$\hat{F}$ [B]	F_e [C]
46	*Est*-4	0·82	0·06	0·10	—	0·02	0·10	0·79	0·82
52	*Est*-4	0·77	0·05	0·18	—	—	0·12	0·83	0·78
46	*Got*-3	—	—	0·97	0·02	0·01	0·12	0·49	0·78

[A] See text for a description of the estimation procedure for t_m.
[B] $\hat{F} = 1-\hat{R}/2\hat{q}(1-\hat{q})$; $\hat{R}$ = observed level of heterozygosity; $\hat{q}$ = frequency of the most common allele.
[C] $F_e = (1-\hat{t}_m)/(1+\hat{t}_m)$.

Between-race Variation

Despite the remarkable lack of intraracial allozyme variation in *X. strumarium*, there were genetic differences between races. At several of the loci (e.g. *Est*-4, *Lap*-1, *Got*-3, *Adh*-2) some races are fixed for one allele, while others are fixed for alternate alleles. Thus, in *X. pennsylvanicum*, all plants are homozygous for the S allele at the *Lap*-1 locus while the other three races are fixed for the N allele. Similarly, *X. cavanillesii* is fixed for the S allele at *Got*-3 while the other races are homozygous

Table 3. Estimates of the normalized identity of genes (I) and genetic distance (D) among the four races of *X. strumarium* (after Nei 1972)

The values above the diagonal are estimates of D, those below the diagonal are estimates of I

Race	*X. pennsylvanicum*	*X. cavanillesii*	*X. chinense*	*X. italicum*
X. pennsylvanicum	—	0·33	0·33	0·183
X. cavanillesii	0·72	—	0·26	0·23
X. chinense	0·72	0·77	—	0·14
X. italicum	0·83	0·80	0·87	—

(except for the low level of polymorphism in population 46) for the N allele. Considering all races as part of *X. strumarium* in Australia, the proportion of polymorphic loci is 0·31. However, this value has little populational meaning—with the present distribution of the four races on the mainland and their different short-day photoperiodic requirements (McMillan 1975), only *X. italicum* and *X. chinense* can possibly hybridize in the field.

To measure the relative genetic similarity of the four races of *X. strumarium* we have employed Nei's (1972) indices of genetic distance (D) and normalized identity of genes (I). The calculated coefficients are based on allele frequencies at both

polymorphic and monomorphic loci (Table 3). These estimates show that although the genetic identity between races is quite high there are differences in genetic distances among races. *X. chinense* and *X. italicum* would appear to be the most closely related on the basis of the seed isozymes used in this study.

Discussion

The results of the present study contrast sharply with the results of the majority of previous electrophoretic surveys of soluble proteins which have shown that most species populations of plants and animals contain remarkable stores of genetic variation (see reviews in Allard and Kahler 1972; Harris and Hopkins 1972; Lewontin 1974; Selander 1976). They provide additional support for the suggestion of Jain *et al.* (1970) that a wide range of population structures is encountered in predominantly inbreeding plant species. Populations monomorphic, or nearly so, for all loci, such as those found in *X. strumarium*, represent one extreme, while populations in which no two individuals appear to be genetically similar, such as those of *Avena fatua* in California (Imam and Allard 1965; Jain and Marshall 1967), represent the other extreme.

There is no evidence to suggest that an unusual breeding system, for example apomixis as in *Taraxacum officinale* (Solbrig 1971), or a rare chromosome system, for example permanent translocation heterozygosity as in *Oenothera biennis* (Levin 1975*b*), is responsible for the maintenance of the low levels of allozyme variation within and between races of *X. strumarium*. Two main hypotheses can therefore be erected to account for the lack of allozyme variation in Australian populations of *X. strumarium*. The first is that populations of *X. strumarium* normally contain little variation even in those areas of the New World where the species is indigenous and that the introductions into Australia represent the ancestral situation. The second hypothesis is that populations of *X. strumarium* are normally much more variable in the New World but only a fraction of this variation was introduced into Australia (founder effect) or, if this variability was introduced, it was lost via drift or natural selection during the early colonizing period of the species in Australia.

At present we favour the first hypothesis on the grounds that, if populations of *X. strumarium* are indeed highly polymorphic in the New World, it is unlikely that the same, or very similar, genotypes of each race would have been introduced into Australia from four different areas and on four different occasions by chance alone. Further, given the wide distribution of *X. strumarium* in Australia and the broad range of environments it occupies, it also seems improbable that, if polymorphism is an important mode of adaptation in this species, natural selection would favour the same or similar genotypes over its entire range of distribution. Nevertheless, critical studies on the population structure of *X. strumarium* in its indigenous habitats are obviously required to verify or falsify this viewpoint.

However, regardless of the reason for the poverty of allozyme variation in Australian populations of *X. strumarium*, it is clear that allozyme polymorphism has played only a minor role in the adaptation of this species to Australian environments. It is also clear that the differences in colonizing ability of the four races cannot be attributed to differences in levels of genetic variation among the races and, hence, to differences in populational buffering, at least at the enzyme level. We can only surmise, therefore, that the differences in colonizing ability among the races of *X. strumarium* in Australia are due either to differences in the levels of within-race genetic variation for quantitative,

morphological and physiological traits, in which case we would be forced to conclude that there is little relationship between the level of allozyme variation and adaptive variation for quantitative characters in *X. strumarium*, or to differences in the level of individual buffering. Indeed the enzyme data do point to some genetic differentiation between the races and such fixation of different genes may be necessary to account for different levels of individual buffering. Estimates of phenotypic plasticity, an important component of individual buffering, and quantitative variability in *X. strumarium* will be reported in the second paper of this series.

Finally, it should be emphasized that our results have important practical implications with respect to the biological control of *X. strumarium* in Australia. Past experience suggests that biological control programs against introduced weeds and pests are more likely to be successful if the organism to be controlled is relatively uniform genetically [e.g. *Opuntia* sp. in Australia and *Hypericum* sp. in Western U.S.A. (Levin 1975*a*)]. In the light of our findings the possibility of controlling *X. strumarium* biologically would appear to merit serious consideration.

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