Amer. J. Bot. 76(7): 1048–1059. 1989.

# SEX EXPRESSION, BREEDING SYSTEM, AND POLLEN BIOLOGY OF RICINOCARPOS PINIFOLIUS: A CASE OF ANDRODIOECY?[1]

JAMES D. THOMSON, K. R. SHIVANNA,[2]
J. KENRICK,[3] AND R. B. KNOX[3]

Department of Ecology and Evolution, State University of New York, Stony Brook, New York 11794; [2] Department of Botany, University of Delhi, Delhi-110 007, India; and [3]Plant Cell Biology Research Centre, Department of Botany, University of Melbourne, Parkville, Victoria 3052, Australia

ABSTRACT

A population of 54 *Ricinocarpos pinifolius* (Euphorbiaceae) plants contained male plants, which produced only staminate flowers, and hermaphrodites, which produced staminate and pistillate flowers. The fraction of pistillate flowers ranged continuously from 0 to 0.68. Insect pollination was effective and fruit set virtually complete except for losses to herbivores. Self pollen, outcross pollen from male plants, and outcross pollen from hermaphrodites were all equivalent in viability, germination, tube growth, ovule penetration, and fruit setting ability. Inbreeding depression was manifested as late abortion of some selfed seeds. Geitonogamous selfing is largely prevented by temporal separation of male and female functions within plants. This temporal separation, combined with population-wide synchrony of flowering, may create unusual conditions allowing male plants at low frequency to match hermaphrodites in reproductive success.

ANDRODIOECY, the presence of male-only and hermaphroditic plants in a population, is a rare and controversial form of sex expression in angiosperms. Some workers consider it a key intermediate stage in the evolution of dioecy from cosexuality (Willson, 1979; Bawa, 1980; Bawa and Beach, 1981; Ross, 1982). Others contend that the rarity of androdioecy argues against the importance of such a pathway to dioecy (Lloyd, 1975; Charlesworth and Charlesworth, 1978), and Charlesworth (1984) even questions the existence of any cases of functional androdioecy. She points out that in the best-studied cases, the morphologically hermaphroditic plants have no male function, a result of indehiscent anthers or nonfunctional (often inaperturate) pollen. She concludes that most reported cases of androdioecy are functionally dioecious systems, with the putative hermaphrodites reproducing only as females. In some other reported cases, male plants are simply small or young individuals that presumably gain female function as they age (Charlesworth, 1984; Willson, 1983, p. 75). In the remaining cases, there is a 1:1 ratio of males to hermaphrodites, which Charlesworth also takes as evidence of functional dioecy, with the hermaphrodites being male-sterile.

After observing apparently male and hermaphroditic plants in a population of the Wedding Bush, *Ricinocarpos pinifolius* Desf. (Euphorbiaceae), we began a study with three goals: to document the patterns of sexual expression, both within plants and at the population level; to examine the pollination ecology and breeding system; and to test the viability and fertilization ability of pollen from both male and hermaphrodite plants.

*Ricinocarpos pinifolius* plants are woody bushes, usually single-stemmed, that produce hundreds to thousands of unisexual flowers. Hermaphroditic plants generally produce staminate and pistillate flowers at different times, so that sequential observations are necessary to characterize sex expression fully. We use such observations to characterize the phenotypic gender (Lloyd, 1979, 1980) of a plant, i.e., the fraction of all flowers produced that are pistillate. Throughout, we describe flowers as staminate or pistillate, but refer to plants using the functional terms male or hermaphrodite, emphasizing their functional roles and providing conformity with the theoretical literature that stimulated the study.

MATERIALS AND METHODS—*Field data*—We conducted field studies from September to De-

[1] Received for publication 16 May 1988; revision accepted 26 January 1989.

We thank T. Slater, P. Taylor, B. Thomson, and M. Tuohy for their assistance. J. Taylor and G. Bertram provided access to the field site. This research was supported by Visiting Research Fellowships to JDT and KRS at the Plant Cell Biology Research Centre, University of Melbourne, and a grant to RBK from the Botanic Gardens Research Trust, Melbourne. J. Brunet, D. Charlesworth, D. M. Calder, and D. Lloyd provided helpful comments. This is contribution 721 from Ecology and Evolution at SUNY–Stony Brook.

cember 1987 at the Cranbourne Annexe of the Royal Botanical Gardens of Melbourne, at Cranbourne, Victoria. The vegetation is native shrubby heath, on nutrient-poor, sandy soils derived from old dunes. At our site, sparsely scattered 5–8 m Manna Gum trees (*Eucalyptus viminalis*) overtopped a dense, 2–3 m tall thicket of Prickly Tea Tree (*Leptospermum juniperinum*), *Ricinocarpos pinifolius,* and other shrubs. We used an unpaved access road as the centerline of a 122 m transect, and mapped all flowering *R. pinifolius* bushes located on both sides of the road, penetrating into the thicket about 5 m. Shallow excavations around two individuals showed no suggestion of connections among plants or of common clonal origins.

To describe gender and to assess the possibility of size-gender correlations, we assigned an identification number to each of the 54 mapped plants, measured the height of the top of the crown, and measured the diameter of all stems approximately 10 cm above ground. All plants had $\leq 3$ stems except for two extremely bushy individuals which were excluded from the correlations because they appeared to have sustained recent damage. On 42 of these, we chose a typical flowering branch, bearing about 100 flowers, and on 28 October 1987 counted the total number of staminate and pistillate flowers produced by the branch. Any unopened buds were dissected for sex determination. On four plants that finished flowering earlier than the others, it was apparent that some staminate flowers had abscised before the census; thus, our data underestimate the number of staminate flowers on these plants. Virtually no pistillate flowers abscised. On the remaining twelve plants of the 54, we sampled five branches instead of one, and examined floral sex ratios at intervals throughout the bloom. Six of these plants were chosen a priori as predominantly male, six as predominantly female in gender. Flower counts from the sampled branches gave us an estimated floral gender (EFG) for each plant (pistillate flowers divided by all flowers). Thus, EFG describes phenotypic gender as a continuous variable potentially ranging from 0 (complete maleness) to 1.0 (complete femaleness). For all plants, we estimated by eye the proportional contribution of the sampled branches to the total number of flowers on each plant; thus we could calculate rough estimates of the total numbers of both sexes of flowers. If the sampled branch was entirely male, we searched the whole plant for pistillate flowers. To see whether gender was patchily distributed in space, which could suggest edaphic influences, we looked for spatial autocorrelation of EGF, considering the plants as if they were arranged linearly along the road and using the autocorrelation procedure (ACF) of the Minitab statistical package. On 27 September 1987 we netted insect flower visitors whose size and behavior suggested that they were pollinators. On 2, 8, and 13 October 1987, we examined haphazard samples of stigmas for pollen grains, which are easily seen by $10\times$ hand lens.

*Breeding system*—Because we wished to assess self-compatibility, we chose as seed parents three hermaphroditic plants (162, 163, and 200) in which the typical temporal separation of male and female function had been disrupted by the advanced flowering of one or more branches. We chose pistillate buds that were within 1 day of opening, prised the petals back, and covered the entire flower with a friction-fitting excluder cap. Each cap was made from a 2 cm length of plastic drinking straw, split lengthwise, and bound into a scroll by a thin ring sliced from plastic tubing. A wad of polyurethane foam in the mouth of the tube allowed gas exchange while keeping out pollen. Five or six days later, we pollinated the capped flowers by drawing freshly dehisced anthers over the entire stigmatic surface. We examined stigmas after each pollination to verify that at least 10 grains had been transferred to each of the three stigmas. Pollinations included self, outcross from other hermaphrodite plants, and outcross from predominantly male plants. Some capped flowers were left unpollinated to test for spontaneous apomixis. We scored fruit set on 11 November, then returned on 17 December to harvest and weigh seeds.

*Pollen and pistil function*—To address the question of pollen function in hermaphrodites, and to examine the compatibility system, we carried out standard physiological tests, typically comparing pollen from hermaphrodites vs. predominant males, or self vs. outcross pollen. We brought fresh branches to the lab in plastic bags, and kept them in vases at room conditions. When possible, we used the same plants employed in the breeding system study. Standard techniques used included localization of functional stigmatic surfaces via the esterase reaction (Heslop-Harrison, Heslop-Harrison, and Barber, 1975; Bernhardt, Knox, and Calder, 1980; Mattsson et al., 1974); pollen cytology (binucleate vs. trinucleate) via DAPI preparations (Coleman, Maguire, and Coleman, 1981; Heslop-Harrison and Heslop-Harrison, 1984); and pollen viability and membrane activity via the fluorochromatic reaction (FCR) test (Heslop-Harrison and Heslop-Har-

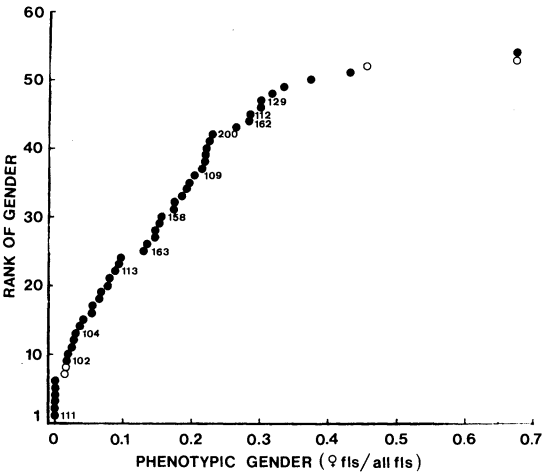


Fig. 1. The rank of estimated floral gender (EFG) vs. estimated floral gender for 54 plants of *Ricinocarpos pinifolius*. Open circles indicate four plants for which the EFG is known to be an overestimate. Plants that were used in experiments are indicated by identification number.

rison, 1970; Heslop-Harrison, Heslop-Harrison, and Shivanna, 1984). We tried to germinate pollen in Brewbaker and Kwack (1963) medium with sucrose concentrations ranging from 5 to 25%, but did not achieve satisfactory germination, whether the grains were previously hydrated or not; therefore, all conclusions about pollen function came from in vivo tests. For these tests, we microscopically examined stigmas of cut flowers and discarded those bearing pollen; picked up freshly dehisced anthers with forceps and carefully brushed pollen across the entire stigmatic surface; kept flowers under laboratory conditions for 6–48 hr, depending on the experiment (details below); fixed pistils in 1:3 acetic acid: ethanol for 1–6 hr, followed by 50% ethanol (30 min), overnight clearing in 10 M NaOH, and an aqueous rinse; mounted the pistils in decolorized aniline blue with a drop of 50% glycerine; and examined via fluorescence microscopy for pollen germination, pollen tube growth, and penetration of ovules.

In some experiments (details below), we separately pollinated the three stigma lobes of a single flower with pollen from three donors. After an interval for pollen tube growth, we separated the three carpels, with their styles and stigmas intact, by making longitudinal cuts along the carpel sutures. Each carpel was then fixed and processed as above. In other experiments, we used buds and flowers of different ages, judging the age of preanthesis buds by their size and appearance.

We squashed whole anthers in basic fuchsin-tinted glycerine jelly to count pollen grains.

TABLE 1. *Spearman rank correlations of estimated gender (EFG) and plant size variables. Four plants whose EFG's were subject to doubt (see text) have been excluded, as have two additional plants with more than three stems, thus N = 48. For two- and three-stemmed plants, stem area is computed as the sum of the cross-sectional basal areas of the stems. The stem area correlations in parentheses exclude all multistemmed plants (N = 43). Asterisks denote significant correlations (P < 0.05)*

| | EFG | Plant height | Stem area |
|---|---|---|---|
| Plant height | −0.29* | | |
| Stem area | −0.14 | 0.54* | |
| | (−0.23) | (0.46) | |
| Flower number | 0.10 | −0.06 | 0.27 |

RESULTS—*Whole-plant sex expression*—In our population, estimated floral gender ranged from 0 to 0.68, with a mean of 0.16. With the exception of two strongly female plants, one of which had an inflated EFG caused by early abscision of staminate flowers, there is continuous variation in gender (Fig. 1). Thus, the population is not divided into two distinct groups of males and hermaphrodites. The plants that we chose as "males" for the pollen and breeding system studies did not all completely lack female flowers, but had extremely low EFG values (≤0.03), as indicated in Fig. 1.

As Table 1 indicates, EFG was weakly negatively correlated with plant size, measured as cross-sectional basal stem area or height, but uncorrelated with estimated total flower production. No spatial patterning of EFG was apparent in the field, and there was no significant autocorrelation at any scale tested (lags 1–10), confirming that EFG varies unsystematically throughout the population, rather than being driven by local patchiness in soil nutrients, moisture, fire history, etc.

*Floral biology*—Flowers of both sexes have five white petals (Fig. 2, 4). Pistillate flowers are approximately 24 mm in diameter; staminate flowers vary considerably within plants, with early flowers being larger (~26 mm) than later ones (~15 mm). Our impression is that the smallest flowers on predominantly male plants are typically smaller than the smallest staminate flowers on hermaphrodites. Flowers of both sexes produce nectar from large, discrete yellow nectaries (Fig. 3, 5). Male flowers bear 15–35 anthers, the filaments of which are united into a staminal column (Fig. 3). Anthers dehisce gradually and sequentially, from the apex to the base of the staminal column. Dehiscence of all of the anthers of a single flower

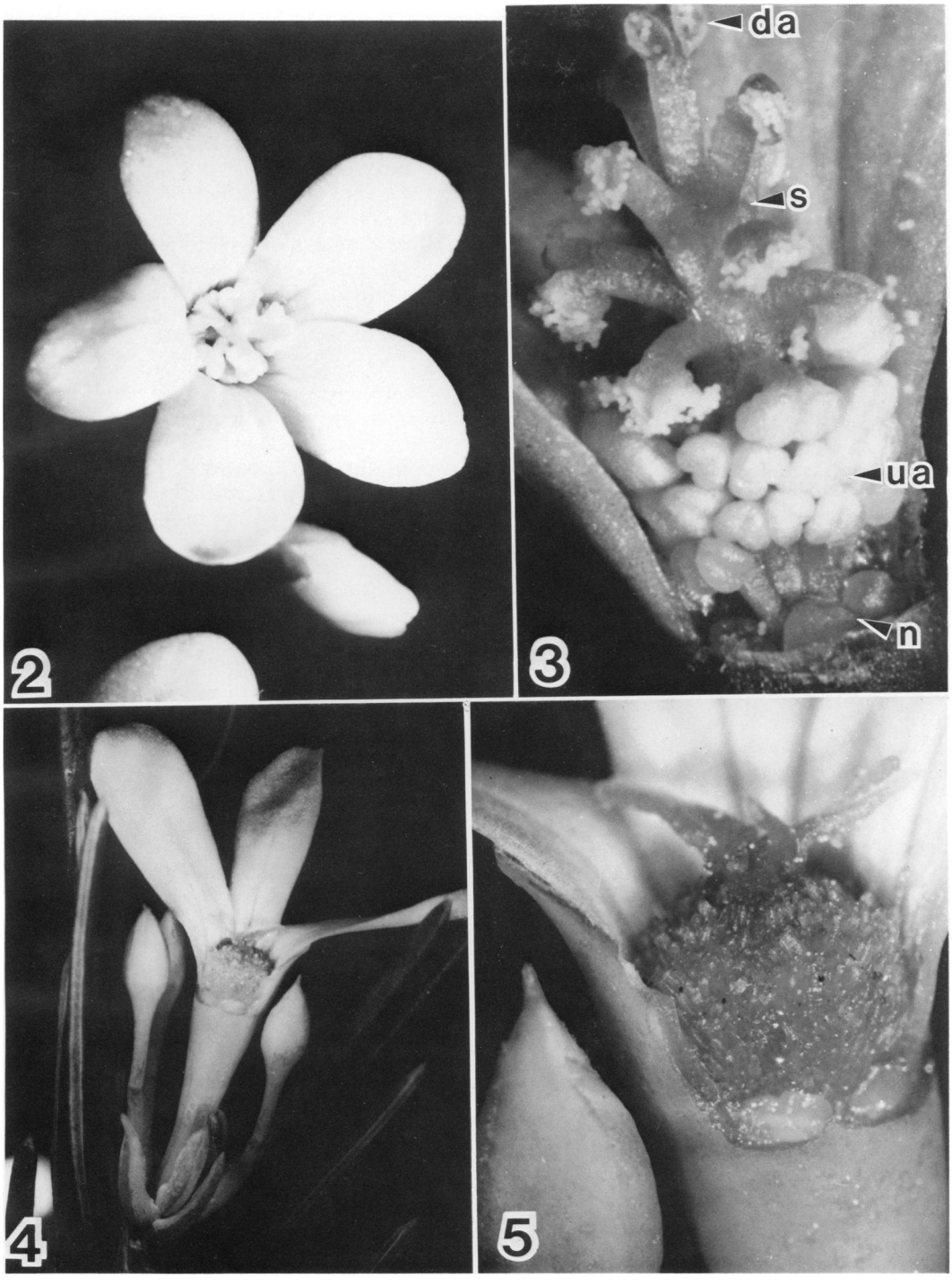


Fig. 2–5. **2.** Staminate flower in midanthesis. Note unopened bud of later-opening flower (also staminate) in same inflorescence. **3.** Staminate flower in midanthesis with petals cut away to reveal: s, column of fused stamens, with da, dehisced (apical) anthers, ua, undehisced basal anthers, and n, nectaries. **4.** Inflorescence showing open terminal pistillate flower flanked by several later-opening staminate flowers. **5.** As in Fig. 4, petals removed to show stigma lobes, ovary, and nectaries.

TABLE 2. *The number of pollen grains in single anthers taken from the central portion of the staminal column of eight flowers of* Ricinocarpos pinifolius. *The flowers were chosen to span a range of flower sizes and plant genders (EFG = the proportion of pistillate flowers on a plant)*

| Plant | EFG | Anthers per flower | Grains per anther |
|---|---|---|---|
| 111 | 0 | 32 | 202 |
| 104 | 0.030 | 21 | 196 |
| 113 | 0.089 | 29 | 186 |
| 158 | 0.155 | 33 | 221 |
| 109 | 0.203 | 19 | 153 |
| 162 | 0.283 | 25 | 182 |
| 112 | 0.286 | 29 | 212 |
| 112 | 0.286 | 39 | 216 |

takes approximately 2 weeks under field conditions; for example, one marked flower had its first anther dehisce on 15 October; on 21 October, 16 anthers had dehisced, nine of which appeared brownish and retained no pollen, seven of which were still yellow and polliniferous; by 28 October, all 29 anthers had dehisced, and only the last four contained fresh-looking pollen. By this time the corolla bore brownish stains and the petal tips had started to shrivel. Similar timed observations of six flowers from a predominantly male plant (102) and five from a hermaphrodite (163) revealed no obvious differences in floral longevity or in the timing of anther dehiscence.

The amount of pollen per anther varied with anther position. In two flowers, it was higher in the middle than in the apical or basal anthers. In two other flowers, pollen production declined monotonically from apex to base. When the apical anthers were ready to dehisce, pollen maturation in the basal anthers still appeared incomplete, rendering simultaneous grain counts difficult. Based on a sample of 26 anthers, taken from 7 plants and from stratified positions in the staminal column, the mean pollen production per anther is 166.8 ($s = 42.6$). Considering only anthers from the middle rank of the staminal column (Table 2), pollen production is uncorrelated with the plant's floral gender, but positively correlated with anther number ($r = 0.79$, $N = 8$, $P < 0.05$). Thus, pollen production, per anther and per flower, varies with flower size. Therefore, we can only roughly estimate pollen production (4,000–5,000 for a typical staminate flower). Pollen grains are large, spherical, and largely free of ornamentation. Pollen grains (from plants 111 (EFG = 0) and 158 (EFG = 0.155)) were uniformly two-celled. The generative nucleus showed intense DAPI fluorescence; the vegetative nucleus fluoresced only faintly.

Pistillate flowers have three carpels with one ovule each. Three elongated, bifid stigma lobes arise from a short style (Fig. 6, 7). The stigma is green, dry, and smooth (nonpapillate) (Heslop-Harrison and Shivanna, 1977). In receptive flowers (see below) esterases are present in the form of a pellicle on the entire surface of the stigma lobes (Fig. 8). Thus the entire adaxial surface of the lobes is receptive, which was confirmed by direct observation of pollen germination and penetration along the entire stigmatic surface.

*Stigma receptivity*—Timed pollinations, followed by aniline blue staining for pollen germination and tube growth, show that stigmas support little germination prior to anthesis, but that considerable germination can occur on the day of anthesis and at least 7 days later, although the stigmas become dry, brown, and senescent by 14 days after anthesis (Fig. 14–16, Table 3). In a few cases, seven-day stigmas show brownish necrotic patches, especially at the lobe-tips. These areas show specks of callose deposition and do not support pollen germination. Similar specks appeared in bud-pollinated stigmas.

*Pollination*—Casual observation of over 100 pistillate flowers, on several plants and on several days throughout flowering, revealed pollen grains on virtually all mature stigmas. This was an unexpectedly high rate of pollination, given that pollinators did not seem abundant, and that pollination usually required interplant movement. Wind pollination is unlikely; the morphology of stigmas and the size and stickiness of pollen grains are inappropriate. The primary diurnal flower visitors are flies and bees. All of the insects, which were caught at hermaphrodite plants in male phase, carried *Ricinocarpos* pollen. We observed no vertebrate visits, although New Holland Honeyeaters (*Phylidonyris novaehollandiae*) commonly fed on other species in the vicinity. We made no nocturnal observations, but flowers showed no signs of the damage that would be expected if mammals visited.

*Timing of sex expression*—The basic flowering pattern was an initial wave of female flowering, from single terminal buds, followed by male flowering, from numerous buds subtending each pistillate flower (Fig. 4). In some plants, there was a second, usually small, wave of female flowering after the male phase was

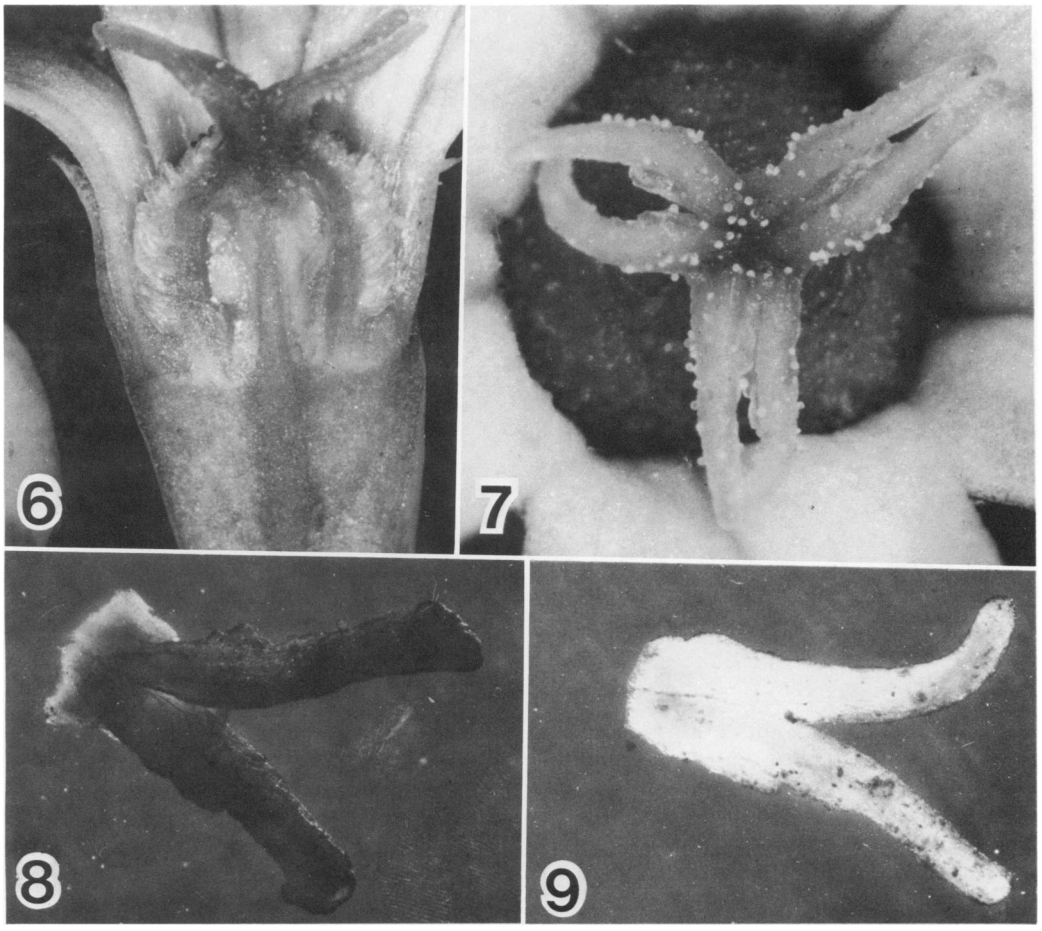


Fig. 6–9. **6.** As in Fig. 5, ovary sectioned to show two of three carpels with one ovule each. **7.** Pistil showing three styles with two stigma lobes each, with pollen grains. **8.** Receptive stigma showing (black regions) positive esterase test. **9.** Control stigma for esterase test (without esterase substrate).

completed. In the plants that we selected as males, the first female wave was reduced to extremely few or no flowers. In some of these (e.g., plant 111), there was no second wave; others produced modest numbers of second-wave pistillate flowers. In all but four plants, the sex shifts occurred synchronously, so that there was little within-plant overlap of male and female functions. In each of those four, a single asynchronous branch was responsible for most of the overlap. Because of the long life of pistillate flowers, later-opening ones of the first wave could in principle be pollinated geitonogamously, even on well-synchronized plants, but at the observed levels of pollen delivery they would usually have been outcrossed first. Thus, the temporal separation of sexes effectively promoted outcrossing, except when intraplant synchrony broke down (see Thomson and Barrett, 1981; Bawa, Webb, and Tuttle, 1982).

We could not collect enough data to give a detailed description of floral sex ratios at the population level (cf. Thomson and Barrett, 1981). However, it was obvious from the twelve plants given detailed study, and from observations of the population as a whole, that the relative availabilities of staminate and pistillate flowers changed drastically during the flowering season (Fig. 17).

*Pollen function*—Fresh pollen (samples ranging from 123 to 363 grains, from eight plants on 2 October and from seven plants on 14 October) showed uniformly high viability, as indicated by the FCR test (mean fraction viable = 0.88, $s = 0.03$). There was no difference between days, and there was no correla-

TABLE 3. *Germination of outcross pollen on stigmas of* Ricinocarpos pinifolius *as a function of flower age. The 0, −1, and −2 day flowers were collected as buds and pollinated; the 7 and 14 day flowers were given excluder caps in bud stage and left on the plant until the day before pollination. All pairwise comparisons are significant ($P < 0.001$) except day 0 vs. day 7 (by Mann-Whitney tests treating each stigma as a single datum)*

| | Flower age | | | | |
|---|---|---|---|---|---|
| | Days before anthesis | | | Days after anthesis | |
| | 2 | 1 | 0 | 7 | 14 |
| Number of stigmas examined | 9 | 6 | 9 | 6 | 8 |
| Total grains applied | 346 | 334 | 441 | 263 | — |
| Grains germinated | 21 | 84 | 248 | 161 | 0 |
| Percent germination | 6.1 | 25.2 | 56.2 | 66.2 | 0 |

TABLE 4. *Germination and growth of different pollens on stigma lobes of flowers of* Ricinocarpos pinifolius *plant 163. Tabled values are grains germinated/grains applied. Tube growth was visually equivalent in all cases. Asterisks indicate preparations in which pollen tube penetration of the ovule could be seen*

| | Paternal plant (EFG) | | |
|---|---|---|---|
| Flower | 163-self (0.131) | 158 (0.155) | 102 (0.018) |
| 1 | 20/22 | 40/52 | 14/21 |
| 2 | 22/23* | 71/91* | 49/56* |
| 3 | 55/70* | 44/44* | 40/54* |
| 4 | 68/75* | 48/56 | 55/62 |
| 5 | 61/65* | 36/62* | 43/68* |
| Overall percent germination | 89% | 78% | 77% |

tion between plant EFG and FCR score, although the plants were chosen to span the full range of genders. Pollen retained some viability following exposure in the anthers. In one flower in which nearly all anthers had dehisced, fresh pollen taken directly from the basal anthers was 84% viable ($N = 252$ grains); approximately four day old pollen from the middle rank of anthers gave 50% viability ($N = 196$); and approximately eight day old apical pollen gave 13% ($N = 135$). Parallel experiments measuring in vivo germination gave similar results for the three age classes of pollen: 56, 25, and 14% ($N = 441$, 480, and 249 grains on 9, 12, and 9 stigmas, respectively). Prior hydration of the same pollen at 100% RH (Shivanna and Heslop-Harrison, 1981) did not increase the FCR scores (86, 44, 11%, $N = 333$, 276, 263, respectively). (This flower was brought from the field to the lab after the middle ranks of anthers had dehisced.) Germination, tube growth, and penetration of ovules were roughly equivalent for self pollen, outcross from male plants, or outcross from hermaphrodites (Table 4, Fig. 10–12). In similar experiments, pollen from 158 and 109 (EFG = 0.203) grew well in styles of both 112 and 162, although we did not record percent germination. Also, five flowers of plant 163 were selfed for additional observations. Pollen tubes grew about ¾ of the way to the ovary in the first 24 hr, and had reached the ovary by 48 hr. In some cases two to four tubes entered the ovule (Fig. 13), and there was no indication of incompatibility reactions.

*Breeding system*—Loss of fruits to herbivores, branch breakage, and limited pollen availability compromised the balance of the cross design, but it is apparent from the pooled data (Table 5a) that *Ricinocarpos pinifolius* is fully self-compatible, and that pollen from hermaphrodites is as effective for fruit set as pollen from predominantly male plants. Both results are consistent with our studies of pollen tube growth. All unpollinated flowers abscised ~3 wk after opening, demonstrating the efficacy of the exclusion caps and the absence of spontaneous apomixis.

Some fruits appeared normal externally, but contained one aborted seed (plus two healthy ones). These abortions occurred after considerable growth had occurred, judging by the large size of the empty locule and the considerable amount of shrivelled seed tissue. Such fruits were significantly more likely to be selfed than outcrossed (Table 5b), suggesting that the death of the developing seeds constituted inbreeding depression. Overall, fresh weights (minus elaiosome) of selfed ($\bar{x} = 0.0247$ g, $N = 24$) and outcrossed ($\bar{x} = 0.0278$ g, $N = 40$) seeds did not differ significantly (Mann-Whitney test).

DISCUSSION—*Separation of sexes*—The large family Euphorbiaceae includes a great variety of pollination systems, from wind and generalized insect pollination to specialized systems involving bees, moths, birds, bats (Perkins et al., 1975; Steiner, 1983), and possibly others. Additionally, mating systems and sex expression show various types of dicliny, and several recent authors have studied the reproductive biology of monoecious taxa (e.g., Perkins, Estes, and Thorp, 1975; Bawa et al., 1982; Reddi and Reddi, 1983). Reproduction in *Ricinocarpos pinifolius* is similar in many respects to that described in detail for the Central American

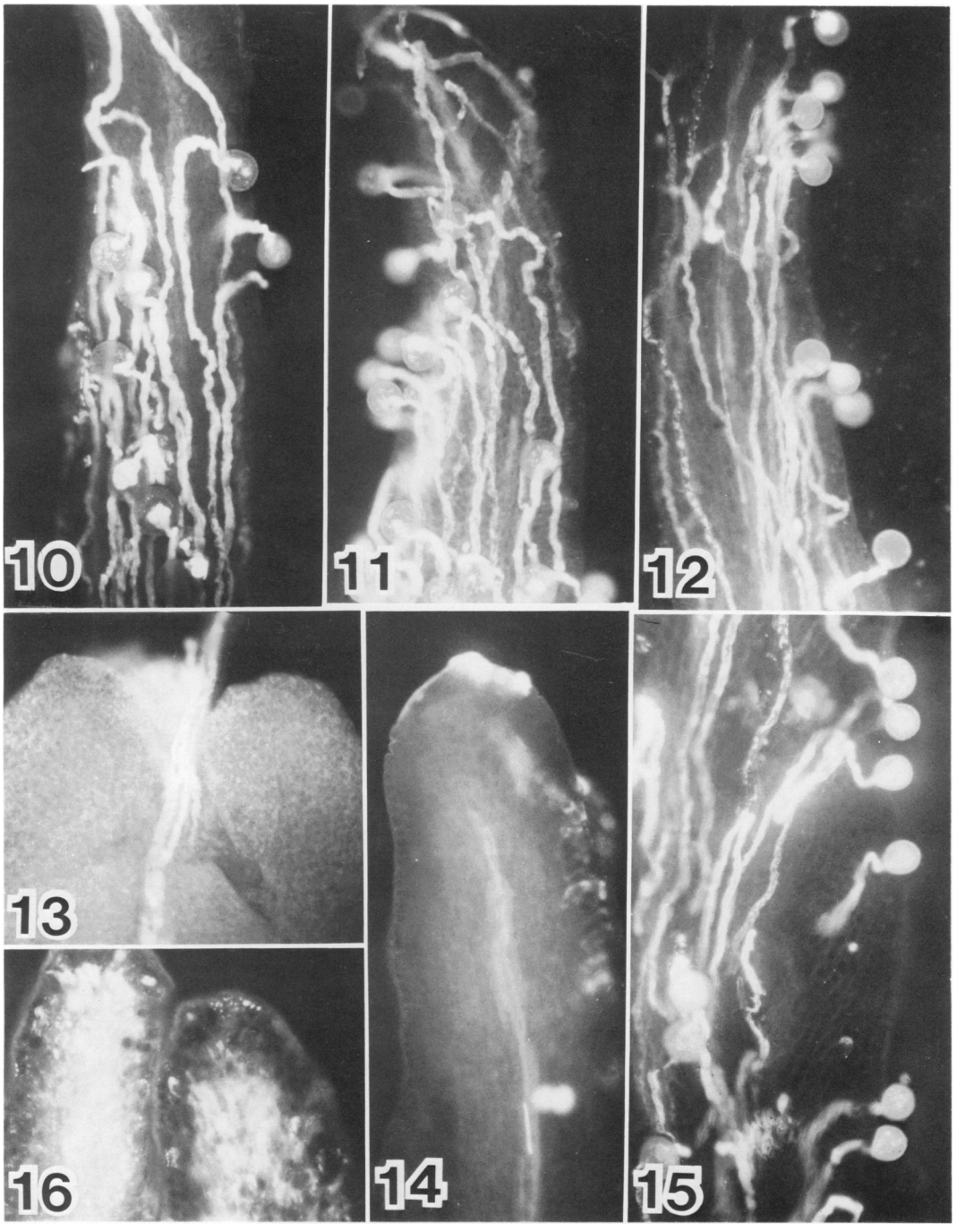


Fig. 10–16. **10.** Pollen germination and growth in *Ricinocarpos pinifolius* stigma lobe, pollinated 1 day after anthesis, fixed after 48 hr, and stained for callose with decolorized aniline blue. Self pollination. **11.** As in Fig. 10, but outcross pollen from a hermaphrodite pollen parent. **12.** As in Fig. 10, but outcross pollen from a male pollen parent. **13.** As in Fig. 10, but showing penetration of ovule by self pollen tubes. **14.** Bud pollination, two days prior to anthesis, preparation as in Fig. 10. Pollen does not adhere. **15.** As in Fig. 10, outcross pollination 1 week after anthesis. Germination and growth are equivalent to undelayed pollinations. **16.** As in Fig. 15, but with two weeks' delay. Stigma is senescent; pollen does not adhere.

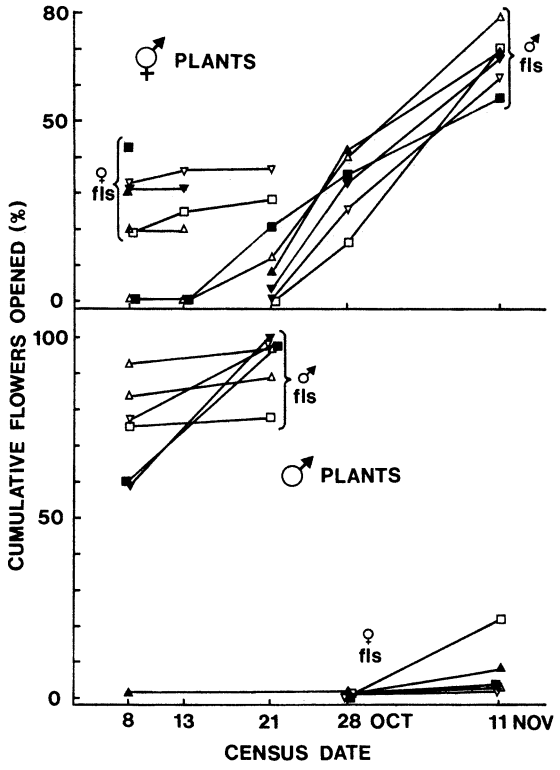


Fig. 17. Temporal patterns of staminate and pistillate flower production by six hermaphrodite plants (upper panel) and six predominantly male plants (lower panel) of *Ricinocarpos pinifolius*. Within each panel, different symbols indicate different plants. For each sex of flowers, the points show the *cumulative* number of flowers produced by each census date, expressed as a percentage of the total number of flowers produced by the plant. Line segments terminate on the date after which no more flowers were produced. Note that the hermaphrodite plants produce their pistillate flowers when the predominantly male plants are producing staminate flowers (and vice versa, to the extent that predominant males produce a few pistillate flowers late in the season). Plant identities, upper panel: ▽, 103; △, 109; □, 112; ▼, 124; ▲, 129; ■, 161. Lower panel: ▽, 102; △, 104; □, 107; ▼, 111; ▲, 113; ■, 115.

euphorbiaceous herb *Cnidoscolus urens* by Bawa et al. (1982). In both species, staminate and pistillate flowers are similarly arranged in inflorescences; male and female functions are largely temporally separated within plants, with an initial wave of female flowering followed by a period of male flowering; pollination (mostly by insects) is effective and nearly all pistillate flowers set fruit. *Ricinocarpos pinifolius* differs in having longer lasting flowers and more anthers per staminate flower; in having a few plants that produce no pistillate flowers; in having a 5:1 population-wide ratio of staminate : pistillate flowers, as compared to ~16:1 for *C. urens*; and in some plants showing a second wave of female function after the main period of staminate flowering. This female-male-female sequence has not been previously reported in monoecious plants with synchronized dichogamy. The range of genders is far greater in *R. pinifolius* (0–0.68) than in the 19 inflorescences of *C. urens* sampled by Bawa et al. (0.023–0.128). (S. Ducker [personal communication] found entirely female plants near Anglesea, Victoria, but these populations have since been decimated by fire.)

Although monoecy has generally been thought to promote outcrossing (Grant, 1975; Frankel and Galun, 1977), the relationship between monoecy and outcrossing is problematic (Primack and Lloyd, 1980; Bawa et al., 1982; Willson, 1983). As these authors point out, the segregation of male and female functions into separate flowers need not in itself reduce self-fertilization, which may still occur through geitonogamy. Bawa et al. (1982) consider that the particular manifestation of monoecy displayed by *Cnidoscolus urens* does promote outcrossing, but that this function is achieved through the temporal, not spatial, segregation of the sexes. Monoecy in *Ricinocarpos pinifolius* achieves the same result in the same way. Synchronized dichogamy may be common in monoecious Euphorbiaceae (D. Lloyd, personal communication), but few accounts exist. *Jatropha gossypiifolia* (Reddi and Reddi, 1983), shows the same sort of sexual progression (pistillate flowers early, staminate late), but there is substantial temporal overlap, even within inflorescences. Reddi and Reddi conclude that most of the visitors effect both geitonogamy and xenogamy. The staminate : pistillate flower ratio in *J. gossypiifolia* is 11:1. *Ricinus communis* displays the same temporal patterns, but Shifriss (1956) does not clearly report the degree of synchrony.

In addition to the outcrossing advantage, Bawa et al. propose that intrasexual competition for mates and selection for mating success have been important influences on sex expression in *Cnidoscolus urens*: by separating male and female functions, the proportion of male : female flowers and their nectar amounts, longevities, etc. can be adjusted to maximize mating success through one sexual mode, without the compromises necessary when one flower fulfills both roles. Bawa et al. (1982) interpret the high ratio of staminate : pistillate flowers and the extended period of male bloom in *C. urens* as responses to such selection pressures. Although similar trends occur at the population level in *R. pinifolius,* the much greater range of genders means that only some plants invest more in staminate flowers or have

TABLE 5. *Summary of crosses.* ***a.*** *Tabled values are fruits set/flowers pollinated. Flowers attacked by herbivores have been eliminated. Fruit set was scored in the field, on 11 November 1987*

| | Paternal parent (EFG) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Males | | | Hermaphrodites | | | |
| Maternal parent | 102 (0.018) | 104 (0.030) | 111 (0) | 162 (0.283) | 163 (0.131) | 200 (0.230) | Unpollinated control |
| 162 | 4/4 | 1/1 | 3/5 | 4/6 | 2/3 | 0/2 | — |
| 163 | 6/11 | 3/6 | 7/9 | 3/4 | 11/15 | 3/5 | 0/9 |
| 200 | 1/4 | 2/4 | 0/1 | 1/4 | 2/3 | 1/3 | 0/1 |
| 129 | — | — | — | — | — | — | 0/9 |

Percentage fruit set, pooled by paternal type: self 67% (16/24); outcross male 60% (27/45); outcross hermaphrodite 52% (11/21).

***b.*** *Results of fruit dissections done 17 December 1987. Number of fruits is smaller than above due to loss of labels. Fruits are categorized by cross type and by whether one seed had aborted*

| | Number of fruits | |
|---|---|---|
| | No abortion | Single seed abortion |
| Self | 4 | 6 |
| Outcross | 15 | 2 |

Fisher's exact test, two-tailed, $P = 0.025$

longer male phases of bloom; thus, any sexual selection arguments must be more complex.

*Androdioecy*—Should *R. pinifolius* be considered androdioecious? In a technical sense, the coexistence of males and hermaphrodites in a population would support such a description (Darwin, 1877, p. 12), especially because Charlesworth's (1984) additional criteria are met: hermaphrodites produce fully competent pollen, males are not merely young or small individuals, and the male : hermaphrodite ratio is not 1:1. There remain two important caveats. First, we have no idea as yet whether EFG's are constant from year to year. If individuals change from one category to the other, androdioecy is clearly an inappropriate term. Second, some authorities feel that "androdioecy" should be restricted to situations in which the frequency distribution of floral gender is discontinuous (D. Charlesworth, personal communication) or at least bimodal (D. Lloyd, personal communication). Although it is conceivable that further sampling could indicate a bimodality of functional gender in *R. pinifolius,* this seems unlikely, and the distribution is clearly not discontinuous. Thus, gender is monomorphic rather than dimorphic, and these authors would categorize *R. pinifolius* as simply monoecious, albeit with an extremely wide adjustment of phenotypic gender.

On the other hand, regardless of the terminology used to describe it, the range of gender all the way to maleness does correspond to the evolutionarily interesting situation described (as androdioecy) by Willson (1983, p. 56), in which "most individuals are hermaphroditic, but there are some male individuals." It is this situation, in which nearly or completely female-sterile plants appear capable of invading a population of hermaphrodites, that is controversial. Existing mathematical models (Charlesworth and Charlesworth, 1981; Charlesworth, 1984) consider that female-steriles may compensate for their loss in female function through 1) lowered mortality, and 2) increased male function following reallocation of resources formerly required for female function. Charlesworth (1984) concludes that those benefits alone are unlikely to render female-steriles as fit as hermaphrodites, hence the rarity of androdioecy. Thus, even though gender in *R. pinifolius* is monomorphic, it is still of interest to consider 1) how males are maintained in the population, and 2) whether the present situation can be considered an intermediate condition in the evolution of dioecy, as proposed by Willson (1979) and Bawa and Beach (1981).

Indeed, the temporal separation of sexual functions within *R. pinifolius* plants, coupled with a reasonable level of population-wide synchrony in blooming, may set up special circumstances in which great gains in male reproductive success would accrue to rare female-steriles. Although we would need much more complete floral censuses to model this effect accurately, it appears (Fig. 17) that male plants and hermaphrodites begin blooming in rough synchrony, and that in this species a gender shift toward maleness comes about through earlier cessation of female flower pro-

TABLE 6. *Estimation of reproductive success of males and hermaphrodites when the population sex ratio varies with time. We consider a synthetic population of the six males and six hermaphrodites for which repeated floral censuses are available (Fig. 17). We assume that all pistillate flowers set fruit. For each interval we calculate how many fruits would be sired by male pollen parents and how many would be sired by hermaphrodites, assuming that the two types of pollen parents sire fruits in direct proportion to the numbers of staminate flowers they produce (i.e., if 25% of the staminate flowers are on male plants during an interval, 25% of that interval's fruits are assumed to be sired by male pollen parents). Total offspring production by males and hermaphrodites is roughly equal, but the two groups emphasize different sexual modes*

| Pollen parent | Flower type | 1987 Intervals | | | | |
|---|---|---|---|---|---|---|
| | | ≤8 Oct | 8–21 Oct | 21–28 Oct | 21 Oct–11 Nov | >11 Nov |
| Six male plants | ♂ | 22,770 | 6,204 | 0 | 0 | 0 |
| | ♀ | 112 | 0 | 148 | 1,550 | 0 |
| Six hermaphroditic plants | ♂ | 12 | 3,200 | 8,496 | 12,015 | 8 |
| | ♀ | 9,758 | 622 | 0 | 16 | 0 |
| Fruits sired by males | | 9,864.8 | 410.3 | 0 | 0 | 0 |
| Fruits sired by hermaphrodites | | 5.2 | 211.7 | 148 | 1,566 | 0 |

| Seasonal totals | Offspring produced | | |
|---|---|---|---|
| | As pollen parents | As seed parents | Total |
| Males | 10,275.1 | 1,810 | 12,085.1 |
| Hermaphrodites | 1,930.9 | 10,396 | 12,326.9 |

duction. Under such circumstances, rare female-steriles could have access to far more ovules than hermaphrodites have (Pellmyr, 1987). As an illustration, consider a synthetic population comprising only the twelve plants whose flower production was studied in detail (with the important caveat that these were not randomly selected). If we assign each fruit a paternity based on the relative numbers of staminate flowers produced by all potential fathers during each portion of the blooming season (see Thomson and Barrett, 1981), the paternity of virtually all the fruits produced early in the season would be attributed to the six male plants (Table 6). Indeed, total reproductive success for the male group would be 98% of that of the hermaphrodite group. A single rare male would be even more effective; through similar calculations, the completely male plant 111, in company with the same six hermaphrodites, would have 5.5-fold greater reproductive success than the average hermaphrodite. Such considerations suggest that the type of synchronous dichogamy in *R. pinifolius,* which is probably maintained by selection against geitonogamous selfing, is a special circumstance that allows extraordinarily wide gender adjustments, because reductions in female function coincidentally confer exceptional access to mates through male function. Because the advantage to female-steriles is frequency dependent, this type of "androdioecy" could be stable, and need not constitute an intermediate stage in the evolution of dioecy (cf. Willson, 1979; Bawa and Beach, 1981). Nonetheless, it would be interesting to investigate sex expression in other populations of *R. pinifolius* or of the other (ca. 14) species of *Ricinocarpos.* Because the temporal separation of sexes can easily be weakened (as in *Jatropha gossypiifolia*), a group of species displaying synchronized dichogamy might be expected to show interesting variability in selfing rate, inbreeding depression, allocation to the sexes, and relative reproductive successes of gender variants.

## LITERATURE CITED


BAWA, K. S. 1980. Evolution of dioecy in flowering plants. Annual Rev. Ecol. Syst. 11: 15–39.

———, AND J. H. BEACH. 1981. Evolution of sexual systems in flowering plants. Ann. Missouri Bot. Gard. 68: 254–274.

———, C. J. WEBB, AND A. F. TUTTLE. 1982. The adaptive significance of monoecism in *Cnidoscolus urens* (L.) Arthur (Euphorbiaceae). Bot. J. Linn. Soc. 85: 213–223.

BERNHARDT, P., R. B. KNOX, AND D. M. CALDER. 1980. Floral biology and self incompatibility in some Australian mistletoes of the genus *Amyema* (Loranthaceae). Austral. J. Bot. 28: 437–451.

BREWBAKER, J. L., AND B. H. KWACK. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Amer. J. Bot. 50: 859–865.

CHARLESWORTH, B., AND D. CHARLESWORTH. 1978. A model for the evolution of dioecy and gynodioecy. Amer. Naturalist 112: 975–997.

CHARLESWORTH, D. 1984. Androdioecy and the evolution of dioecy. Biol. J. Linn. Soc. London 22: 333–346.

———, AND B. CHARLESWORTH. 1981. Allocation of resources to male and female functions in hermaphrodites. Biol. J. Linn. Soc. London 15: 57–74.

COLEMAN, A. W., M. MAGUIRE, AND J. R. COLEMAN. 1981. Mithramycin and 4′,6′-diamidino-2-phenylindole (DAPI) staining for fluorescence microspectropho-

tometric measurement of DNA in nuclei, plastids and virus particles. J. Histochem. Cytochem. 29: 959–968.

DARWIN, C. 1877. The different forms of flowers on plants of the same species. University of Chicago Press, Chicago.

FRANKEL, R., AND E. GALUN. 1977. Pollination mechanisms, reproduction and plant breeding. Springer-Verlag, Berlin.

GRANT, V. 1975. Plant speciation. Columbia, New York.

HESLOP-HARRISON, J., AND Y. HESLOP-HARRISON. 1970. Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. Stain Technol. 45: 115–120.

———, AND ———. 1984. The disposition of gamete and vegetative cell nuclei in the extending pollen tube of a grass species *Alopecurus pratensis* L. Acta Bot. Neerl. 33: 131–134.

———, ———, AND J. BARBER. 1975. The stigma surface in incompatibility responses. Proc. Roy. Soc. Lond. B 188: 297.

———, ———, AND K. R. SHIVANNA. 1984. The evaluation of pollen quality, and a further appraisal of the fluorochromatic (FCR) test procedure. Theor. Appl. Genet. 67: 367–375.

HESLOP-HARRISON, Y., AND K. R. SHIVANNA. 1977. The receptive surface of the angiosperm stigma. Ann. Bot. 41: 1233–1258.

LLOYD, D. G. 1975. The maintenance of gynodioecy and androdioecy in angiosperms. Genetica 45: 325–339.

———. 1979. Parental strategies in angiosperms. New Zealand J. Bot. 17: 595–606.

———. 1980. The distribution of gender in four angiosperm species illustrating two evolutionary pathways to dioecy. Evolution 34: 123–134.

MATTSSON, O., R. B. KNOX, J. HESLOP-HARRISON, AND Y. HESLOP-HARRISON. 1974. Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions. Nature 247: 298–300.

PELLMYR, O. 1987. Multiple sex expressions in *Cimicifuga simplex*: dichogamy destabilizes hermaphroditism. Biol. J. Linn. Soc. 31: 161–174.

PERKINS, G., J. R. ESTES, AND R. W. THORP. 1975. Pollination of *Cnidoscolus texanus* (Euphorbiaceae) in south-central Oklahoma. Southw. Naturalist 20: 391–396.

PRIMACK, R. B., AND D. G. LLOYD. 1980. Andromonoecy in the New Zealand montane shrub Manuka, *Leptospermum scoparium* (Myrtaceae). Amer. J. Bot. 67: 361–368.

REDDI, E. U. B., AND C. S. REDDI. 1983. Pollination biology of *Jatropha gossypiifolia* (Euphorbiaceae). Proc. Indian Acad. Sci., Pl. Sci. 92: 215–231.

ROSS, M. D. 1982. Five evolutionary pathways to dioecy. Amer. Naturalist 119: 297–318.

SHIFRISS, O. 1956. Sex instability in *Ricinus*. Genetics 41: 265–280.

SHIVANNA, K. R., AND J. HESLOP-HARRISON. 1981. Membrane state and pollen viability. Ann. Bot. 47: 759–770.

STEINER, K. E. 1983. Pollination of *Mabea occidentalis* (Euphorbiaceae) in Panama. Syst. Bot. 8: 105–117.

THOMSON, J. D., AND S. C. H. BARRETT. 1981. Temporal variation of gender in *Aralia hispida* Vent. (Araliaceae). Evolution 35: 1094–1107.

WILLSON, M. 1979. Sexual selection in plants. Amer. Naturalist 113: 777–790.

———. 1983. Plant reproductive ecology. John Wiley, New York.