

Alternations of sexes in a deciduous tree: temporal dioecy in *Bridelia retusa*

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Bridelia retusa (Euphorbiaceae), a monoecious tree species at Bhimashankar, Maharashtra, was found to exhibit temporal dioecy – a system of sex expression in which there is little or no overlap between the production of male and female flowers within an individual tree during the flowering season. There was variation between individuals and between populations in the number of sex alternations. Some individuals that were phenotypically more male were functionally more female relative to the gender of other individuals in the population. The possible advantages of this breeding system are discussed and experiments to confirm these postulations are suggested.

ANGIOSPERMS present a bewildering array of sexual systems ranging from hermaphroditism to dioecy. Hermaphrodites either have perfect hermaphroditic flowers or are monoecious with separate male and female flowers on the same individual. In dioecious species, male and female flowers occur in separate individuals. Between hermaphroditism and dioecy are sexual systems incorporating such sex expressions as gynomonoecey (coexistence of hermaphroditic and female flowers on a single plant), andromonoecey (coexistence of hermaphroditic and male flowers on a single plant), gynodioecy (coexistence of hermaphrodites and female individuals in a population), and androdioecy (coexistence of hermaphrodites and male individuals in a population).

Although these sexual systems were recognized and named by Linnaeus and Darwin, the selective forces responsible for their origin, evolution and maintenance are still unresolved¹⁻⁴. Avoidance of inbreeding, optimal allocation of reproductive effort between male and female function, sexual selection, and the interaction of these forces with ecological factors have been proposed as explanations for the variety of sexual systems in plants⁵⁻⁸. The understanding of the evolution of sexual systems is further complicated by the fact that many species also possess traits such as genetic self-incompatibility mechanisms, heterostyly, cleistogamy, and dichogamy. Traits such as dichogamy, in which there is a temporal separation of male and female phases within a flower, effectively convert a morphologically

perfect-flowered hermaphroditic individual into a plant bearing functionally unisexual flowers at any given time.

The interplay between morphology and functionality is of considerable interest in the evolution of sexual systems such as temporal dioecy. The term temporal dioecy was coined by Cruden and Hermann-Parker⁹ to describe a breeding system in which there is temporal alternation of male and female function within an individual during a single flowering season. It describes a flowering pattern in which the staminate and pistillate phases of an individual are so distributed that there is little or no overlap between the male and female phases of an individual hermaphroditic plant at any given time. In plants with perfect flowers, this can be achieved by synchronous dichogamy via successive episodes of either synchronized protandry or protogyny¹⁰. In monoecious plants, it can be arrived at by second-order dichogamy¹¹ in which the production of male and female flowers alternates through time within an individual plant.

Although temporal dioecy was first mentioned by Darwin¹², it has usually been thought of as rare and anomalous and has been neglected as a breeding system¹³. There has been almost no theoretical investigation of the conditions responsible for the evolution of temporal dioecy relative to true dioecy¹⁴. Temporal dioecy can also occur with variable frequency among individuals of a population, with some individuals exhibiting temporal dioecy while others do not¹⁴. There is also intra-population variation in the number of sex alternations and in the success of the same sexual phase at different times during a single flowering period^{13,15}.

In this paper we report the occurrence of temporal dioecy in *Bridelia retusa* (Euphorbiaceae) and record variation in the frequency of sex alternations among individuals in two sample populations. We speculate on the advantages of this breeding system and we suggest experimentation to confirm these postulations.

Methods

Bridelia retusa is a monoecious, deciduous tree. We sampled the flowering phenology of 23 trees (13 in a

forest patch named Adhal – Population 1; 10 in a forest patch named Hindola – Population 2) at the Bhimashankar Wildlife Sanctuary, Pune District, Maharashtra. We collected a minimum of two flowering twigs from each of these trees on the following dates: 22 April, 28 April, 4 May, and 26 May 1996 (henceforth referred to as Times I–IV). From these samples, for each tree, we counted the number of open male and open female flowers. We dissected flower buds and noted the presence of male and female buds and their relative sizes. We recorded the presence and relative sizes of fruit. For each sample day, we calculated the phenotypic gender (P_{id}) of each tree by the formula $P_{id} = D_{id}/(D_{id} + L_{id})$, where D_{id} is the total number of open female flowers in the sample from the i th tree on day 'd', and L_{id} is the total number of open male flowers in the sample from the i th tree on day 'd'¹⁶. This measure can vary from 0 (male phase only) to 1 (female phase only). For each sample day, for each tree, we also calculated S_{id} – the standardized phenotypic gender following Lloyd and Bawa¹⁷. S_{id} weights investment in male function according to the relative availability of pollen and ovules in the entire population.

$$S_{id} = \frac{D_{id}}{D_{id} + (L_{id} E_{id})}$$

E_{id} is an equivalence factor and is given by $E_{id} = (\sum(D_{id}/L_{id}))/i$. It is the average ratio of female to male flowers within the samples for all trees sampled on a given day. S_{id} also varies from 0 (male phase only) to 1 (female phase only).

Results

The flowering and fruiting season of *Bridelia retusa* had already commenced when we started collecting samples. By 26 May, 1996 (Time IV), flowering had nearly ended with samples from only 5 trees in Population 2 bearing open male flowers (2–6 flowers) which were considerably smaller than the male flowers of earlier cycles. At Time IV, samples from only 2 trees (one in each population) had a few, small male buds. There was no evidence that any further buds of either sex would arise on any of the sampled individuals. We, therefore, calculated phenotypic gender only for Times I, II and III. The pattern of change in the phenotypic gender varied among individuals and between the two populations (Table 1). In Population 1, the change was mostly from female to male with the exception of Tree # 7 which remained all-male. This tree did not produce any open female flowers, female buds or fruits throughout the observation period. In Population 2, there were complete reversals from male to female to male phases (4/10 individuals) within the short duration of 12 days (22 April–4 May), as well as male to female alternations

Table 1. Phenotypic gender (P_{id}) and standardized phenotypic gender (S_{id}) of individuals of *Bridelia retusa*

Tree no.	P_{id}			Time	S_{id}		
	I	II	III		I	II	III
Population 1 (Adhal site)							
1	0.35	0.02	0.00	0.13	0.94	0.00	
2	0.88	–	0.25	0.66	–	0.21	
3	–	0.00	0.00	–	0.00	0.00	
4	1.00	0.00	–	1.00	0.00	–	
5	1.00	0.00	0.00	1.00	0.00	0.00	
6	0.00	0.00	–	0.00	0.00	–	
7	1.00	–	0.00	1.00	–	0.00	
8	0.94	0.00	0.00	0.82	0.00	0.00	
9	1.00	1.00	0.00	1.00	1.00	0.00	
10	0.00	0.00	0.00	0.00	0.00	0.00	
11	0.50	–	0.00	0.22	–	0.00	
12	0.89	–	0.40	0.69	–	0.90	
13	0.88	1.00	0.00	0.66	1.00	0.00	
Population 2 (Hindola site)							
1	0.08	1.00	0.00	0.86	1.00	0.00	
2	0.00	0.00	0.63	0.00	0.00	0.62	
3	0.00	0.00	–	0.00	0.00	–	
4	0.00	1.00	0.00	0.00	1.00	0.00	
5	0.00	1.00	0.00	0.00	1.00	0.00	
6	0.00	1.00	0.00	0.00	1.00	0.00	
7	0.03	0.00	0.00	0.65	0.00	0.00	
8	0.00	0.00	0.00	0.00	0.00	0.00	
9	0.03	0.00	0.00	0.70	0.00	0.00	
10	0.00	0.00	0.90	0.00	0.00	0.90	

Times I, II and III are 22.4.96, 28.4.96 and 4.5.96 respectively. Blanks indicate samples with no open flowers of either sex. Values of both P_{id} and S_{id} range from 0 (male phase only) to 1 (female phase only).

(2/10 individuals). Two plants were recorded with only open male flowers during the sample period, although they did produce fruit. There were no all-male trees in Population 2.

The standardized phenotypic gender measures indicate that in Population 1, two trees which were phenotypically mostly male were 'functionally' more female when compared to the gender of the population at that time (Tree # 1 at Time II, and Tree # 2 at Time III; Table 1). Similarly, Tree # 1, 7, and 9 of Population 2 were 'functionally' female when phenotypically mostly male at Time I (Table 1).

Since our sampling of the flowering phenology did not begin at the onset of the flowering season, there were already fruit present in some samples taken at Time I. Also, after Time III, there was a 22-day interval during which no samples were collected. Based on the presence and size of fruit in the samples collected at Time I and on the sex of the buds available in the samples at Time III, we inferred the presence of a female or male phase prior to Time I and after Time III (Table 2). Based on these projections, there appear to be mostly female to

male transitions in Population 1 but several cycles of transitions in Population 2.

Discussion

Unlike animals which can usually respond to unfavourable environmental conditions or unfavourable sex ratios by moving between environments or populations, plants are sessile, and can only respond to adverse situations by complex patterns of growth and reproduction. In animals, low or no mobility as in sessile forms, has been advanced as an important factor in the evolution of simultaneous hermaphroditism in which both sexes are simultaneously expressed and could self-fertilize if necessary¹⁸. The size-advantage hypothesis has been usually invoked for explaining sequential hermaphroditism in animals, in which individuals start out as either male or female but switch sexes as they become larger and/or older¹⁸. These animal-based ideas were the origin of theories for the evolution of hermaphroditism versus dioecy in plants wherein the law of diminishing returns would influence a plant's decision to invest reproductive effort in combined or separate sexes⁵. Avoidance of self-fertilization, optimal allocation of reproductive effort via maleness or femaleness, local mate competition, and sexual selection, coupled with environmental conditions conducive for male or female reproduction are some of the current explanations for the variety of sexual systems found in plants^{6, 19-21}.

Table 2. Alternations of sexes in *Bridelia retusa* individuals based on phenotypic gender, fruit and bud sex

Population 1 (Adhal site) (n = 13)	
Alternation	Number of individuals
F → M	10
M → F	1
F → M → F	1
All M	1
Population 2 (Hindola site) (n = 9*)	
Alternation	Number of individuals
F → M	2
M → F	1
F → M → F	1
M → F → M	2
F → M → F → M	2
M → F → M → F	1

Alternations based on phenotypic gender at Times I, II, and III and on presence of fruit at Time I (indicative of at least one female phase prior to Time I), and of bud sex at Time III (indicative of identity of sex phase after Time III).

* In case of Tree # 3, there were no fruit prior to Time I, no female flowers at Times I-III, but mature fruit at Time III. The possible sequence of sex alternations in this tree could not be determined, resulting in n = 9 instead of n = 10 in Population 2 in this case.

Temporal dioecy is reported to occur in 122 genera of at least 37 angiosperm families and is believed to be strongly associated with monoecy and self-compatibility¹³. Temporal dioecy is considered to be adaptive because it can maximize fitness by minimizing geitonogamy and maximizing outcrossing¹³. After investigating a large sample of hermaphroditic and monoecious angiosperms, Bertin²², however, argued that self-fertility may be independent of sexual system; for instance, dichogamy is equally common among self-compatible and self-incompatible species. Whether *B. retusa* is self-compatible remains to be determined.

Temporal dioecy has also been suggested as a bet-hedging strategy in plants (sensu Stearns²³) by several workers^{15,16,24}. By alternating sexes throughout the flowering season, it is believed that temporally dioecious plants can counteract the negative effects of factors such as local mate competition, and can optimize reproductive effort through either male or female function given the unpredictable nature of the sexual state of neighbouring individuals, i.e. the mating environment of flowers (sensu Brunet and Charlesworth²⁵), and thereby the unpredictability of reproductive success. From this perspective, it would also be interesting to determine whether individual plants are plastic or fixed in the duration and number of sex alternations over successive flowering seasons, and in relation to their neighbours. For example, in *Atriplex canescens*, some clones have fixed gender expressions while in others, gender varies, and the magnitude and direction of sex change is both genetically and environmentally determined²⁶.

In our limited investigation of the flowering of *Bridelia retusa*, we found evidence for several alternations of sexes in some individuals and at least one individual which was all-male. We do not know if the sequence and durations of sex alternations observed in these two populations will be repeated during subsequent flowering seasons, and whether the all-male tree will continue to remain unisexual. It is possible that this year, the all-male tree was merely exhibiting an extreme in a continuum of intra-tree sex ratios possible within a monoecious species²⁷. Yet, recent studies have revealed the presence of stable sexual polymorphisms within populations of plant species which may include a combination of males, females and hermaphrodites some of which are temporally dioecious^{14,28}. Continued monitoring of marked *Bridelia* individuals over several flowering seasons will be necessary to determine the stability of sex expression in this species.

We also found that some plants which were phenotypically more male were 'functionally' more female relative to the gender of other individuals in the population. The phenotypic gender of other individuals in the population can cause the uncoupling of phenotypic and functional gender of an individual²⁹. In the temporally dioecious *Aralia hispida*, Thomson and Barrett¹⁵ found

that the reproductive success of a ramet through pollen was negatively correlated with the degree of flowering synchrony between the ramet and the population.

In this study we have observed differences between two *Bridelia* populations, albeit on a short time scale. Population 2 appeared to have more types of sex alternations than the other. It is possible that the relation between local environment and sex expression also needs to be investigated. Consistent differences between *Bridelia* populations at the two sites over several flowering seasons may be indicative of an environmental effect. Directional sex change in plants is known to be a function of environmental conditions such as soil fertility where greater fertility causes more femaleness or where higher temperatures cause maleness^{8,30,31}. Females appear to be produced when stress is lacking.

Can the *Bridelia retusa* system be used to address questions regarding the lengths, number and success of the different sexual phases within individual trees? For example, a plant that goes through more than one female phase may be able to evaluate pollen donors from the first phase relative to the second phase, and abort fruits developing from lower quality pollen in the first phase in an attempt to hedge bets and obtain better quality pollen in the second phase. Fruit abortion is an established mechanism employed by plants in mate choice³². Alternatively, if the Bateman Principle is operating and if maternal fitness is determined only by resource availability while paternal fitness is limited by access to ovules, the male phase should have a more variable distribution in the flowering phenology of an individual, and consequently more variable reproductive success at different times^{15,16}.

Determination of true male reproductive success is extremely difficult. Thomson and Barrett¹⁵ attempted to overcome this difficulty by assuming that male reproductive success at any given time was a function of the proportion of open male flowers on an individual plant relative to the population. Assumptions about panmixia and sizes of the mating neighbourhood have also to be made. In order to evaluate female reproductive success, experiments are possible in which flowers are pollinated with self- versus cross- pollen, or if self-incompatible, with outcross pollen from single donors versus mixed pollen from multiple donors. The judicious design of such experiments in different female cycles on the same set of individuals may indicate whether there is any effect of such treatments on the occurrence and success of subsequent female phases. One major shortcoming of an experimental approach with a medium-sized tree like *Bridelia* is that we do not know whether localized experimentation on a few branches is sufficient to alter sex expression on these branches. Although Richardson and Stephenson³³ showed that removal of pollen hastened the onset of the female phase in the protandrous *Campanula rapunculoides*, there is only scant knowledge of

the feedback systems between and within modules in a plant.

There is much to be understood about sex expression in hermaphroditic plants and about the proximal and ultimate causes for specific strategies³⁴⁻³⁶. Considering the absence of sufficient empirical evidence and the mushrooming of theoretical models, Givnish⁷ despaired of the often selective invocation of factors to explain evolutionary scenarios, and thereby the use of arguments by *deus ex machina* instead of rigorous testing of a few models. It is now also recognized that the assumption that hermaphroditic individuals function half as female and half as male is no longer valid³⁷. Sexual asymmetry is widespread and is in need of investigation³⁸. Goldman and Willson⁸ highlight the seeming chaos in the understanding of sex allocation in hermaphroditic plants. They advocate rigorous empirical observation and experimentation since only an augmented data base will provide the large within- and between-species sample sizes necessary to find the patterns and understand the underlying processes. Experimentalists need to find innovative ways of addressing these problems. Systems like those exhibited by *Bridelia retusa* provide opportunities for adding to the general understanding of sex allocation as exhibited in a temporally dioecious species.

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Adaptive evolution and the footprints of history

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The genetic structure of a population at any given time is a reflection of the combined effects of many factors such as past selection history, ongoing selection, ancestry and chance, in the form of random divergence among lineages derived from a common ancestor. In this paper, I describe results from two experiments on the bacterium *Escherichia coli* and the fruit-fly *Drosophila melanogaster*, respectively, in which the contribution of these various factors to adaptive evolution was quantified in rigorous laboratory experiments. In both species, current ongoing selection obliterated the effects of past selection and ancestry on fitness-related traits. In the asexually reproducing *E. coli*, the effects of history on traits

not related to fitness were still important after 1000 generations, whereas in the sexually reproducing *D. melanogaster*, traits uncorrelated with fitness showed most variation (~95%) to be among individuals within populations. When an effect of history was seen, it was largely due to past selection experienced by a population rather than ancestry *per se*. The time scale of adaptive evolution was much faster in *D. melanogaster*, and the obliteration of the effects of history much more complete, suggesting that genetic recombination may play a major role in removing historical constraints and facilitating adaptive evolution in sexually reproducing species.

BIOLOGICAL systems are characterized by the two inter-linked attributes of variation and evolution¹⁻⁵. Although much of the observed variation in populations is undoubtedly environmentally generated, a substantial part of the variation among individuals is due to genetic differences. It is this genetic variation that not only constitutes the raw material for agents of evolutionary change to act upon, but also reflects the outcome of any evolutionary change⁴. In fact, it would not be inaccurate to say that evolution largely consists of the reshaping of patterns of genetic variation within and among populations.

Evolution, it has been said, is what makes biology a different sort of subject from physics, and a key element in evolution is that of historicity⁶. The genetic structure of a population at any given time is a reflection of the combined effects of many factors. The selection pressure faced by a population in the recent past constitutes a directional force that is responsible for the adaptive effects of evolutionary change: natural selection tends to favour the increase in frequency of those genetic variants that make an individual better adapted to the present environment. At the same time, natural selection is constrained by the range of genetic variation available in