

BREEDING SYSTEMS, GENE FLOW AND LEVEL OF GENETIC DIFFERENTIATION IN PLANT POPULATIONS

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Genetic variation and its distribution among plant populations are primarily determined by their breeding system and the level of gene flow among them (Bawa *et al.* 1985, Bawa *et al.* 1990, Rocha & Aguilar 2001b). It is well known that mating systems influence the amount, as well as the distribution of the genetic variation within and among populations (Wright 1921, Stebbins 1950, 1957, Hamrick *et al.* 1991). The net result of the operation of the various breeding systems is the regulation of the outcrossing rates, which may vary among populations and among geographical regions (Bateman 1956, Grant 1958, Rocha & Aguilar 2001a). Therefore, the study of plant breeding systems, and their effect on the genetic structure of populations is of fundamental importance for the study of plant evolution and population genetics (Richards 1986).

Plants show extensive variation in their sexual expression (Willson 1983, Richards 1986). The vast majority of flowering plants are hermaphrodites; i.e., they bear both male and female functional parts in the same flower, and are capable of contributing genes to the next generation through both functions. Other forms of sex expression, such as monoecy and dioecy, are also common in plants. Monoecious plants also bear both male and female functional parts, but they are borne on different flowers. In a broad sense, monoecious plants are also hermaphrodites. In contrast, only one sex function is present in dioecious plants. Other forms of sex expression result from the combination of different the sex types on flowers of the same individual.

The reproductive biology of tropical forest plants has been studied by several authors (Bawa & Hadley 1990). Bawa *et al.* (1985) studied the sexual systems of tropical rain forest trees in Costa Rica. They reported that most trees are hermaphrodites and found

no significant difference between canopy and sub-canopy habitats in the distribution of the various sexual systems (Table 1). More recently, Kress & Beach (1994) examined the sexual systems in 507 species from the lowland rain forest of La Selva, Costa Rica. They reported that 70% of species were hermaphrodites, 12.4% were monoecious, and 17.4% were dioecious (Table 2). Similarly, in the premontane rain forest of Costa Rica, most of the 501 species examined are hermaphrodites (69.7%). Only about 11% of the species are dioecious and 9% are monoecious (Ferrufino & Rocha, unpublished) (Fig. 1). Among trees, the proportion of species with different sexual systems found in the premontane forest is similar to that described by Bawa *et al.* (1985) and Kress & Beach (1994) in the lowland rain forest; 64.1% are hermaphrodites, 9.6% are monoecious and 18.6% are dioecious.

The relationship between sexual systems and breeding systems is not always clear (Table 3). Hermaphroditic plants may be capable of selfing; but many species exhibit mechanisms to avoid self-fertilization. Reducing the level of selfing, in turn, enhances the movement of genes within and between populations (Willson 1983, Richards 1986). For example, in some species, plants change from one sex to the other once or more during their lifetime. Typically, plants initiate sexual reproduction producing only male flowers, and later stop producing male flowers and begin to produce only female flowers. This phenomenon is known as sequential hermaphroditism, and occurs when the fitness gain of each sexual role changes with age or size of the individual.

Self-incompatibility systems are mechanisms to avoid self-fertilization (Nettancourt 1977). These mechanisms usually ensure obligate outbreeding, and result from failure of self-pollen to adhere to, or ger-

Table 1: Floral sexuality and canopy position in 333 lowland tropical rain forest tree from Costa Rica (Taken from Bawa *et al.* 1985).

Flower sexuality	% species in		
	Canopy	Sub-canopy	% species overall
Hermaphroditic (N=218)	63.2	66.4	65.5
Monoecious (N=38)	9.5	12.2	11.4
Dioecious (N=77)	27.4	21.4	23.1

Table 2: Sexual systems of flowering plants in the lowland rainforest at La Selva, Costa Rica (taken from Kress & Beach 1994).

	Forest stratum						All strata	
	Understory		Sub-canopy		Canopy			
	N	%	N	%	N	%	N	%
Hermaphroditic	168	74.7	124	67.4	64	65.3	356	70.2
Monoecious	35	15.5	18	9.8	10	10.2	63	12.4
Dioecious	22	9.8	42	22.8	24	24.5	88	17.4
Total species	225	100	184	100	98	100	507	100

minate on the stigma, or the failure of self-pollen to penetrate the stigma, or to grow down the style (Richards 1986). Two major mechanisms of self-incompatibility systems have been studied in detail, and they operate before fertilization: gametophytic (GSI) and sporophytic (SSI) self-incompatibility. Gametophytic self-incompatibility results from the expression of genes in the haploid male gametophyte, the pollen grain. In sporophytic self-incompatibility, pollen failure is determined by the expression of genes in the diploid sporophytic producer of pollen. Bawa *et al.* (1985) showed that 24 out of 28 lowland

rain forest species subjected to controlled pollination showed the presence of self-incompatibility. More recently, Kress & Beach (1994) showed that 16 out of 19 canopy and sub-canopy taxa (84.2%) in the lowland rain forest of La Selva are self-incompatible. They also showed that 13 out of 38 understory taxa (34.2%) are self-incompatible.

The amount of selfing also depends on the degree of separation that occurs between male and female parts in time (dichogamy) and space (herkogamy) within the flowers. Sometimes, flowers are functionally male after anthesis, and later shift to be functionally female (protandry). In such species, the rate of selfing is explained by the amount of time occurring between anther dehiscence and the start of stigma receptivity within the flower (Schoen 1982a, 1982b). In contrast, in other species flowers are functionally female after anthesis, and later shift to be functionally male (protogyny). The rate of selfing may also be determined by the distance between the male and the female parts within the flower. For example, in many species of orchids the pollinium release occurs before the stigmatic cavity is receptive (protandry). In addition, the stigmatic cavity is usually hidden with respect to the pollinia in such a way that within flower pollination is impossible (herkogamy).

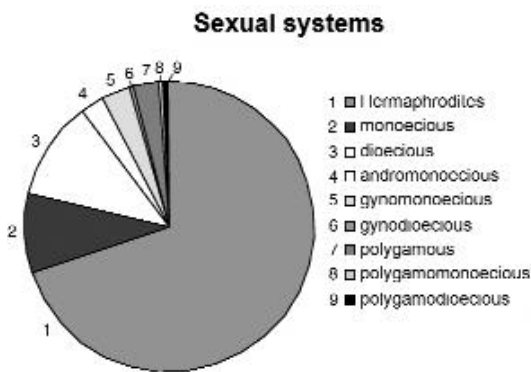


Figure 1: Percentage of species by sexual system in a premontane rain forest of Costa Rica.

Table 3: Relationship between sexual systems and breeding systems (modified from Richards 1986, and Willson 1982). CX= male-female, C=female only, X= male only

Sexual system	Distribution of sex parts		Breeding system
	Within a flower	Within a plant	
Hermaphrodites	CX	CX	From full selfing to full outcrossing
Monoecy	C or X	CX	Enhances outcrossing
Dioecy	C or X	C or X	Enforces outcrossing
Gynomonoecy	CX or C	CX	Enhances outcrossing, but allows selfing
Gynodioecy	CX, C or X	CX or C	Enhances outcrossing
Andromonoecy	CX or X	CX	Enhances outcrossing, but allows selfing
Androdioecy	CX, C or X	CX or X	Not enough information
Polygamy	CX or C or X	CX, or C or X	Enhances outcrossing, allows some geitonogamy and some autogamy

Overall, there is little information available about these mechanisms in tropical plants.

The majority of tropical rain forest species investigated so far appeared to be outcrossers with extensive gene flow (Ashton 1969, Bawa *et al.* 1985, Hamrick & Murawski 1991, Hamrick *et al.* 1991, Hall *et al.* 1994a, Hall *et al.* 1996, Doligez & Joly 1997). Isozyme studies conducted to determine the mating system of these species further support the predominance of outcrossing among tropical rain forest trees (O' Malley & Bawa 1987, O' Malley *et al.* 1988, Doliguez & Joly 1997, Nason & Hamrick 1997, James *et al.* 1998). Doliguez & Joly (1997) and Nason & Hamrick (1997) reviewed the outcrossing rates reported for 28 and 36 species of tropical forest trees in natural populations, respectively. They found that most of these species have estimates of outcrossing rates higher than 0.80. Therefore, the outcrossing rates could be used to explain the levels of genetic variation and differentiation between populations.

Few studies have examined the variation in the outcrossing rates of tropical plants (Murawski & Hamrick 1990, 1991, 1992, Escalante *et al.* 1994, Pascarella 1997, James *et al.* 1998, Rocha & Aguilar 2001a). For example, it has been reported that the outcrossing rate did not vary significantly among wild and cultivated populations of *Phaseolus coccineus* in Mexico (Escalante *et al.* 1994). However, the same study also revealed great variation in the

outcrossing rates among families within each population. In contrast, Pascarella (1997) showed that outcrossing rates varied significantly among four populations of the tropical shrub *Ardisia escallonioides* in south Florida. He also showed that outcrossing rates were not correlated with the number of flowering plants within a population. His finding contrasted with those of Murawski & Hamrick (1991) who reported a significant decrease in the outcrossing rates of *Cavanillesia platanifolia* as the density of flowering trees declined.

Spatial and temporal variation in the outcrossing rates of tropical rain forest trees has been examined by Murawski & Hamrick (1991, 1992), Hall *et al.* (1994), Hall *et al.* (1996) and Murawski *et al.* (1994). For example, it has been reported that the outcrossing rates of two populations of *Cavanillesia platanifolia* in Panama were different (Murawski & Hamrick 1992). In addition, this species also showed significant variation in the outcrossing rates in two consecutive years in the population on Barro Colorado Island (Murawski & Hamrick 1991). They found that the outcrossing rate was directly correlated to the density of flowering trees (Murawski & Hamrick 1991, 1992). In contrast, Hall *et al.* (1994b) did not find differences in the outcrossing rates among nine populations of *Carapa guianensis* in Costa Rica. They concluded that the high population density and synchronous flowering contributed to the high outcrossing

rates. The difference between these two studies could be due to the fact that one species (*C. platanifolia*) is self-compatible while *C. guianensis* is self-incompatible (J.L. Hamrick, pers. comm.). Because of that, the later would be expected to have much less flexibility in the amount of outcrossing/selfing.

In another study, Hall *et al.* (1996) reported that outcrossing rates for *Pithecellobium elegans* did not differ across two consecutive years, while the proportion of flowering trees was significantly different between years. Similar findings were also obtained for *Shorea trapezifolia* in Sri Lanka, in two consecutive years the estimates of outcrossing rates only varied from 54 to 62 % (Murawski *et al.* 1994). The authors argued that the variation in outcrossing rates among individual trees suggested that the rate of self-incompatibility in this species is also variable. Rocha & Aguilar (2001a, 2001b) showed little variation in the outcrossing rate of *Enterolobium cyclocarpum* in Costa Rica. However, they also showed significant variation in the mean number of trees that father the seed crop in trees from different populations, and from one year to the next within a given population.

The genetic structure of tropical plants has been examined using genetic markers (Buckley *et al.* 1983; Hamrick & Loveless 1989, Hamrick 1993). Most of these studies revealed that trees and shrubs usually have high levels of genetic variation, most of which is within populations, and subsequently there is little genetic differentiation within populations (Heywood & Fleming 1986, Murawski & Hamrick 1990, 1991, Pérez-Nasser *et al.* 1991, Hamrick 1993, Chase *et al.* 1995). For example, there is little genetic differentiation between five populations of *Enterolobium cyclocarpum*, the guanacaste tree, where only 3.9% of the genetic variation was found between populations (Rocha & Lobo 1996). Rocha & Aguilar (2001a, 2001b) have shown that guanacaste tree is a predominantly outcrossing species with extensive gene flow. In contrast, Hall *et al.* (1994a) examined the distribution of the genetic variation for *Pentachlethra macroloba* in Costa Rica. They reported that genetic differentiation between populations accounted for 21.9% of the total genetic variation. Similar findings were

obtained by Moran *et al.* (1989a, 1989b) for three tropical species of *Acacia* from natural riverine forest and open savannas in Australia and New Guinea. They found also found low levels of genetic diversity and high levels of population differentiation.

Maquet *et al.* (1996) studied the genetic structure in wild populations of the short lived perennial predominantly inbreeder lima bean, *Phaseolus lunatus*. They found that a significant proportion of the genetic variation is found between populations of this vine. They argue that such level of genetic differentiation between populations is due to the high rates of self-fertilization and biparental inbreeding that result in low rates of gene flow. Similarly, Murillo & Rocha (1999) studied the levels of genetic differentiation between 17 populations of *Alnus acuminata*, a monoecious wind pollinated tree species, in Costa Rica and Panama. They found levels of population differentiation that ranged between 0.14 and 0.28, and found some evidence of isolation by distance and weak gene flow between populations from different geographical regions. In addition, the same authors also found that this is a predominantly outcrossing species; but their data revealed that most of the seed crop of each tree is sired by one or two pollen donor trees (Murillo & Rocha, unpubl.).

In summary, sexual systems have an important role in determining the rate of outcrossing. Self-compatibility in hermaphrodite flowers may have been originally advantageous to assure reproduction. When reproductive assurance improved, outcrossing may have become selectively advantageous, and led to the evolution of different sexual systems. In addition, new features, such as dichogamy, herkogamy, and self-incompatibility system, which promote outcrossing may have also been favored by natural selection. Selection for genetic recombination may be strong in environments where biotic interaction with competitors, pathogens, parasites and predators are intense (Bawa *et al.* 1985). Overall, an increase in the level of outcrossing increases genetic diversity, and it might also enhance gene flow counteracting genetic differentiation between populations (Hartl 1980).

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