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# The post-fire effects on the outcrossing rate of a Brazilian savannah shrub, *Helicteres sacarolha* A.St.-Hil.

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**ABSTRACT –** (The post-fire effects on the outcrossing rate of a Brazilian savannah shrub, *Helicteres sacarolha* A.St.-Hil.). The effect of post-fire, plant density and number of flowers per plant on outcrossing rates was examined in a Brazilian savannah shrub, *Helicteres sacarolha*. Data on number of flowering plants and flowers per plant were collected during the flowering season in January and February of 1994. In October of 1994, a fire swept through the studied area after 30 years of fire absence. The burnt plants of *H. sacarolha* resprouted, producing flowers and fruits in 1995. Seeds from several plants in both years were collected. Allozyme markers were used to estimate the multilocus outcrossing rates for 1994 and 1995 progenies. After the fire, outcrossing rates increased. In 1995, plants flowered vigorously, increasing flower density and probably pollinator activity. *Helicteres sacarolha* seems to be fire resistant, like other plant species of the Brazilian savannah, but several plants tagged in 1994 were not found after the fire, and may have died. Thus, although genetic diversity and outcrossing rates increased following the fire in 1994, repeated events of fire may decrease drastically the population size of *H. sacarolha*, leading to a diminished genetic diversity and outcrossing rates.

Key words - cerrado, flower density, *Helicteres*, plant outcrossing rate, post-fire effect

**RESUMO –** (Os efeitos pós-fogo na taxa de cruzamento de um arbusto do cerrado, *Helicteres sacarolha* A.St.-Hil.). Os efeitos do fogo, da densidade de plantas, do número de flores por planta na taxa de cruzamento foram checados para uma espécie de arbusto do cerrado, *Helicteres sacarolha*. Dados de número de plantas e flores por planta foram coletados durante o período de floração de janeiro a fevereiro de 1994. Em outubro de 1994, uma queimada ocorreu na área de estudo após 30 anos de ausência de fogo. No entanto, as plantas queimadas de *H. sacarolha* brotaram, produzindo flores e frutos em 1995. Sementes de várias plantas foram coletadas em 1994 e 1995. Marcadores aloenzimáticos foram utilizados para estimar a taxa de cruzamento para as progênies de 1994 e 1995. A taxa de cruzamento aumentou após a ocorrência do fogo. Em 1995, as plantas floresceram vigorosamente, aumentando a densidade de flores e, provavelmente, a atividade dos polinizadores entre as plantas da população. Como muitas espécies do cerrado brasileiro, *H. sacarolha* é tolerante ao fogo, contudo várias plantas marcadas em 1994 não foram encontradas após o fogo e podem ter morrido queimadas. Desta forma, eventos repetidos de fogo podem levar a uma drástica diminuição do tamanho da população de *H. sacarolha* e, conseqüentemente, a um decréscimo em suas taxas de diversidade genética e de cruzamento.

Palavras-chave - cerrado, densidade de flor, efeito do fogo, *Helicteres*, taxa de cruzamento

## Introduction

Mating systems strongly influence the establishment and maintenance of genetic diversity and structure in populations (Wright 1946, Brown *et al.* 1989, Barrett & Eckert 1990, Barrett 1995, Charlesworth & Charlesworth 1995). The degree of genetic variation in a population affects its response to selection (Darwin 1876, Vasek & Harding 1976, Gregorious *et al.* 1987, Mitton 1993, Sharma *et al.* 1993, Hall & Bawa 1996, Lu *et al.* 1997) and therefore its viability. The mating system of a species may be measured through its outcrossing rate. This rate is influenced by several ecological factors, such as plant density, number of flowers per plant, and pollinator movements (Franceschinelli & Bawa 2000). Outcrossing rate increases with plant density (Farris & Mitton 1984, Burdon *et al.* 1988, Wolff *et al.* 1988, Murawski *et al.* 1990, 1994, Murawski & Hamrick 1991, 1992a, b, Van Treuren *et al.* 1993). Large plants that produce many flowers can induce the pollinator to visit high number of flowers per plant, thereby increasing the probability of selfing (Burdon *et al.* 1988, Wolff *et al.* 1988). However, pollinators may be induced to visit higher number of flowers per plant in populations that are less dense regardless of the plant size (Franceschinelli & Bawa 2000).

Little is known about the effect of abiotic factors such as fire on the outcrossing rates of plants. Sampson

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*et al.* (1994) suggested that low outcrossing rates found for populations of *Banksia brownii* Baxter ex R. Br. could be associated with frequent events of fire. The occurrence of fire in the Brazilian savannah ("cerrado") is very common. Natural fire and burning due to human activity occur mainly during the dry season in the Brazilian savannahs. Although the effects of fire on vegetation and plant population dynamics have been studied (Figueira 1998, Hoffman 1999, De Castro & Kauffmann 1998), the effect of fire on the outcrossing rates of a "cerrado" plant species has not been examined.

*Helicteres sacarolha* A.St.-Hil. is a shrub of open Brazilian savannahs ("cerrado aberto"). In 1994, we studied the effect of plant and flower densities on the outcrossing rate of this species. Also, we compared the outcrossing rates of *H. sacarolha* with its cogeneric *H. brevispira* A.St.-Hil which has populations with higher plant and flower densities (Franceschinelli & Bawa 2000). The studied population of *H. sacarolha* occurs in an area that has been protected against fire for more than 30 years until the end of 1994, when a deliberate fire lit by unknown people occurred. We used this opportunity of a fire event to examine the post-fire effect on the outcrossing rate of *H. sacarolha*.

## Material and methods

Study site and species – The fieldwork was conducted at the Mogi Guaçú Ecological Reserve in the São Paulo State, Brazil, at 22°18' S and 47°13' W. This reserve comprises 980 ha of riparian semi-deciduous forest and savannah vegetation. Part of the savannah area was burnt in October of 1994, after having been protected from fire for more than 30 years.

*Helicteres sacarolha* A.St.-Hil. is a small shrub, up to 1.5 m tall, which occurs in the open Brazilian savannah of the central-western and southeastern Brazil. Populations of *H. sacarolha* are usually clumped in the studied area, but the plant density is not very high. The plants loose all their leaves during the dry period (from July to the beginning of September). They have xylopodes, which resprout in the beginning of the raining season. From the same xylopode more than one stem may arise. However, it is easy to distinguish an individual plant, since the plant density is low. *Helicteres sacarolha* flowers during the months of January and February in the studied area (Franceschinelli 1989). Generally, only one to 12 flowers are open per day, though some large plants may produce 15 to 35 flowers a day during the flowering peak in January. The flowering was synchronous among plants within the studied population, although some plants did not flower. The fruits are small capsules (2-3 cm in length) with 10 to 30 seeds. At the study site, this species was visited and pollinated by the hummingbirds *Chlorostilbon aureoventris* D'Orbigny & Lafresnaye and *Amazilia lactea* Lesson (Franceschinelli 1989).

*Helicteres sacarolha* is hermaphrodite and self-compatible (Franceschinelli 1989), and the species may have a mixed mating system. This model of mating system is the main assumption of the method used here for calculating the outcrossing rate (Ritland 1990).

Data collection – In January of 1994, every plant was tagged and had their open flowers counted weekly during the four weeks of the flowering peak. All the flowered plants in the population were mapped (figure 1). After a few months, the mature fruits of 18 plants for the mating system analyses were collected. The average number of seeds per plant used in the mating system analysis in 1994 was 18. The locations of the sampled plants for this analysis are in the figure 1. Plant density was estimated as the average number of neighbouring plants within a 10 m radius of each sampled plant.

In 1995, after the fire, the tagged plants were relocated and mature fruits were collected partly from the same plants used in the mating system analysis in 1994 (figure 2). Mature fruits of 13 plants were collected for the mating system analysis. The average number of analysed seeds per plant in 1995 was 17. Open flowers per plant were counted only for 12 plants in 1995. In this year, pollinator foraging behaviour was also observed on those 12 plants. The number of visited flowers at each pollinator visit to a plant was counted.

Electrophoresis – The mating system analyses were done using allozymes as genetic markers. Sample sizes for these analyses are in table 1. Seeds were put in hot water at 70 ºC for five minutes to break dormancy, and were germinated in the greenhouse. When the seedlings had three to four pairs of leaves, they were ground in liquid nitrogen and the powdered tissue was mixed with extraction buffer (1 mM EDTA, 10 mM KCl, 20 mM MgCl2, 0.03 mM NADP, 0.03 mM NAD, 4% PVP-40, 10% glycerol, and 0.1 mM NaPO4, pH 7.5) and absorbed onto filter paper wicks. The staining procedure followed the techniques of O' Malley *et al.* (1980).

Twenty-six enzyme systems were screened with three electrophoretic buffers. Eight enzyme systems showed simple banding patterns and could be reliably scored: phosphoglucomutase (PGM, EC 5.4.2.2), uridine diphosphoglucose pyrophosphorylase (UGPP, EC 2.7.7.9), aconitate hydratase (ACO, EC 4.2.1.3), isocitrate dehydrogenase (IDH, EC 1.1.1.41), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), triose-phosphate isomerase (TPI, EC 5.3.1.1), 6-phosphogluconic dehydrogenase (6PG, EC 1.1.1.44), and aspartate aminotransferase (GOT EC 2.6.1.1). Phosphoglucomutase, UGPP, ACO, IDH, and 6PG were resolved on a morpholine citrate buffer system at pH 7.8 (Clayton & Tretiak 1972). Glucose-6-phosphate isomerase, GOT, and TPI were assayed on a lithium borate buffer at pH 8.3 (Soltis *et al*. 1983). Loci banding patterns were consistent with typical subunit structures (Weeden & Wendel 1989).

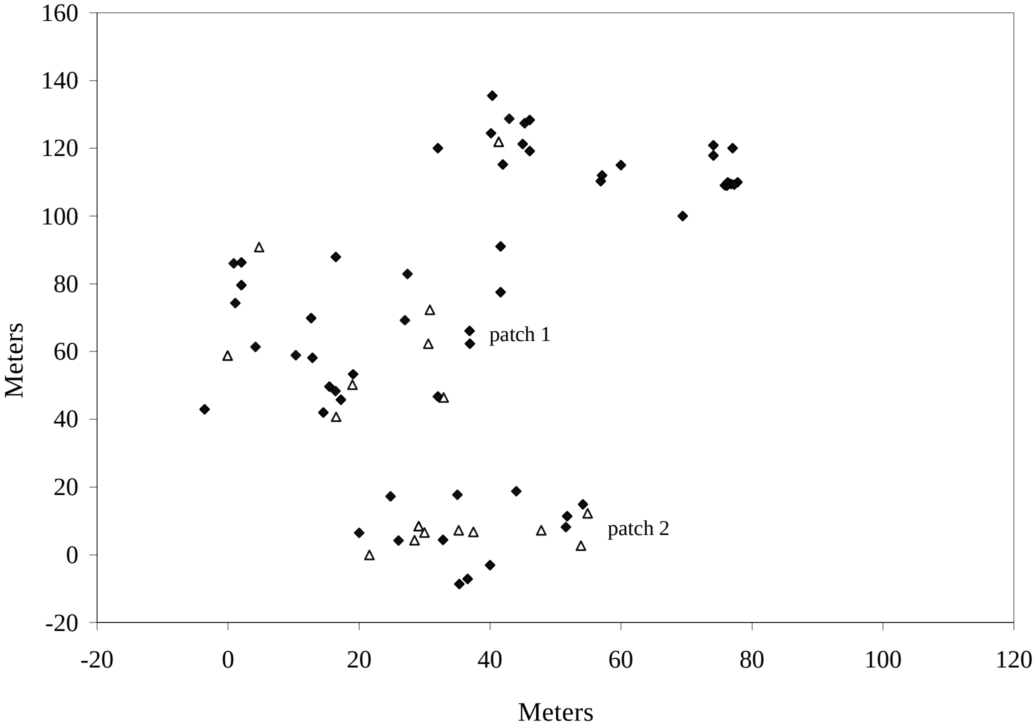

Figure 1. Map of the flowering plants of the studied population of *Helicteres sacarolha* in 1994. ♦ = flowering plants in 1994 in the studied area, Δ = sampled plants for the mating system analysis.

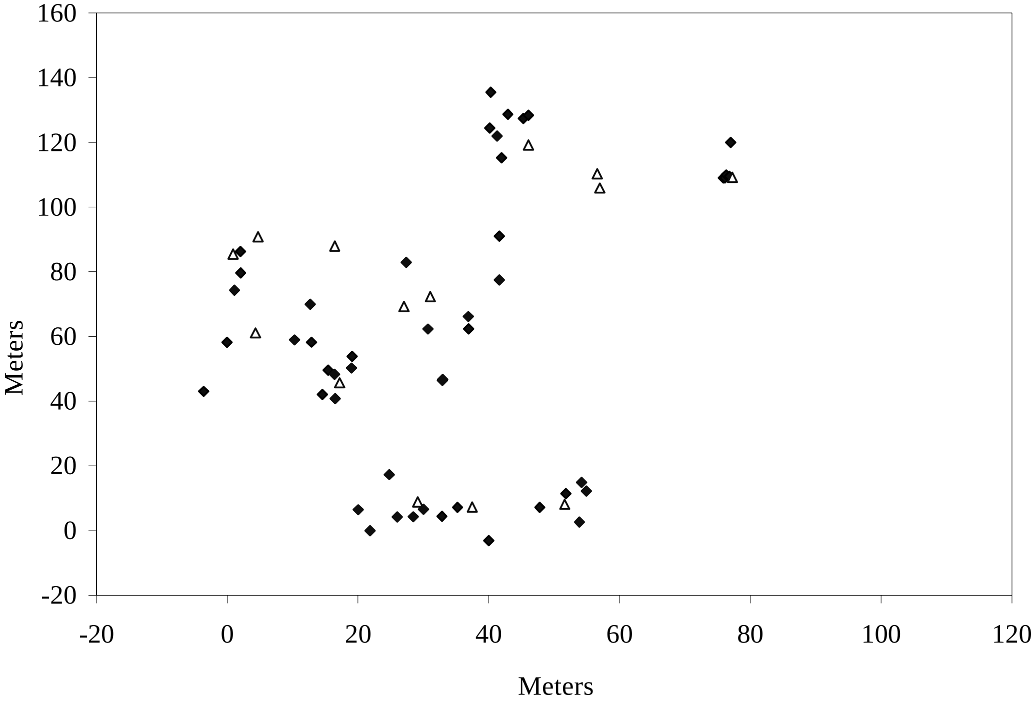

Figure 2. Map of the flowering plants of the studied population of *Helicteres sacarolha* in 1995. ♦ = flowering plants in 1995 in the studied area, Δ = sampled plants for the mating system analysis.

Table 1. Outcrossing rates for *Helicteres sacarolha* in 1994 and 1995. Density of flowering plants is the average number of neighbouring plants within 10 m radius of the sampled plants. F is the average single-locus inbreeding coefficient of maternal parents. N is the number of sampled plants. Number of seeds is in parentheses. Outcrossing rates ($t_m$ and $t_s$) and correlated mating ($r_p$) parameters from 1994 and 1995 are significantly different from each other (T-test, $P < 0.01$).

| Year | Density of flowering plants (SD) | N | $t_m$ (SD) | $t_s$ (SD) | $t_m$-$t_s$ (SD) | $r_p$ | F |
|---|---|---|---|---|---|---|---|
| 1994 | 3.78 (1.3) | 18 (312) | .48 (.07) | .45 (.07) | .04 (.03)$^{ns}$ | 0.50 (.06) | 0,01 |
| 1995 | 3.75 (2.1) | 13 (230) | .62 (.09) | .59 (.10) | .02 (.03)$^{ns}$ | 0.17 (.05) | 0,01 |

Polymorphic loci conformed to Mendelian expectations for segregation in the analysed progeny arrays.

Data analysis – Mating systems were analysed by examining the segregation patterns of allozymes in the open-pollinated progenies. Ritland's multilocus mixed mating model (Ritland & Jain 1981, Ritland 1990) was used to estimate the following parameters: 1) tm and ts - multilocus and average single locus outcrossing rates (calculated by the Newton-Raphson method), 2) $r_p$ the correlation of outcrossed paternity within progeny arrays (or the probability that a randomly chosen pair of progeny from the same array are full sibs), 3) F - the average single locus inbreeding coefficient of maternal parents. These parameters were computed for 1994 and 1995 progenies. Multilocus outcrossing rate (tm) was also calculated for each family. Here, pollen frequency was fixed at the population estimate. Variances of the above parameter values were estimated using bootstrap methods (Ritland & Jain 1981), where the unit of resampling was the individual seedling. Bootstrapping was done 1000 times. All these estimations were done with the software "multilocus mating system program" (MLTR) provided by Ritland (1996). Deviations of the progeny genotype frequencies from model expectations were tested using the single locus chi-square goodness of fit of tm to the data. As suggested by Ritland (1996), loci with highly significant chi-square values were excluded from the multilocus estimates (see below).

## Results

Figure 1 shows the map of the flowered plants of *H. sacarolha* in 1994 in the studied site is the figure 1. In 1994, 96 plants were tagged and had their flowers counted. Twenty three plants did not flower, 44 had one to three flowers open per day, and only five plants set more than nine flowers a day (figure 3).

In 1995, 62 plants could be found in the population after the fire (figure 2). All 62 relocated plants flowered. Almost every plant that flowered both years, presented higher number of open flowers per day in 1995 than in 1994 (table 2).

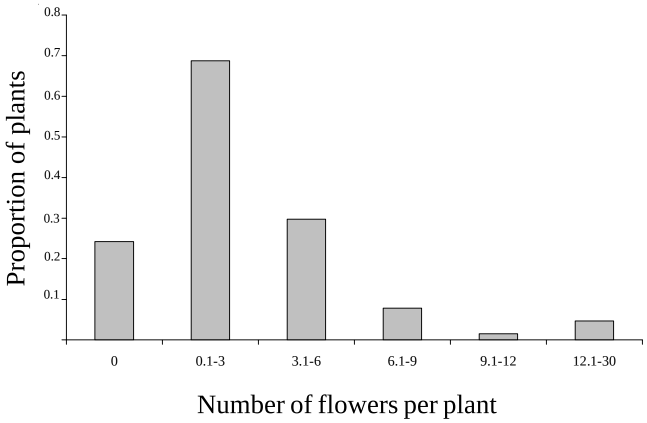

Figure 3. Proportion of plants with different numbers of open flowers. Data collected for the studied population of *Helicteres sacarolha* during the flowering peak of 1994.

The outcrossing rate was significantly higher in 1995 than in 1994 (table 1). The single and multilocus outcrossing rate estimates did not differ significantly (table 1). The fixation index was low for both years. Two loci had highly significant deviation of the progeny genotype frequencies from the model expectations and were not included in the analyses (TPI for 1994 and IDH for 1995 sample). The correlation of outcrossed paternity was significantly higher in 1995 than in 1994 (table 1).

The population of *Helicteres sacarolha* showed significant variation in outcrossing rates for two different plant density patches in 1994 (table 3). At plant level, the 1994 sample showed a positive correlation between the number of flowers per plant and their outcrossing rate, but this correlation was non significant ($r = 0.20$, $N = 18$, $p = 0.43$). For the 1995 sample, this correlation was higher, positive, but also non significant ($r = 0.48$, $N = 12$, $p = 0.11$). This correlation was significant only when samples of 1994 and 1995 were included in the analysis ($r = 0.45$, $N = 30$, $p = 0.012$).

Table 2. Average number of open flowers per day per plant during the flowering period of 1994 (absence of fire) and 1995 (after fire) for *Helicteres sacarolha*.

| Plant | 1994 | 1995 |
|---|---|---|
| 39 | 0.0 | 8.0 |
| 40 | 7.0 | 14.0 |
| 41 | 9.0 | 8.0 |
| 42 | 8.5 | 5.0 |
| 43 | 3.5 | 12.0 |
| 44 | 6.0 | 13.0 |
| 65 | 7.5 | 16.0 |
| 67 | 3.5 | 16.0 |
| 68 | 0.0 | 8.0 |
| 92 | 10.0 | 11.0 |
| 94 | 2.0 | 8.0 |
| 95 | 1.0 | 12.0 |
| 41A | 1.0 | 3.0 |
| Average number of flower | 4.5 | 10.3 |

The correlation between individual plant outcrossing rate and numbers of flowering neighbours within 10 m (plant density) was also positive and almost significant only for the 1995 sample ($r = 0.10$, $N = 18$, $p = 0.66$ for 1994 sample; $r = 0.55$, $N = 12$, $p = 0.06$ for 1995 sample; $r = 0.32$, $N = 30$, $p = 0.88$ for 1994 and 1995 samples). A multiple regression analysis, including the number of flowers per plant and plant density as independent variables, had a regression rate significant only when samples of 1994 and 1995 were included in the analysis ($r= 0.524$, $F_{2,27} = 5.1$, $p = 0.013$).

*Clorostilbon aureoventris* female was the most common hummingbird visitor. These hummingbirds showed traplining behaviour when foraging in the studied population. They may present generalist behaviour either in the beginning or in the end of flowering season, visiting few flowers and plants of *H. sacarolha*, moving soon to other flower species. *Amazilia lactea* and *Clorostilbon aureoventris* males also visited the flowers as generalists, but not very often. On a rare occasion, *Amazilia lactea* defended a territory comprising few large plants of *H. sacarolha*. This happened for two or three days during the flowering peak of 1995, when the number of flowers per plants and flowering plants were very high. Two of those plants had high rates of outcrossing (plant 41, tm = 0.81 and plant 43, tm = 0.84).

There was a highly significant positive correlation between numbers of flowers visited and numbers of flowers open per day per plant ($r = 0.89$, $N = 12$, $P < 0.01$).

## Discussion

After the fire, the number of plants in the remaining population had reduced from 96 in 1994 to 62 in 1995. Others 32 plants either died or simply could not be found in the area. *Helicteres sacarolha* has xylopodes. If the xylopodes do not resprout after the fire or produce small branches with few leaves, it becomes very difficult to locate plants in the field and to know if plants are dead or not because the xylopodes may be alive and resprout latter.

Although the number of plants in the studied population was apparently lower after fire, the population outcrossing rate was higher in 1995 than in 1994. This may be due mainly to variation in the number of flowers per plant and total number of flowers. After the fire, the resprouted plants produced new branches with many flowers. Out of the 96 plants observed in 1994, 23 had no flowers during the flowering season, 32 had 1 or 2 flowers open per day, and only 15 plants produced more than 5 flowers per day on average during this flowering period. By contrast, in 1995, all 62 relocated plants flowered and most of them set higher number of flower

Table 3. Outcrossing rates of *Helicteres sacarolha* for patches 1 and 2 in 1994. The density in each patch is the average number of neighbouring plants within 10 m radius of the sampled plants. N is the number of sampled plants. Number of seeds is in parentheses. Outcrossing rates ($t_m$ and $t_s$) of patches 1 and 2 are significantly different from each other (T-test, $P < 0.001$). The correlated mating parameters ($r_p$) are significantly different from each other (T-test, $P < 0.01$). The plant density in the area 1 is significantly different from the area 2 (T-test, $N = 15$, $P < 0.05$). The average number of flower per plant in the area 1 is significantly different from the area 2 (T-test, $N = 95$, $P < 0.01$).

| Patch | Density of flowering plants (SD) | Flower density (SD) | N | $t_m$ (SD) | $t_s$ (SD) | $r_p$ |
|---|---|---|---|---|---|---|
| 1 | 2.7 (1.0) | 2 (2.2) | 7 (135) | .37 (.06) | .32 (.06) | .51 (.13) |
| 2 | 4.3 (1.2) | 4.1(5.5) | 8 (121) | .58 (.07) | .56 (.06) | .40 (.05) |

in 1995 than in 1994 (table 2). On average, these plants produced twice as much flower in 1995 than in 1994. Consequently, the population flower density and pollinator activities increased in 1995. The lower proportion of full sib seeds within progeny in 1995 compare to 1994 corroborates this assertion.

Fire induces several other plant species of the Brazilian savannah to flower (Figueira 1998, Hoffman 1999). This has been seen also for savannah species of Australia (Johnson *et al.* 1994) and Mediterranean species of Israel (Potts *et al.* 2003). The studied population of *Helicteres sacarolha* had been protected against fire for more than 30 years before the fire of 1994 happened. Despite the fact that flower density and outcrossing rate increased after 1994 fire, repeated fire may decrease the population size of *H. sacarolha* if some plants are killed by fire, as the data suggest. A simulation of the effect of spatial and temporal variation in fire regimes on the population viability of a *Banksia* species showed that when fire frequency is high, the population extinction probability increases (Bradstock *et al.* 1996). Sampson *et al.* (1994) found low outcrossing rates for populations of *Banksia brownii* and suggested that frequent events of fire may have decreased plant density and outcrossing rate within population.

Outcrossing rate in different plant density areas – The population of *Helicteres sacarolha* showed significant variation in outcrossing rate for two different plant density patches in 1994 (table 3). Patch 2 had a higher outcrossing rate than patch 1. This may be due to variation in plant density in the population and also to variation in number of flower per plant. Patch 2 had a higher plant density, higher proportion of plants with many flowers, and fewer plants without any flowers than patch 1 in 1994 (figure 4, table 3). When plant and flower densities within population are high, pollinator activity among plants increases and many plants are visited, which enhances pollen exchange among them. This may have happened in patch 2, leading to a lower proportion of full sib seeds compared to patch 1.

Outcrossing rate at plant level – The correlation rate between the number of flowers per plant and their outcrossing rate was positive and significant ($r = 0.45$, $N = 30$, $P = 0.012$). However, this correlation was significant only when samples of 1994 and 1995 were combined for the analysis. When these samples were analysed individually, the correlation rates were positive but not significant ($r = 0.20$, $N = 18$, $p = 0.43$ for 1994 sample and $r = 0.48$, $N = 12$, $p = 0.11$ for 1995 sample). This may be explained by the increased sample size and also by the higher rates of outcrossing and number of flowers per plants in 1995 than in 1994.

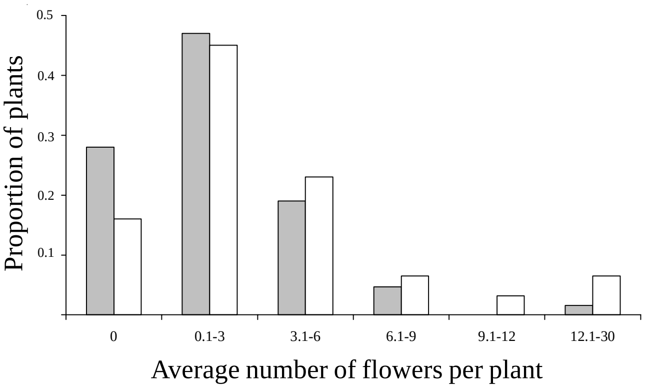

Figure 4. Proportion of plants with different numbers of open flowers for two areas with different plant densities. Data collected for the studied population of *Helicteres sacarolha* during the flowering peak of 1994. ■ = lower plant density, □ = higher plant density.

The correlation between individual plant outcrossing rate and plant density was also higher for the 1995 sample, although not significant. This may be explained also by the increased rates of outcrossing per plant in 1995. Although flowering plant density had not changed from 1994 to 1995, plants in 1995 apparently set more flowers, resulting in increased pollinator activity among plants and their outcrossing rates.

A multiple regression analysis, including as independent variables the number of flowers per plant and plant density, had a regression rate significant only when samples of 1994 and 1995 were combined in the analysis ($R = 0.524$, $F_{2,27} = 5.1$, $p = 0.013$). The number of flower per plant showed higher and significant beta (0.42, $p = 0.017$), while plant density had a low and non-significant beta (0.26, $p = 0.12$). Thus, at plant level, the number of flower per plant seems to have higher influence on the variation of outcrossing rate than plant density.

Comparison with a cogeneric species – In 1994, a sympatric population of a cogeneric species, *Helicteres brevispira,* had higher outcrossing rates (tm = 0.49 to 0.68, Franceschinelli & Bawa 2000) than *H. sacarolha* (tm = 0.37 to 0.58). This may be explained by the lower plant density of *H. sacarolha* population and by the traplining behaviour of the *H. sacarolha* pollinators. These pollinators visited almost every open flower during each visit to a plant, which may increase correlated mating and decrease pollen flow and crossing between distant plants. In the studied population of *H. brevispira*, traplining behaviour is common only in low plant density areas, where outcrossing rates also showed low values (Franceschinelli & Bawa 2000).

The population of *Helicteres sacarolha* has uniformly low plant density compared to the population of *H. brevispira*. However, it seems that a small change in plant density and flower number per plant can have a major effect on the outcrossing rate in *H. sacarolha* as compared to *H. brevispira*. For example, a threefold difference in plant density increased outcrossing rate from 0.49 to 0.68 in *H. brevispira* (Franceschinelli & Bawa 2000) whereas in *H. sacarolha* for a twofold difference the increase was from 0.37 to 0.58 (table 3). A previous study showed that each flower of *H. sacarolha* produces close to three times more nectar than each flower of *H. brevispira* (Franceschinelli 1989). Thus, a small variation in flower number may cause a higher impact on the pollinator activity and outcrossing rate in the population of *H. sacarolha* than in *H. brevispira*.

Franceschinelli (1989) also showed that selective abortion against self-fertilised fruits is stronger in *H. sacarolha* than *H. brevispira*. Other studies have revealed that abortion against self-fertilised fruits becomes stronger as the number of cross-pollinated flowers increase on a plant (Becerra & Lloyd 1992), which might be the case of *H. sacarolha* (Franceschinelli 1989). The higher number of flowering plants and flowers per plant after fire increased the rate of cross-pollination and perhaps the selection against self-fertilised fruits within plants of *H. sacarolha*.

Overall, fire may positively or negatively influence reproduction and outcrossing rates, depending upon its frequency and intensity.

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