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Sexual selection in a hermaphroditic plant

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The ornaments and weapons of male animals are among the showiest and seemingly most wasteful of nature's productions. Darwin's theory of sexual selection showed how such traits could be selected through competition for mates even if they were otherwise detrimental¹. Flowering displays of plants often show a comparable degree of gaudiness and profligacy, but exploration of the role of sexual selection in plants has only just begun²⁻⁵. Bateman⁶ argued that sexual selection is caused by the greater ability of males than females to increase fitness by mating repeatedly, due to the females' greater energetic commitment to gametes or parental care. Similar reasoning applies to hermaphrodites^{2,3}. In hermaphroditic milkweeds, most young fruits are aborted⁷⁻¹⁰ and female reproduction (seeds) is limited more by resources than by pollination¹¹. Sexual selection theory therefore predicts that traits increasing mating success will have evolved because they increase male success through pollen. Here I report that a suite of floral traits of a hermaphroditic plant is best interpreted as having evolved through the male competition component of sexual selection, a result with important implications for evolutionary studies of pollination systems.

I studied *Asclepias exaltata*, a herbaceous, perennial milkweed, in southeastern Michigan. Each hermaphroditic flower has five pollinaria, each of which consists of two pollinia composed of about 180 fused pollen grains. The intricate pollination mechanism is like that of other milkweeds¹². A fissure subtends each pollinarium and the pollinarium is removed when an insect withdraws a leg or other appendage that has slipped into the fissure. The pollinarium, now attached to the insect, dries into an orientation that facilitates the next step¹²⁻¹⁴; if the insect draws the same appendage past another fissure, a pollinium may detach and remain in the fissure, and fertilization follows. Self-pollination is possible but pollinia are usually carried far enough to prevent it (see below).

I estimated the number of pollinaria removed from each umbel (inflorescence) from counts on five randomly chosen flowers. The umbel estimates were summed for each plant to obtain an estimate of relative male success. Female fitness was estimated by counting fruits (seed counts were discontinued when I found that plant fruit number was highly correlated with seed number— $r=0.95$, $n=111$ —and that seed number per fruit did not change when fruit numbers were experimentally reduced).

To simulate the fitness effects of producing fewer flowers, I removed half the flowers from each umbel on randomly chosen plants. In another population, I simulated decreased flower longevity by bagging all umbels of some plants (thereby excluding pollinators) for 4 days of the 7-9-day anthesis period. Table 1 compares these experimental plants with their untreated control groups. Plants in both experimental groups had reduced pollinarium removals but, despite initiating fewer fruits, did not mature fewer.

Thus, plants can raise a full complement of fruits with half their flowers or with flowers open half as long. Unless the male

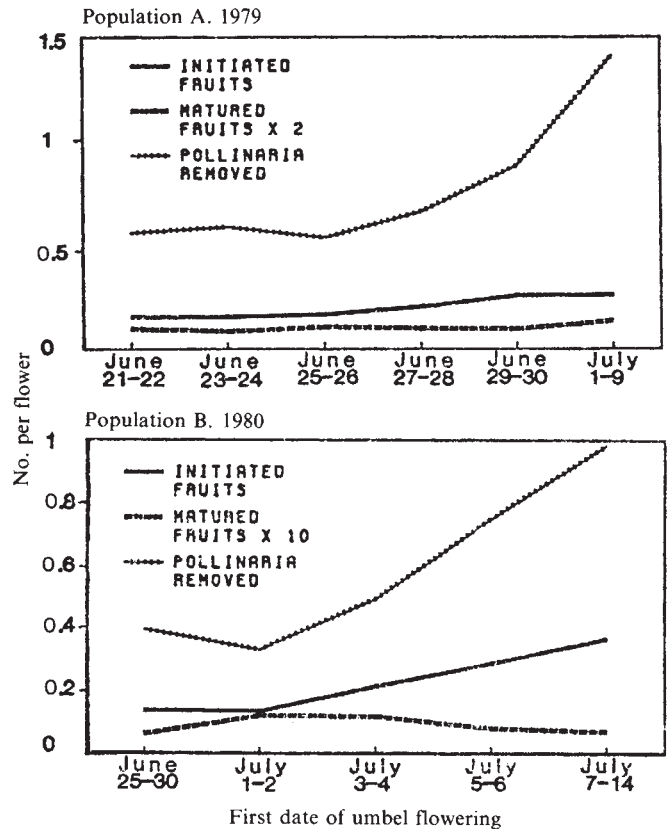


Fig. 1 Seasonal changes in per flower reproductive measures. Pollinarium removal rates vary seasonally, as do fruit initiation rates ($P < 0.0001$ for each in both populations, Kruskal-Wallis test), but fruit maturation rates do not (Kruskal-Wallis or χ^2 for presence of mature fruit). Seeds per flower, measured only in population A and not shown, did not vary either. Numbers of umbels for each period in population A are 69, 71, 70, 60, 76, 25, and for population B, 48, 56, 87, 54, 25. Some dates were grouped to increase sample size.

fitness measure is badly biased, it appears that flower numbers and longevity are maintained to enhance male competition. Because about 95% of pollinator visits were by bumblebees, it is unlikely that such a bias could arise through differences between treatments in pollinators. A bias could exist if there are strongly diminishing returns on pollinarium removals due to saturation of neighbouring plants⁴, but pollinia are probably dispersed far enough to avoid this. The time required for pollinaria to dry into the orientation most suitable for insertion (107 ± 58 s, $n=40$) far exceeds the time pollinators remain on one plant (31.3 ± 29.6 s, $n=105$). Moreover, *Asclepias syriaca* pollinia have been shown to remain on bumblebees for an average of several hours^{15,16}.

A possible alternative is that such traits are selected because, by increasing fruit initiations, they allow choice of superior fruits¹⁷. Although it seems unlikely that any such gain would be as large as the male benefit, the above experiments cannot exclude it. However, it can be excluded for a third 'male' trait, the seasonal distribution of flowers. In terms of male competition, the best time to produce a flower is whenever other flowers produced at the same time have the highest probability of maturing fruit (that is, less pollen competing for more fruit). Selection on the male function should therefore lead to flowers being shifted from times when small fractions of flowers mature fruits to periods of high maturation probabilities. This selection should persist until the fruit/flower ratio is constant throughout the flowering season, at which point no flower gains a male advantage due to its timing. This model makes some assumptions that will be detailed elsewhere. A critical assumption, that early flowers gain no large advantage by pollinating later ones (the

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Table 1 Plant reproductive measures for experimental treatments and controls

| | E1 | C1 | <i>p</i> | E2 | C2 | <i>p</i> |
|--------------------|---------------|----------------|----------|--------------|---------------|----------|
| Pollinaria removed | 6.36 (50) | 12.45 (89) | 0.01 | 18.4 (33) | 33.5 (112) | 0.03 |
| Fruits initiated | 3.44 (50) | 5.01 (137) | 0.06 | 5.39 (33) | 9.02 (112) | 0.02 |
| Fruits matured | 0.320 (50) | 0.234 (137) | — | 1.67 (33) | 2.23 (112) | 0.18 |

Treatments are: E1, half the flowers removed from each umbel; E2, pollinators excluded from each umbel for 4 days; C1 and C2 are the corresponding untreated controls. Sample sizes are in parentheses. Significance levels are for one-tailed Mann-Whitney *U*-tests, so no level is reported when the observed difference is opposite to the hypothesized one.

converse being impossible), is probably valid because the ratio of fruit initiations to pollinarium removals does not increase during the season.

In this model, selection on the female function may determine when fruits are produced, but selection on the male function alone causes flowering to be timed so as to keep the fruit/flower ratio constant. In a species with excess pollinations, like *A. exaltata*, no single flower distribution maximizes fruit number because a plant could shift many of its flowers and still be adequately pollinated. If, on the other hand, there is selection to maximize choice of high-quality fruits, flowers should be concentrated at times when the largest fraction is pollinated.

The data in Fig. 1 confirm the prediction for selection on the male function only. There is no seasonal change in fruits per flower, despite an increase in pollination levels as measured by either pollinarium removals or fruit initiations. The observed pattern is not due to plants spacing out the drain of fruiting on resources because all fruits achieve most of their growth after the end of flowering. The pattern is inconsistent with maximizing choice because few flowers are produced at the best time for pollination. Choice might occur, but it is not important enough to alter the optimal male strategy.

These results suggest that flower number, umbel longevity and the seasonal distribution of flowers are traits selected primarily for their effects on male competition. In other milkweeds, flower number per umbel is another such trait⁸⁻¹⁰. With apologies to Charles Baudelaire, I call this the fleurs-du-mâle syndrome. Since seed production is often resource-limited in plants¹⁸, the fleurs-du-mâle syndrome should be common; traits increasing pollination levels should often be primarily male traits. An important consequence is that the usual measure of fitness in plants, seed production, will often be inappropriate for evolutionary studies of pollination systems.

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Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo

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The widespread use of clomiphene citrate and exogenous gonadotrophins for *in vitro* fertilization (IVF) in humans^{1,2} frequently results in the production of multiple embryos. Replacement of more than two embryos increases pregnancy rate³ but may result in multiple pregnancies with increased pre- and post-natal abnormality. Preservation of embryos for a limited time allows fewer embryos to be replaced on several different occasions and thus the problems of multiple pregnancy can be minimized, the effectiveness of a single IVF procedure increased and embryo replacement in adverse maternal conditions avoided. Preimplantation embryos have been successfully cryopreserved in many animal species. The sensitivity of embryos to cooling and freezing varies between species and stages of embryo development⁴⁻⁶. We report here the cryopreservation procedures that allow a high survival rate of four- and eight-cell human embryos and the establishment of a pregnancy following the freezing and storage of an eight-cell embryo for 4 months in liquid nitrogen. The pregnancy terminated at 24 weeks' gestation due to development of a septic *Streptomyces agalactiae* chorion amnionitis after premature membrane rupture.

Embryos were provided by patients participating in IVF and who had requested embryo preservation. Four- and eight-cell embryos (34-60 h culture *in vitro*) were frozen using either glycerol or dimethyl sulphoxide (DMSO) as cryoprotectant and subsequently thawed at the appropriate time following spontaneous ovulation in the patient. Embryos were transferred to the patient only if at least 50% of the original blastomeres appeared morphologically normal after 4-12 h culture following thawing and removal of the cryoprotectant. A total of 16 embryos were transferred to 15 patients.

Both four- and eight-cell embryos can survive with the majority of cells intact, using a variety of procedures (Table 1). Greater success was achieved using DMSO as cryoprotectant. Early-cleavage-stage human embryos, unlike morulae and blastocysts, equilibrate very slowly in glycerol solution because of the low permeability of glycerol⁷, and low intracellular levels of glycerol may reduce embryo survival, particularly if embryos are cooled slowly to low temperatures. Moreover, it is difficult to remove glycerol after thawing and we have frequently observed rapid hydration of cells during removal of the cryoprotectant at concentrations of glycerol below 0.33 M and excessive swelling of blastomeres. If the embryos are placed in isotonic sucrose this rapid hydration does not occur. We have yet to establish complete embryo viability by establishment of pregnancy after freezing in glycerol, although embryo survival appears to be high when four-cell embryos are frozen by the short technique and thawed rapidly (Table 1). DMSO is more permeable than glycerol and appears to be a suitable cryoprotectant for both rapid and slow thawing (Table 1). All embryos which appeared morphologically normal were replaced *in utero*. No further assessment of these embryos was permitted under the ethical guidelines established for this work.

Of the 15 patients receiving embryos, pregnancy was established in one (Fig. 1). The embryo was one of four obtained from a patient who had suffered from primary infertility for 12 yr because of bilateral tubal blockage. The details of her initial treatment for IVF are given in Fig. 2 legend. Following transfer of three four-cell embryos, pregnancy was initiated but