Functional androdioecy in the flowering plant *Datisca glomerata*

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THE role of androdioecy (the presence of male and hermaphrodite individuals in a breeding population) in the evolution of dioecy has long been the subject of much interest and discussion[1–9]. But no functionally androdioecious species has been previously documented[2] and recent studies have even raised doubt about whether the phenomenon exists at all[3]. Although many cases of androdioecy have been reported, most of these are based on morphological data alone and, when examined in detail, are actually found to be functionally dioecious[10–12]. Here we describe functional androdioecy in the flowering plant *Datisca glomerata* (Presl.) Baill. (Datiscaceae). We suggest that the condition evolved from a dioecious precursor, and not from hermaphroditism as is commonly postulated for the evolution of androdioecy[1–9]. Androdioecy in this case could be a transitional state in the breakdown of a dioecious breeding system towards hermaphroditism.

Datisca glomerata is distributed from northern California to northern Baja California, Mexico. Plants are long-lived tall (1–2.5 m) herbaceous perennials of riparian habitats. Flowering and fruiting occur throughout the summer months. The floral morphology of the species is relatively simple[13,14]. Flowers are apetalous. Calyx lobes are short (1–1.5 mm) and inconspicuous. Male flowers have from six to twenty anthers. At anthesis, the pedicels of male flowers elongate, often reaching lengths of 2 cm or more. In hermaphrodite flowers the ovary is a three-sided capsule with three branched styles. They are protogynous and usually have three anthers. Nectaries are absent. The pendulous male flowers, large styles of the female and hermaphrodite flowers, unornamented pollen[13,14] and the otherwise reduced flowers without nectar, are characteristic of an anemophilous pollination syndrome[15]. Likewise, the height and growth of the plant in an exposed riparian habitat are consistent with anemophily.

We verified the existence of functional androdioecy in *D. glomerata*. Hermaphrodite flowers in this species have fertile pollen (>95% reactive with an enzyme-specific pollen viability stain[16]), dehiscent anthers, and are self-compatible producing as many viable seeds on self-pollination as open pollinated flowers. Hermaphrodite flowers that we emasculated and bagged set no seed, indicating that non-pseudogamous apomixis does not occur. In addition, we examined 23 populations of *D. glomerata* for electrophoretically detectable genetic variation, revealing an inbreeding coefficient of $F = 0.617$ ($P < 0.001$)[17] for six variable loci, indicating high inbreeding[18]. By contrast, we found no deviation from random mating ($F = -0.005$ ($P > 0.995$) for four variable loci) for three populations of the congeneric dioecious species *D. cannabina* L., native to southwestern Asia[17].

To confirm androdioecy in *Datisca*, it must be shown that individual plants are not sexually labile, that is, able to vary their sex according to their physiological condition or environment[19–21]. In a perennial plant with a prolonged flowering season such changes can be temporary and therefore difficult to detect. But flowers of *Datisca* do not abcise after anthesis, and their sex can be readily distinguished in the dry state. A mature inflorescence therefore contains a complete record of the sex of the flowers from the entire growing season. By observing all branches and inflorescences of 397 flowering plants at the end of the 1989 growing season, we found that plants remained either hermaphrodite or male throughout the growing season (Table 1).

If physiological conditions determine the sex of plants, then we would have expected younger or smaller individuals to be male, and mature plants to be hermaphrodite[21]. But 24 plants grown from seed collected in two completely hermaphrodite populations flowered after two years and were all hermaphrodites. We observed three additional plants cultivated at the Rancho Santa Ana Botanic Garden over three growing seasons, and eight plants growing in the Big Tujunga Canyon population, and found no instances of year-to-year sex changes. Long-term observations of marked individuals in the experimental and natural populations are continuing.

We quantified the sex ratios in 10 populations and found that a 1:1 sex ratio, as would be expected in a functionally dioecious plant[2], does not exist between male and hermaphrodite individuals in *D. glomerata* (Table 1). In those populations in which we observed males, their frequency was always <25%. The finding of relatively few non-flowering individuals indicates that there is little potential for significant changes in these ratios due to recruitment.

One reason for the apparent rarity of androdioecious species is that male plants must have a high male fertility, at least double that of hermaphrodites, in order to be maintained by selection[2,5,8]. An even greater advantage is required in partially self-fertilizing populations[2,5,8] as found in *D. glomerata*, because the gain in fitness through having pollen is least when few ovules are available for outcrossing. Consistent with these stringent conditions for androdioecy, we found that male plants of *D. glomerata* have ~3.8 times as many fertile anthers as do hermaphrodites (male mean $x = 11.3 \pm 2.4$, $N = 97$; hermaphrodite $x = 3.0 \pm 0.6$, $N = 100$; both sexes have about equal numbers of flowers).

Despite the evidence for androdioecy in *D. glomerata*, this breeding system is very rare, and is not generally involved in the evolution of dioecy from hermaphroditism[2,3]. In *Datisca*, it seems that androdioecy is in fact derived from dioecy. Evidence for this is found in the Datiscaceae, where the three species other than *D. glomerata* in the family are all dioecious. This includes *D. cannabina* and the presumed primitive members of the family, *Octomeles* and *Tetrameles*, forest trees of south-east Asia[13,14]. A chloroplast DNA-based phylogeny of the family is now being reconstructed to test the hypothesis that androdioecy in the Datiscaceae is a derived condition.

It is interesting in this context that of 17 putatively (but undocumented) androdioecious taxa listed by Charlesworth[2], all but two have dioecious relatives. In two cases where nearly androdioecious species are thought to exist but no dioecy has

TABLE 1 No. individuals of each sex in populations of *D. glomerata*

Population	Hermaphrodite	Male	Non-flowering
Alder Creek	54	11	14
Baughman Spring	135	8	15
Big Tujunga Canyon	8	0	3
Cedar Springs Dam	30	1	2
Cloudburst Summit	3	0	0
Little Rock Dam	10	0	0
Mt Islip I	45	14	6
Mt Islip II	37	0	9
Tie Canyon I	26	2	0
Tie Canyon II	13	0	1

All populations are in the Angeles National Forest, Los Angeles County, California, USA, except for Cedar Springs Dam which is in the San Bernardino National Forest, San Bernardino County, California, USA. Populations were surveyed at the end of the flowering season to determine if sex changes had occurred over the course of the season as discussed in the text. The frequency of hermaphrodites and males in all populations shows a highly significant deviation from the 1:1 ratio expected with dioecy ($P < 0.001$). Mt, Mount.

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been reported, heterostyly is present[22-24]. Heterostyly, like dioecy, represents an extreme form of outbreeding, and subandrodioecy seems to be the outcome of a breakdown of the heterostylous condition[22-24]. We suggest that functional androdioecy in *Datisca* is the result of an analogous breakdown of the dioecious breeding system. The breakdown of dioecy could have resulted from pollination limitations due to low population densities (D. Charlesworth, personal communication).

In *D. glomerata*, female plants have already been replaced by self-compatible hermaphrodites. In this situation male individuals are at a strong reproductive disadvantage. Perhaps the large number of anthers and high pollen production of the males relative to the hermaphrodites has allowed the androdioecious condition to persist. Nevertheless, four of the populations of *D. glomerata* that we examined for sex ratios had already lost all male individuals (Table 1), and it seems likely that this process will continue, leading to a totally hermaphrodite breeding system. As such, androdioecy in *Datisca* probably represents a rare and transient state in the breakdown of dioecy. □

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Programmed death of autoreactive thymocytes

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T LYMPHOCYTES bearing high-affinity T-cell receptors (TCR) for self-antigens are clonally deleted during thymus development[1]. Several recent studies[1,4-10] have identified variable domains of the β-chain of the TCR that are specifically deleted *in vivo* in mouse strains that express major histocompatibility complex class II molecules in addition to poorly defined self-antigens, including those encoded by the *Mls-1*^{a} and *Mls-2*^{a} loci. Deletion of autoreactive cells in these systems occurs in the thymus, and antibody blocking experiments *in vivo* have implicated the phenotypically immature CD4$^+$CD8$^+$ 'cortical' subset as the target population for clonal deletion[11,12]. Similarly, studies with transgenic mice bearing autoreactive TCR have provided independent evidence that clonal deletion occurs at the CD4$^+$CD8$^+$ stage of development[13]. But none of these studies directly identified dying autoreactive cells, and the circumstances leading to deletion remain unclear. Here we report that neonatal thymus contains a significant population of phenotypically mature CD4$^+$CD8$^-$ cells bearing autoreactive TCR. When placed in short-term culture, a large proportion (60%) of these autoreactive cells die selectively. Furthermore, their death can be prevented by inhibitors of macromolecule (RNA and protein) synthesis, as is the case for glucocorticoid-induced death of thymocytes[2,3]. These data indicate that physiological clonal deletion of autoreactive cells involves 'programmed' cell death, and that it can occur in cells with a mature (CD4$^+$CD8$^-$) surface phenotype.

While investigating the ontogeny of expression of autoreactive β-chain of TCR (TCR Vβ), we have previously observed[14] that Vβ6$^+$ cells with a mature (CD4$^+$CD8$^-$) phenotype could be transiently detected in the early postnatal thymus of BALB.D2.Mls^a (Mls-1^a) mice, although such cells were virtually absent from the thymus and periphery of adult animals. Further analysis of these CD4$^+$ Vβ6$^+$ thymocytes revealed that they expressed approximately twofold fewer TCR than age-matched BALB/c (Mls-1^b) control mice, indicating that they could be undergoing receptor down-regulation as a consequence of interaction with a putative self-antigen[14]. This interpretation is greatly strengthened by the data presented in Table 1. When we placed CD8$^-$ thymocytes isolated from 4-day-old BALB.D2.Mls^a mice *in vitro* at 37 °C in the absence of any deliberate stimulus, a reproducible fraction (~60%) of the CD4$^+$ Vβ6$^+$ cells died within 20 h. We established by kinetic studies that death was detectable after 4 h, and we saw no increase in the fraction of dying Vβ6$^+$ cells by prolonging the culture period from 20 h to 48 h (Fig. 1). Death of CD4$^+$ Vβ6$^+$ thymocytes was selective, because few (≤10%) CD4$^+$ cells died during this culture period and the levels of CD4$^+$ cells expressing another TCR β-chain (Vβ8) were unchanged (Table 1). Furthermore, CD4$^+$ Vβ6$^+$ thymocytes from neonatal or adult BALB/c (Mls-1^b) mice did not die under comparable experimental conditions (Table 1).

Cell death in a variety of developmental systems involves chromatin condensation, membrane blebbing and fragmentation of DNA after the activation of a calcium-dependent endogenous endonuclease. These events have been collectively referred to as apoptosis[15] and are believed to represent a programmed (as

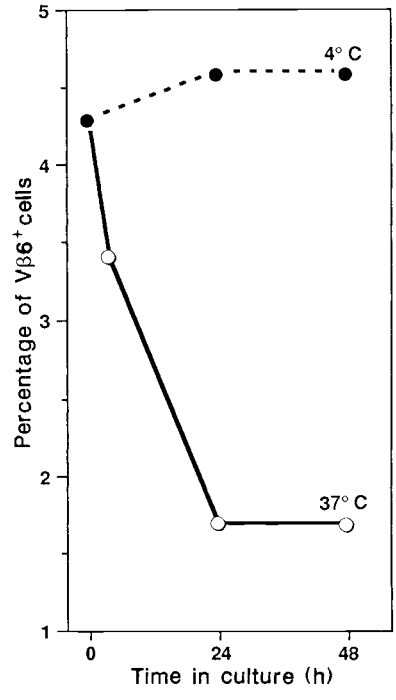

FIG. 1 Kinetics of death of autoreactive thymocytes *in vitro*. CD8-depleted thymocytes from 4-day-old BALB.D2.Mls^a mice were cultured at 4 °C or 37 °C. At various times, recovered viable cells were assessed for expression of Vβ6 (see Table 1). No significant decrease in Vβ8$^+$ cells was observed at 37 °C at any time (Table 1, and data not shown).