

Evolution Towards Dioecy in Heterostylous Populations

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Abstract: The effective gender of a plant is defined as the proportion of the plant's genes that are transmitted through pollen (its maleness) or through ovules (its femaleness). Formulae are derived that enable the average effective gender of the morphs of a heterostylous population and the gender of individual plants to be estimated. Estimates require a knowledge of the morph ratio and the seed set that results in each morph from self-fertilization and from legitimate and illegitimate cross-fertilizations. If no illegitimate fertilizations occur, the average gender of the morphs can be estimated from their seed production alone; in this situation the average femaleness of one morph is exactly equal to the average maleness of the other morph. The average gender of long- and short-styled morphs is calculated for populations of five *Cordia* species (*Boraginaceae*) from published data of OPLER & al. (1975). In two species, both morphs transmit their genes equally through pollen and ovules. In the other species, the long-styled morph acts predominantly (or in one species exclusively) as an ovule parent and the short-styled morph succeeds predominantly (or exclusively) as a pollen parent.

The features of the evolutionary pathway from heterostyly to dioecy and the selective forces that may be responsible are outlined.

In heterostylous species, the two or three floral morphs in a population have hermaphrodite flowers and yet they are as dependent upon each other for cross-fertilization as are the males and females of dioecious species (DARWIN 1877). Besides pointing out the significance of heterostyly as a mechanism promoting outbreeding, DARWIN postulated that in several groups heterostyly had evolved gradually into dioecy. Since then VOGEL (1955), BAKER (1958), ORNDUFF (1966) and others have proposed additional examples of the evolution of heterostyly, or more particularly distyly, to dioecy. (Tristyly is not known to evolve directly to dioecy and will not be further considered here.) The evolution of distyly towards dioecy implies that the two morphs no longer make

equal contributions to their sexual offspring through their pollen and ovules. One morph, either the long- or short-styled one, comes to act predominantly or even exclusively as a pollen parent and the other morph conversely specializes as an ovule parent. This gender specialization results when one direction of fertilization, characteristically that from the short-styled morph as pollen parent to the long-styled morph as ovule parent, becomes more important than the reverse direction. There have, however, been no previous attempts to measure exactly how far any population has moved along this evolutionary pathway.

In this paper, I propose to show how measurements of the frequencies of the two morphs and of their levels of seed production and self-fertilization can provide estimates of their sexual nature or gender. I have suggested elsewhere that exact descriptions of gender should employ quantitative and functional measures estimating the relative success of a plant as a pollen and an ovule parent (LLOYD, in prep. a, b). It was recommended there that the best measures of such "effective gender" are the proportions of a plant's genes that are transmitted through pollen (its maleness) or through ovules (its femaleness). To obtain estimates of this nature, one must somehow estimate the androecial or pollen fitness of a plant, a_i , the number of offspring contributed to the next adult generation via pollen, and the gynoeceal fitness, g_i , the number of offspring contributed via ovules. The gynoeceal fitness can be estimated from either ovule production (ovule fitness) or, preferably, seed production and quality (seed fitness). The total fitness of a plant is the sum of its androecial and gynoeceal fitnesses, i.e. $w_i = a_i + g_i$.

Then the femaleness of a plant,

$$G_i = \frac{g_i}{g_i + a_i}, \quad (1a)$$

and its maleness,

$$A_i = \frac{a_i}{g_i + a_i}. \quad (1b)$$

If the pollen of all plants competes at random for the fertilization of all cross-fertilized ovules, it is possible to estimate effective gender by calculating an equivalence factor that weights the expected fitness of a single pollen grain so that it is commensurable with the expected fitness of a single ovule or seed (LLOYD, in prep. a). But in heterostylous populations, the floral dimorphism and the incompatibility reaction associated with it place a restriction on the success of pollen in cross-fertilization. Pollen succeeds exclusively or predominantly in "legitimate" cross-pollinations between the two morphs. One must assess the pollen fitness of the two morphs differently by taking into account the number of ovules that each morph can fertilize legitimately and illegit-

imately. It is therefore not possible to compare pollen and ovule contributions with the aid of a single equivalence factor for all members of a population.

The next section devises special methods for estimating the gender of the morphs of distylous populations from measurements of the frequencies of the two morphs and their seed production and patterns of fertilization. Under certain conditions, the effective gender of the morphs can be estimated with remarkable ease. These conditions are satisfied in *Cordia* species, and in the subsequent section the effective gender of five *Cordia* species is estimated from data of OPLER & al. (1975).

Theory

Let the number of long- and short-styled plants in a population or in a representative sample be n_l and n_s respectively. The number of seeds produced by a long-styled plant, d_l , may vary from plant to plant. A variable fraction, s_l , of these seeds are self-fertilized and the remaining fraction, t_l , are cross-fertilized; of the cross-fertilized seeds, a variable fraction c_l result from legitimate (compatible) fertilization by pollen from short-styled plants while the remaining fraction r_l result from illegitimate cross-fertilization by pollen from other long-styled plants. Hence $s_l + t_l(c_l + r_l) = 1$ for any long-styled individual. Similarly, $s_s + t_s(c_s + r_s) = 1$ for the d_s seeds produced by a short-styled individual. All cross-fertilized seeds from either legitimate or illegitimate fertilizations are considered to have a fitness of one, while seeds from self-fertilization have a relative inbred fitness, i_l for a long-styled plant and i_s for a short-styled plant, where $0 \leq i \leq 1$. Then the total seed fitness of all long-styled plants combined,

$$\sum g_l = \sum d_l (t_l + s_l i_l).$$

A parallel expression can be written for short-styled plants, but to save space henceforth expressions will generally be written only for long-styled plants.

The total pollen fitness of all long-styled plants is the sum of their pollen success from legitimate pollinations of short-styled plants, illegitimate cross-fertilizations of other long-styled plants and self-fertilizations. That is,

$$\sum a_l = \sum d_s t_s c_s + \sum d_l t_l r_l + \sum d_l s_l i_l.$$

The combined (pollen plus seed) fitness of all long-styled plants,

$$\sum w_l = \sum g_l + \sum a_l = \sum d_l [t_l (1 + r_l) + 2 s_l i_l] + \sum d_s t_s c_s.$$

Thus the average femaleness of all long-styled plants,

$$\bar{G}_l = \sum g_l / (\sum g_l + \sum a_l) = \frac{\sum d_l (t_l + s_l i_l)}{\sum d_l [t_l (1 + r_l) + 2 s_l i_l] + \sum d_s t_s c_s}. \quad (2)$$

To calculate the effective gender of the two morphs requires a formidable amount of information on the morph ratio, the numbers of seeds the two morphs produce, the pollen parents of the seeds and the effects these have on seed fitness. Fortunately, it is often possible to reduce the burden of data-gathering by introducing simplifying assumptions that do not greatly reduce the accuracy of gender estimates.

One such assumption, that illegitimate fertilizations are absent or of negligible frequency, simplifies the formulae required and leads to some remarkable properties. If there are neither self-pollinations nor illegitimate cross-pollinations of the ovules of both morphs, then for all individuals $t = 1$, $s = 0$, $c = 1$ and $r = 0$. Equation 2 then reduces to

$$\bar{G}_l = \frac{n_l \bar{d}_l}{n_l \bar{d}_l + n_s \bar{d}_s}. \quad (3a)$$

Similarly,

$$\bar{G}_s = \frac{n_s \bar{d}_s}{n_l \bar{d}_l + n_s \bar{d}_s}. \quad (3b)$$

In the absence of illegitimate fertilizations, the effective gender of the morphs can be calculated simply from the total seed production of the two morphs in the whole population or sample. If one could further assume that individuals of the two morphs are either equally frequent or produce equal numbers of equivalent seeds, then effective gender estimates could be obtained from either the seed numbers per plant or the morph ratio alone. It is scarcely possible to obtain a simpler method for estimating the effective gender of subclasses of a population.

If all successful fertilizations are legitimate cross-fertilizations between long-styled and short-styled plants, the total pollen fitness of either morph is equal to the total seed fitness of the other morph. That is,

$$\sum a_l = \sum g_s, \text{ and } \sum a_s = \sum g_l. \\ \therefore \bar{G}_l = \frac{\sum g_l}{\sum g_l + \sum a_l} = \frac{\sum a_s}{\sum g_s + \sum a_s} = \bar{A}_s = 1 - \bar{G}_s. \quad (4a)$$

Similarly,

$$\bar{G}_s = \bar{A}_l = 1 - \bar{G}_l. \quad (4b)$$

The average femaleness of one morph is exactly equal to the average maleness of the other morph. The evolution of heterostyly towards dioecy is therefore characterized (in the absence of illegitimate self-fertilizations) by the two morphs always approaching unisexuality to exactly the same degree. The specialization of one morph as a pollen

parent is exactly matched at the same time by reciprocal specialization of the other morph as an ovule parent. This is not generally true of evolution towards dioecy by other pathways (LLOYD, in prep. b).

The gender of an individual plant may depart from the morph average. The seed fitness of an individual long-styled plant,

$$g_l = d_l (t_l + s_l i_l).$$

If f_l is the fraction of all long-styled pollen that is produced by one particular long-styled plant, the pollen fitness of that individual,

$$a_l = f_l (\sum d_l t_l r_l + \sum d_s t_s c_s) + d_l s_l i_l,$$

assuming that all pollen grains of long-styled plants have equal chances of cross-fertilizing an ovule. The femaleness of a long-styled plant is then,

$$G_l = \frac{d_l (t_l + s_l i_l)}{d_l (t_l + 2 s_l i_l) + f_l (\sum d_l t_l r_l + \sum d_s t_s c_s)}. \quad (5)$$

The distribution of gender among a sample of long-styled plants could be obtained by applying equation 5 (or a simplified version of it if illegitimate self- and/or cross-fertilizations are of negligible importance).

Two general features of the methods derived above for obtaining gender estimates for heterostylous populations should be pointed out. First, the estimates do not depend on knowledge of the inheritance of heterostyly or on whether the morph ratio of a population is stable. They do, however, require accurate estimates of the proportions and seed performances of the morphs. To be complete, information on the seed fitness of the morphs should ideally extend beyond seed production to the fate of the seeds up to sexual maturity of the next generation. Second, the formulae obtained (except for equation 5 for individuals) do not contain expressions for pollen production. Estimates of the effective gender of angiosperm populations generally require the pollen fitness of individuals to be estimated from their pollen production (LLOYD, in prep. a, b). Fortunately, the overall pollen fitness of heterostylous morphs can be obtained indirectly from a knowledge of seed production of the morphs and the sources of pollen which fertilized the seeds.

The formulae derived above for heterostylous species could also be applied to other non-heterostylous populations which consist of two morphs that are habitually or exclusively fertilized by each other. These include the heterodichogamous populations of *Acer* (DE JONG 1976), and avocado populations which have two protogynous morphs that begin anthesis at different times of the day when the flowers of the other morph present their pollen (STOUT 1927).

Heterostylous *Cordia* Species, Worked Examples

There are no data known to the author which could be substituted in equations 2 and 5 to obtain the average gender and individual gender respectively of heterostylous morphs when illegitimate fertilizations occur at an appreciable frequency. The simpler situation when there is no illegitimate fertilization may be used, however, to demonstrate the use of estimates of effective gender.

In the genus *Cordia* (*Boraginaceae*), species show a range of conditions from heterostyly without gender specialization to dioecy without inconstancies i.e. complete gender specialization. For five *Cordia* species, OPLER & al. (1975) have provided the data necessary to obtain estimates of average gender of the two morphs. They obtained the frequencies of long- and short-styled plants in counts of between 105 and 427 plants of each species (Table 1). They also recorded the average number of fruits per infructescence in the two morphs of the same species (Table 1); it is assumed here that these figures provide unbiased estimates of the seed fitnesses of the morphs. Illegitimate fertilizations are likely to be absent or negligible in all five species. Except for one homostylous species not considered here, all *Cordia* species are either heterostylous and self-incompatible or they are dioecious (OPLER & al. 1975). Two of the five species, *C. dentata* and *C. pringlei*, yielded abundant seed after legitimate cross-pollinations and few (possibly inviable) or no seeds after illegitimate cross- and self-fertilizations (OPLER & al. 1975). Hence equations 3a and 3b are applicable.

The calculations of the effective gender of the morphs of the five species (Table 1) show that little or no gender specialization of the morphs has developed in *C. dentata* and *C. pringlei*. It is not possible to say whether the slight departures from equal contributions through pollen and ovules are significant, since the distributions of ovule fitness among plants of the same morph are not known. There is little doubt, however, that the morphs of both *C. inermis* and *C. collococca* have specialized in gender to a considerable degree. In both species, only 4 or 5 per cent of genes are estimated to be transmitted through pollen in the case of the long-styled plants and through ovules among the short-styled plants. It would be equally appropriate to describe these two species as either heterostylous or subdioecious and the morphs as either long- and short-styled or as female and male. *C. panamensis* has evolved strict dioecy, with the long-styled plants transmitting their genes solely through ovules and the short-styled plants acting as constant (strictly unisexual) males.

The calculations of gender in Table 1 estimate only the average behaviour of the morphs in each species. Conspecific individuals of one

Table 1. Estimates of the frequencies, fruit production and effective gender of long- and short-styled plants of *Cordia* species. The proportions of long- and short-styled individuals and their fruit production are from data presented in OPLER & al. (1975). The average effective femaleness (estimated proportion of genes transmitted through ovules) of the morphs are calculated

from the formulae, $\bar{G}_l = \frac{n_l \bar{g}_l}{n_l \bar{g}_l + n_s \bar{g}_s}$, and $\bar{G}_s = \frac{n_s \bar{g}_s}{n_l \bar{g}_l + n_s \bar{g}_s}$, explained in the text

Species	Long-styled plants			Short-styled plants		
	Frequency in population	Fruit per infructescence	Gender (Female-ness)	Frequency in population	Fruit per infructescence	Gender (Female-ness)
	$\frac{n_l}{n_l + n_s}$	\bar{g}_l	\bar{G}_l	$\frac{n_s}{n_l + n_s}$	\bar{g}_s	\bar{G}_s
<i>C. dentata</i>	0.457	17	0.443	0.543	18	0.557
<i>C. pringlei</i>	0.440	17	0.471	0.560	15	0.529
<i>C. inermis</i>	0.654	9.5	0.947	0.346	1	0.053
<i>C. collococca</i>	0.401	33	0.957	0.599	1	0.043
<i>C. panamensis</i>	0.321	19	1.000	0.679	0	0.000

morph need not be exactly identical in their pollen and seed behaviour. OPLER & al. (1975) mentioned that in *C. inermis* only 17 per cent of short-styled individuals (males) produced any fruit, that is, are inconstant. The occurrence of constant and inconstant individuals of the same sex together in one population also occurs in gender-dimorphic populations derived from monoecy and non-heterostylous hermaphroditism (LLOYD 1975, 1976, in prep. b).

Discussion

The taxonomic distribution of related heterostylous, subdioecious and dioecious species and their floral morphology indicate that evolution towards dioecy has occurred in a number of heterostylous groups besides *Cordia* (DARWIN 1877, VOGEL 1955, BAKER 1958, 1962, ORNDUFF 1966, 1970, REDDY & BIR BAHADUR 1976). In some groups, intermediates between normal heterostyly and strict dioecy are not known (e.g. ORNDUFF 1966). In other groups a range of intermediate conditions suggest that the morphs have become gradually specialized in their gender, as DARWIN (1877) postulated. It would be worthwhile calculating the extent of the progress of heterostylous species towards dioecy in genera

other than *Cordia* with the aid of the equations provided above, but the required information about natural populations is available for few species.

The evolutionary pathway from heterostyly to dioecy differs in several respects from the two more common pathways to dioecy from a monoecious condition and from non-heterostylous hermaphroditism (these pathways are compared in LLOYD, in prep. b). The heterostyly pathway to dioecy is distinguished in the first instance by its origin from a population which is already dimorphic—in floral structure although not in gender. The specialization in gender proceeds in the heterostyly pathway, as in the pathway from hermaphroditism through gynodioecy, by the sterilization or reduced utilization of the pollen of one morph and of the ovules of the other morph. But only in the heterostyly pathway is the loss of function in one morph exactly matched by a reciprocal reduction in the other morph—in the absence of illegitimate fertilizations. The heterostyly pathway also contrasts with the gynodioecy pathway (but not with the pathway from monoecy) in the two morphs being approximately equal in frequency throughout the course of evolution.

It should not be thought, however, that all heterostylous populations are evolving gender specialization. Only a few heterostylous groups have dioecious or subdioecious representatives. DARWIN (1877, p. 258) and BAKER (1958, 1962) have pointed out that most heterostylous species are not evolving towards dioecy. It is known that in some heterostylous populations the seed production is not significantly different in the two morphs (e.g. ORNDUFF 1966, 1975, GANDERS 1975). It follows from the argument of the previous section that both morphs of these populations transmit their genes equally through pollen and ovules. There may be a slight tendency towards gender specialization in other heterostylous species, however. In experimental pollinations of some heterostylous species of Rubiaceae, pin (long-styled) plants produced more seeds than thrum (short-styled) plants (BIR BAHADUR 1970).

The selective forces that have caused the morphs of some heterostylous populations to adopt specialized roles as sexual parents are not well known. The positions of the pollen and stigmas of the two morphs in some species may make the transfer of pollen easier in one direction than the other (BAKER 1958). But this could be interpreted as the result of gender specialization as much as its cause. The behaviour of pollinators is important in determining whether the transfer of pollen is equally likely in both directions. ROBERTSON (1892) noted that butterflies visiting *Houstonia purpurea* L. var. *calycosa* GR. are only adapted

to pollinate the short-styled form. He suggested that "a monopoly of the flowers by them would probably result in functional dioecism, characterized by long-styled staminate and short-styled pistillate flowers". Recently, ORNDUFF (1975) has proposed that different pollinator species are largely responsible for pollinating the long- and short-styled flowers of *Jepsonia heterandra* EASTW.

A quite different selective force may have caused evolution to dioecy in *Nymphoides*. The heterostylous species *N. peltata* KUNTZE has large open flowers and incomplete self-incompatibility. Illegitimate pollinations are probably frequent, and dioecy may have arisen in *Nymphoides* as a mechanism promoting cross-pollination (ORNDUFF 1966). If some selfing occurs, the morph with the higher frequency of self-fertilization would have its seed fitness reduced more, and it would therefore succeed better as a pollen parent. Further gender specialization might arise from increased emphasis in each morph on the more profitable type of gamete.

Gender specialization involves reducing either pollen or ovule capabilities of a morph. In no case is it known exactly how this increases the fitness of individuals. Nor is it known why gender dimorphism has evolved in some heterostylous populations but not in others with similar floral syndromes. Further knowledge of the natural history of heterostylous species is required before the selective forces can be identified more closely. Accurate estimates of the effective gender of heterostylous morphs would be a valuable addition to this knowledge. The effective gender estimates advocated here are an ideal vehicle for discussing selective forces since they are directly based on the components of individual fitness, the success of a plant as a pollen parent and as a seed parent.

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