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Ecological genetics of breakdown in tristyly

1. INTRODUCTION

Tristyly is a genetic polymorphism in which plant populations contain three mating groups (L, M, S) differing principally in style length, anther height, pollen size and incompatibility behaviour. The polymorphism is reliably reported from eight genera in three Angiosperm families: Lythraceae, Oxalidaceae and Pontederiaceae (Ganders 1979). Since the families are unrelated, it seems probable that the origin of this complex outbreeding system is polyphyletic and the similarities in sub-characters of the syndrome the product of convergent evolution. Little is known about the evolution of tristyly and species groups exhibiting stages in the build-up of the polymorphism have not been found. The genetic control of tristyly is similar in the three families and is based on a two-locus system *(S, M)*. Variations in linkage relationships, epistasis and polyploidy result in different patterns of inheritance within and among the three families (Fisher & Mather 1943; Weller 1976a; S.C.H. Barrett, unpubl. data). How the constituent genes that control the components of tristyly are organized and whether a supergene is involved is not known (see Charlesworth 1979).

While the evolution of tristyly is poorly understood, more information is available on the breakdown of the polymorphism. There is good evidence from the three tristylous families that trimorphic incompatibility has been replaced by a range of derivative breeding systems involving both outcrossing and selfing (Lewis & Rao 1971; Ornduff 1972; Weller 1976b; Barrett 1979). The commonest pathway involves the evolution of self-fertilization via semi-homostyle formation. Here, relaxation in the strength of self-incompatibility and modification in the positions of reproductive organs give rise to phenotypes with one set of anthers adjacent to the stigma. This ensures automatic self-pollination. Since populations of tristylous species often exhibit variation in incompatibility behaviour and floral form, they can provide useful experimental material for the study of natural selection on the mating system.

Here I review some of our work on the ecological genetics of tristyly in the Pontederiaceae. This includes elucidation of the genetic mechanisms and selective processes responsible for the breakdown of tristyly in *Eichhornia*. I begin by briefly discussing the distribution of breeding systems in the genus and then describe some recent investigations concerned with the distribution and relative frequencies of style morphs in populations of *Eichhornia paniculata* (Spreng.) Solms. These data provide some insights into the effects of selection and drift operating on the style morphs and enable the formulation of a model of the breakdown process.

2. TRISTYLY IN THE PONTEDERIACEAE

The Pontederiaceae is a small monocotyledonous family of eight genera and 34 species of aquatic and palustrial herbs. Tristyly occurs in three of the seven *Eichhornia* species and four of the five species of *Pontederia*. The tristylous species have showy floral displays and are pollinated by a range of insects, particularly specialized, long-tongued, solitary bees. In *Pontederia*, tristyly is associated with strong self-incompatibility, markedly trimorphic pollen and populations which usually contain the three style morphs (Barrett 1977a; Price & Barrett 1982, 1984; Barrett *et al.*, 1983; Glover & Barrett 1983). In contrast, in tristylous species of *Eichhornia*, monomorphic population structure frequently prevails and self-fertility and weak pollen trimorphism occur in *E. crassipes* and *E. paniculata* (Barrett 1977b, 1980, 1985). Self-pollinating, semi-homostylous variants are reported in each of the tristylous species of *Eichhornia* (Barrett 1978, 1979, 1985). The non-tristylous members of *Pontederia* and *Eichhornia* are primarily self-pollinating, exhibit residual tristylic characters, and are most probably semi-homostylous derivatives of tristylous ancestors.

Segregation data from controlled crosses of the style morphs in *E. crassipes* and *E. paniculata* are consistent with a two locus model for the inheritance of trisyly. The *S* locus is epistatic to *M*, and in both species the loci are apparently linked. In *E. paniculata*, which is diploid, genotypes for the morphs can be written as follows: S morph-*Ssmm, SsMm, SsMM*; M morph-*ssMm, ssMM*; L morph-*ssmm*. Since the species is self-fertile, homozygosity at the S locus can occur, although to date *SS*--leave space genotypes have not been recovered from natural populations. More complex inheritance patterns and genotype arrays are evident in *E. crassipes* which is tetraploid and self-fertile.

3. STYLE MORPH FREQUENCIES

Where fitness differences among the style morphs are absent, an isoplethic equilibrium (1:1:1) is the only possible condition in large populations of tristylous plants under legitimate mating (Heuch 1979). Surveys of style morph frequency in *E. crassipes* and *E. azurea* indicate that deviations from isoplethy frequently occur owing to founder effects and rampant clonal propagation (Barrett 1977b, 1978; Barrett & Forno 1982). *Eichhornia paniculata* is a short-lived perennial or annual of seasonally inundated sites and regenerates primarily by seed. Our recent unpublished surveys of style morph frequency in populations of this species provide important clues to the breakdown process and suggest that fitness differences among the style morphs occur under different ecological and demographic conditions.

The major centres of distribution of *Eichhornia paniculata* are in N.E. Brazil and the Caribbean (Cuba and Jamaica). Scattered populations are also reported from Argentina, Paraguay, W. Brazil, and Nicaragua. Our survey involved a total of 48 populations from N.E. Brazil and Jamaica of which 22 were trimorphic, 9 dimorphic and 17 monomorphic for style length (Table 1). The S morph does not occur in Jamaica, was under-represented in trimorphic populations, and absent from dimorphic and

Table 1. *Average frequencies of the style morphs in Brazilian and Jamaican populations of* Eichhornia paniculata.

Region	N populations	Frequencies L	M	S
Brazil				
trimorphic	22	.40	.40	.20
dimorphic	6	.34	.66A	0
monomorphic	2	0	1.00B	0
Jamaica				
dimorphic	3	.25	.75B	0
monomorphic	15	0	1.00B	0

A. Four of six populations with self-pollinating M variants.

B. An M plants self-pollinating variants.

monomorphic populations in Brazil. The M morph predominated in dimorphic populations and was the only morph to form monomorphic populations.

It is significant that virtually all M plants from Jamaica are self-pollinating variants with varying numbers of anthers (1-3) from short-level stamens adjacent to the mid-level stigma. In 6 of the 8 non-trimorphic populations from Brazil similar self-pollinating M variants predominated in populations. Interestingly, where the L and M morphs co-occur within Brazilian and Jamaican dimorphic populations, modifications increasing self-pollination were not evident in the L morph. A similar pattern involving modification of the M morph but not the L morph has been reported in *E. crassipes* (Fig. 2; Barrett 1979). Among tristylous populations of *E. paniculata* variants exbibiting modifications to stamen (M, S morphs) and style (L morphs) position occur rarely.

The populations containing self-pollinating M variants were small (average population size 50.8 plants, range 26-108) and four of the six populations were geographically isolated from the main population centres of *E. paniculata* in N.E. Brazil. Trimorphic populations were, in contrast, frequently large and geographically concentrated. Population size in *E. paniculata* is usually associated with the reliability of moisture, with permanent marshes containing large populations and seasonal pools and ditches smaller populations. Rainfall patterns in N.E. Brazil are extremely unpredictable and the region has one of the highest coefficients of variation in total rainfall in the world (Nimer 1972). Such unpredictable rainfall in an arid region is likely to have a major influence on the availability of aquatic habitats for *E. paniculata*. Fluctuations in population size and local extinction would therefore be expected to play an important role in the population biology of the species.

Stochastic influences on population size in conjunction with random dispersal may explain the loss of the S morph from populations of *E. paniculata*.

Since the species is highly self-fertile, polymorphic populations can arise from selfing and segregation of genotypes heterozygous at the *S* and *M* loci. Since the dominant *S* allele is only carried by the S morph separate introductions of this morph would be necessary for it to become established in populations. In contrast, the *m* allele can be carried by all three morphs and the *M* allele by the M and S morphs. Computer simulation studies by Heuch (1980) on the effects of random fluctuations of population size in tristylous systems verify that the S morphs is most often lost from populations. The same processes, on a neighbourhood scale, may also account for the low average frequency of the S morph in many trimorphic populations (Table 1).

Loss of the L morph and fixation of the M morph are most probably associated with the spread of genes modifying the position of short-level stamens in the M morph, since this change results in increased levels of selfing of the M morph (see below). Simulation studies indicate that increased selfing can result in the spread and fixation of the M morph in populations (Charlesworth 1979; Barrett *et al.*, 1983). On Jamaica, where the M morph predominates and the L morph is rare, high selfing rates in the M morph and fluctuations in populations size may eventually lead to loss of the *m* allele and hence the L morph from the island. Fig. 1 depicts the proposed stages in the breakdown from floral trimorphism to monomorphism in *E. paniculata.*

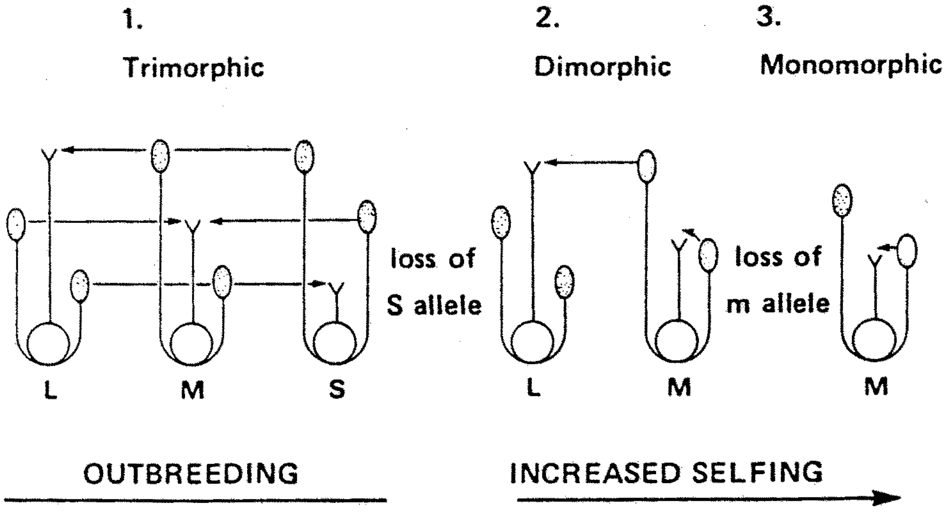

Fig. 1. *Stages in the breakdown of tristyly to semi-homostyly in* Eichhornia paniculata. *Arrows indicate predominant matings. Note modifications in short-stamen position and reduction in flower size in the M morph.*

This process depends in large part on the initial relaxation of self-incompatibility. Self-fertile, tristylous populations are more sensitive to ecological factors influencing mating patterns.

4. MATING SYSTEM ESTIMATES

The breakdown of tristyly in *E. paniculata* is associated with a change in breeding system from outcrossing to selfing. However, quantitative estimates of the magnitude of outcrossing and selfing are required to substantiate various aspects of the model presented in Fig. 1. Some progress has been made towards analysis of the mating system of populations by using the style length loci as genetic markers. Open-pollinated seed families collected from two populations with contrasting structures and style morph representation were grown to flowering and scored for style length. The data were used to estimate outcrossing rates (t) of the style morphs. Population 1 was a large (> 1000 plants), tristylous population containing similar numbers of the three style morphs. Population 2 was smaller (n = 108 plants) and composed of the L and M morphs. The majority of M plants exhibited modified short-level stamens, with a single stamen adjacent to the M stigma promoting self-pollination.

Outcrossing rates for the two populations are presented in Table 2. Despite their high self-fertility, the morphs in population 1 are predominantly outcrossing. The basis of the high outcrossing rates is not known. Floral trimorphism may promote cross-pollination among floral morphs as Darwin (1877) hypothesized or some form of cryptic self-incompatibility system (Weller & Ornduff 1977) may favour inter-form pollinations. We are currently investigating the latter possibility; examination of the former by pollen flow studies is precluded by the absence of strong pollen trimorphism in the species.

Table 2. *Estimates of outcrossing rate (t) of the style morphs in two populations of* Eichhornia paniculata *from N.E. Brazil. S.C.H. Barrett, A.H.D. Brown and J.S. Shore (unpublished data).*

	Population 1			Population 2	
	L	M	S	L	M*
Population frequency	.35	.36	.29	.26	.74
N (families/plants)	21/652	24/721	22/665	6/142	20/554
t	0.904	0.931	0.835	0.657	0.0
Standard error	0.028	0.052	0.077	0.068	0.083

* majority of M plants with modified short-level stamens.

In population 2 significantly different mating patterns were apparent. In the L morph, outcrossing was estimated at 66 per cent whereas no detectable outcrossing was evident in the M morph, presumably as a result of the juxtaposition of anthers and stigma within flowers. With this mating asymmetry the M morph will replace the L morph, provided that fitness differences of progeny from the two morphs are not great. Spread of the M morph would be further augmented by fecundity selection if pollinator service is unreliable (see below).

5. GENETICS OF SEMI-HOMOSTYLY

Homostyle formation in distylous genera such as *Primula* results from crossing-over between loci in the supergene which governs heterostyly (Charlesworth & Charlesworth 1979). In tristylous groups however, the wide diversity in floral modification indicates a more complex genetic basis for semi-homostyle evolution.

In *Eichhornia,* relaxation and eventual loss of self-incompatibility precedes modifications in floral structure. Subsequent alterations occur most commonly, but not exclusively, in the M morph (Barrett 1979, 1985, and unpubl. data). In *E. paniculata,* the modifications proceed by a curious step-wise increase in filament length of the three short-level stamens (Barrett 1985). The variation in filament length is discontinuous implying an underlying developmental regulation involving a threshold response, perhaps to temperature and hormone levels. The variation is further complicated by marked intra-inflorescence instability in the expression of short-stamen position. Self-pollinating M variants frequently display both modified and unmodified flowers. Developmental instability of floral traits in *E. paniculata* is correlated with self-pollination and may result, in part, from a loss in the precision of canalization owing to inbreeding and reduced heterozygosity (Lerner 1954).

Controlled crosses among the range of floral variants in *E. paniculata* indicate that the genes which govern alterations in the position of short-level stamens are recessive and relatively few in number (S.C.H. Barrett, unpubl. data). All progenies of crosses between unmodified style morphs from tristylous populations and selfing M variants are unmodified. Segregation data confirm that semi-homostylous forms are modified M morphs of genotype *ssMM* and not the S morph with an elongated style. Crosses between selfing variants from different geographical areas are presently being undertaken to determine whether homologous genes are involved in floral modification.

The genes controlling stamen position in the M morph have no major phenotypic effects when present in the L and S morphs. As a result, in dimorphic populations the M morph frequently displays the selfing habit whereas the L morph remains unmodified. This observation indicates the importance to the phenotype of the developmental background with which a gene operates. In tristylous systems this interaction is likely to be of paramount importance because of the complex developmental organization of the syndrome.

6. FLORAL MODIFICATIONS ACCOMPANYING SELFING

In addition to their contrasting arrangements of reproductive organs, trimorphic and monomorphic populations of *E. paniculata* differ in several other floral traits associated with the breeding system. Comparisons of Brazilian and Jamaican populations are detailed in Barrett (1985) and describe differences in flower number per inflorescence, size and showiness of perianth parts, nectar guide intensity, degree of pollen heteromorphism, pollen production, and ovule and seed number per flower. In most cases, Jamaican populations exhibit smaller inflorescences containing less showy, smaller flowers with lower reproductive potential. The reduction in floral display of Jamaican populations is paralleled by similar but less dramatic alterations in Brazilian populations which contain self-pollinating variants (Table 3). The differences in the degree of modification are presumably associated with contrasting pollinator service and the evolutionary history of populations in the two regions. Populations in N.E. Brazil may be more recently derived from tristylous ancestors so that selection and drift have not altered floral traits and the reproductive economy of populations to the extent that is evident in Jamaica. The differences in floral biology between trimorphic and monomorphic populations of *E. paniculata* are those that frequently distinguish outbreeding and inbreeding taxa (Ornduff 1969).

Table 3. *Floral traits in Brazilian and Jamaican populations of* Eichhornia paniculata. *Plants grown under uniform glasshouse conditions. Data from Barrett (1985 and unpubl. data); all comparisons involve the M morph.*

Population	Flowers per inflorescence	Flower size (mm)	Pollen heteromorphism^A (μm)	Pollen production	Ovule number	P/O ratio
Brazil (trimorphic)	27.1	24.0	16.8	18,520	113.0	164
Brazil (monomorphic)	19.6	20.1	9.7	11,792	82.4	143
Jamaica (monomorphic)	16.2	16.9	5.7	7,913	77.5	102

A Difference in average size of pollen from long- and short-level anthers.

7. CONCLUSIONS

Comparative studies of the reproductive biology of *Eichhornia* species coupled with investigations of the population biology of species populations provide strong evidence that the major pathway of breeding system evolution in the genus is the breakdown of tristyly to semi-homostyly. A major problem is to identify the selective forces responsible for the evolution of self-fertilization. Data from *E. paniculata* give some clues to the conditions favouring this shift in reproductive mode.

Frequent population bottlenecks resulting from drought and dispersal may play a major role in disrupting the maintenance of tristyly. Loss of the S morph and subsequent fixation of the M morph through the spread of genes modifying stamen position, are best explained by genetic and ecological processes operating in small populations. The recessive genes governing alterations in floral phenotype are more likely to be exposed to selection in small, inbred populations. Furthermore, their spread would be favoured at low density via fecundity selection. This effect is likely to be particularly pronounced in tristylous systems where specialized pollinators may be required to mediate pollination among the morphs (Barrett 1979; Charlesworth 1979). Outside the range of specialized pollinators and in small, newly-founded populations with unreliable, generalist pollinators, self-pollinating variants would be at a selective advantage. Whether this hypothesis has general validity as an explanation for the multiple breakdown of tristyly in the genus as a whole, requires further work.

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8. REFERENCES

Barrett, S.C.H., 1977a - The breeding system of *Pontederia rotundifolia* L., a tristylous species. *New Phytol.*, 78, 209-220.

Barrett, S.C.H., 1977b - Tristyly in *Eichhornia crassipes* (Mart.) Solms. (Water Hyacinth). *Biotropica*, 9, 30-38.

Barrett, S.C.H., 1978 - Floral biology of *Eichhornia azurea* (Sw.) Kunth (Pontederiaceae) *Aquat. Bot.*, 5, 217-228.

Barrett, S.C.H., 1979 - The evolutionary breakdown of tristyly in *Eichhornia crassipes* (Mart.) Solms. (Water Hyacinth). *Evolution*, 33, 499-510.

Barrett, S.C.H., 1980 - Sexual reproduction in *Eichhornia crassipes* (Water Hyacinth). 1. Fertility of clones from diverse regions. *J. Appl. Ecol.*, 17, 101-112.

Barrett, S.C.H., 1985 - Floral trimorphism and monomorphism in continental and island populations of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). *Biol. J. Linn. Soc.*, (in press).

Barrett, S.C.H. & I.W. Forno, 1982 - Style morph distribution in New World populations of *Eichhornia crassipes* (Mart.) Solms-Laubach (Water Hyacinth). *Aquat. Bot.*, 13, 299-306.

Barrett, S.C.H., Price, S.D. & J.S. Shore, 1983 - Male fertility and anisoplethic population structure in tristylous *Pontederia cordata* (Pontederiaceae) *Evolution*, 37, 745-759.

Charlesworth, B. & D. Charlesworth, 1979 - The maintenance and breakdown of heterostyly. *Am. Nat.*, 114, 499-513.

Charlesworth, D., 1979 - The evolution and breakdown of tristyly. *Evolution*, 33, 486-498.

Darwin, C., 1877 - *The different forms of flowers on plants of the same species.* John Murray, London.

Fisher, R.A. & K. Mather, 1943 - The inheritance of style lengths in *Lythrum salicaria. Ann. Eugen.*, 12, 1-23.

Ganders, F.R., 1979 - The biology of heterostyly. *N. Z. J. Bot.*, 17, 607-635.

Glover, D.E. & S.C.H. Barrett, 1983 - Trimorphic incompatibility in Mexican populations of *Pontederia sagittata* (Presl.) Pontederiaceae. *New Phytol.*, 95, 439-455.

Heuch, I., 1979 - Equilibrium populations of heterostylous plants. *Theor. Popul. Biol.*, 15, 43-57.

Heuch, I., 1980 - Loss of incompatibility types in finite populations of the heterostylous plant; *Lythrum salicaria. Hereditas*, 92, 53-57.

Lerner, I.M., 1954 - *Genetic homeostasis.* Oliver and Boyd, London.

Lewis, D & A.N. Rao, 1971 - Evolution of dimorphism and population polymorphism in *Pemphis acidula* Forst. *Proc. R. Soc. Lond. B. Biol. Sci.*, 178, 79-94.

Nimer, E., 1972 - Climatologia da região Nordeste do Brasil. *Rev. Bras. Geogr.*, 34, 3-51.

Ornduff, R., 1969 - Reproductive biology in relation to systematics. *Taxon*, 18, 121-133.

Ornduff, R., 1972 - The breakdown of trimorphic incompatibility in *Oxalis* section Corniculatae. *Evolution*, 26, 52-65.

Price, S.D. & S.C.H. Barrett, 1982 - Tristyly in *Pontederia cordata* L. (Pontederiaceae). *Can. J. Bot.*, 60, 897-905.

Price, S.D. & S.C.H. Barrett, 1984 - The function and adaptive significance of tristyly in *Pontederia cordata* L. (Pontederiaceae). *Biol. J. Linn. Soc.*, 21, 315-329.

Weller, S.G., 1976a - The genetic control of tristyly in *Oxalis* section Ionoxalis. *Heredity*, 37, 387-392.

Weller, S.G., 1976b - Breeding system polymorphism in a heterostylous species. *Evolution*, 30, 442-454.

Weller, S.G. & R. Ornduff, 1977 - Cryptic self-incompatibility in *Amsinckia grandiflora. Evolution*, 31, 47-51.