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The genetics of sex determination in stinging nettle (Urtica dioica)

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Abstract:

Urtica dioica ("stinging nettle") includes both dioecious and monoecious forms. In most sexually dimorphic angiosperm species, the genetic mechanisms of sex determination are completely unknown. The few species that include both monoecious and dioecious forms provide an unusual opportunity to examine the genetic mechanisms that underlie the separation of sexual functions, through crossing experiments and analysis of progeny segregation. Our focus is on the genetic mechanisms distinguishing monoecious and dioecious forms of *U. dioica*. A complicated picture of sex determination in this species has resulted from crosses between dioecious and monoecious subspecies, as well as between dioecious and monoecious forms of the same subspecies. Most significant is evidence for a maternal influence on sex determination and for the possibility of gynodioecy as an intermediate stage in the evolutionary pathway to dioecy.

Key words: sex determination, dioecy, monoecy, cytonuclear sex determination sexual dimorphism

Introduction:

"The genetic study of sex is important…because…it lies at the root of Mendelian heredity itself and is one of the major factors in evolution" (Muller 1932, p. 135). The known genetic mechanisms underlying sex determination in dimorphic angiosperms are extremely diverse (Ainsworth et al. 1998; Barrett 2002; Dellaporta & Calderon-Urrea 1993; Grant 1999; Grant et al. 1994; Irish & Nelson 1989; Lebel-Hardenack & Grant 1997; Westergaard 1958). In most dimorphic species, however, the mechanisms are completely unknown. The few species that include both monoecious and dioecious forms provide an unusual opportunity to examine the genetic mechanisms that underlie the separation of sexual functions. Analysis of crosses between dioecious forms and those with hermaphroditic flowers necessarily confounds genetic mechanisms responsible for separation of sexual function among flowers with those responsible for separation of sexual function among plants. In contrast, crosses between dioecious and monoecious forms allow us to focus our attention on the genetic mechanisms underlying the separation of sexual functions among plants.

Urtica dioica ("stinging nettle") includes both dioceious (subsp. *dioica*) and monoecious (subsp. *gracilis*) forms. Although Richards's 1997 review lists *U. dioica* as having differentiated sex chromosomes, examination of the primary sources does not support this claim (see also Zuk 1970; Woodland et al. 1982). Because so few plant species exhibit both monoecy and dioecy, analysis of crossing data involving these subspecies provides an unusual opportunity for insight into the genetic determinants of sexual dimorphism (Janick & Stevenson 1955; Westergaard 1958; Lloyd 1975; Wolf et al. 2001; Dorken & Barrett 2004). *Urtica dioica* is even more unusual in producing large

numbers of fertile hybrid progeny. In other species with both monoecious and dioecious forms, crosses often produce too few offspring for genetic analysis (Westergaard 1958; Wolf et al. 2001). In *Urtica dioica* as in *Sagittaria* (Dorken & Barrett 2004), however, a dioecious subspecies is easily crossed with a monoecious subspecies. The hybrids are vigorous and fertile, allowing segregation in a variety of backcross and F_1 intercrosses to be included in the genetic analyses. In addition, monoecious individuals are occasionally encountered within subsp. *dioica*, allowing us to determine whether the genetic differences that distinguish monoecious subsp. *gracilis* from dioecious subsp. *dioica* are the same as those distinguishing the two sexual forms of subsp. *dioica*.

Understanding the genetic basis for sex determination is the first step in understanding the evolution of sexual dimorphism, including the intermediate steps that may have occurred along the pathway from hermaphroditism to dioecy. In this paper our focus is on the genetic mechanisms distinguishing monoecious and dioecious forms of *U. dioica*. Specifically, we present evidence that:

- sex determination is under the control of multiple genes.
- maleness and femaleness in subsp. *dioica* may both have more than one genetic basis.
- the genetic basis of monoecy is different in subsp. *gracilis* and subsp. *dioica*.
- monoecious individuals in subsp. *dioica* are not "inconstant" females, but at least some may be "inconstant" males.
- there is a maternal effect on sex determination.

Methods:

We performed eight categories of crosses within *U. dioica* subsp. *dioica* and between subsp. *dioica* and subsp. *gracilis* (Table 1). Because some *dioica* used in crosses were unisexual and others were monoecious, the sex of the *dioica* parent(s) (male, female, or monoecious) is specified for each cross (the epithet "*dioica*," when used alone, refers to subsp. *dioica* throughout). All *gracilis* used in crosses were monoecious; unisexual individuals are not known in *gracilis*. We use the term "reciprocal" to refer to intersubspecific crosses of the same phenotypic gender classes (male, female, and monoecious) that seem to be stably inherited. Many crosses were followed through F_2 and backcrosses (F_2 and backcross data not presented).

All crosses were conducted in research greenhouses at the University of Connecticut. All parental stocks were tetraploid (presumed autoploid; Woodland et al. 1982), from populations in North America. Woodland and colleagues (1982) found that the more common diploid *gracilis* was not cross-compatible with any tetraploid members of the genus, including *dioica*. The results of this study are therefore not affected by ploidy differences among stocks used in crosses, even though most populations of *gracilis* are diploid. Stocks of *dioica* were collected in Montreal (Canada) and Montgomery Co., MD (U.S.A.), and stocks of *gracilis* were collected in Skagit Co., Washington (U.S.A.). No monoecious individuals were observed in either source population of *dioica*, although they have been documented in other natural populations (de Jong et al. 2005; Glawe & de Jong 2005; Pollard & Briggs 1982) and might be found in these two populations on closer examination. Progeny from all crosses were grown to flowering in University of Connecticut greenhouses.

Because of the minute size of the female flowers and because anthesis occurs as soon as the flowers appear at the apex of the growing shoot (before the inflorescence axis has expanded), bagging of individual flowers or even individual inflorescences was impossible. *Urtica dioica* is wind pollinated, and the anthers dehisce explosively, sending a small cloud of pollen floating away from the plant each time an anther dehisces. Thus, we performed crosses either by isolating pairs of plants or by bagging pairs of plants to avoid pollen contamination. When bagging plants, entire flowering shoots of the plants to be crossed were enclosed in Nitex® bags with a pore size of 5 μm, guaranteeing that no pollen (minimum 10 μm diameter, Woodland et al. 1982) entered the bags from outside. Typically, male and female flowers are borne at different nodes on monoecious plants, with several successive nodes often producing flowers of the same sex. When a monoecious plant was used as female parent, only nodes with female flowers were bagged, to prevent self-fertilization. If male flowers opened on the female parent while bagged, the cross was discarded. No such precaution was necessary when monoecious plants were used as male parents. Bags were left on the plants two weeks, then the stems were severed from the plant while still in the bags, and the bags were left on the severed stems a few more days. Seeds were allowed to mature on the severed stems about a week longer, then sown directly onto damp ProMix® BX or a mixture of ProMix® BX and fine vermiculite.

Individual seedlings were potted into 5" pots of ProMix® BX. From October to March, supplemental lighting was used to produce a day length of 15 hours. Scoring was begun as soon as progeny began to flower. Each plant was normally scored three times, recording the sex of flowers produced at each node (male, female, or a mixture). A

minimum of 10 nodes were scored on most plants, and in most cases the shoot was finished flowering, or nearly so, by the final scoring. With a dioecious species that produces a low frequency of monoecious individuals, one can never be completely certain that an individual scored as male or female will not eventually produce some flowers of the opposite sex; floral meristems have the potential to produce organs of both sexes, remaining bipotent even in unisexual plants (Dellaporta & Calderon-Urrea 1993; Lebel-Hardenack & Grant 1997). As a result, our data might slightly under-represent the proportion of monoecious progeny and over-represent the proportion of at least one unisexual class. But even among unisexual plants that were retained for a number of years (for use in crosses) only a few ever produced flowers of the "wrong" sex, and those produced only a tiny percentage of the opposite-sex flowers, a few flowers out of several thousand. Thus, we are confident that very few monoecious individuals could have been missed in our scoring protocol, and only those having such a small proportion of flowers of one sex as to be nearly unisexual would have been missed..

In the case of nodes at which both male and female flowers were produced, a strictly qualitative assessment of gender bias was made. Mixed nodes were therefore recorded as male-biased, female-biased, or unbiased. This made it possible to score each monoecious individual as male-biased, female-biased, or unbiased, based on the total number of male, female, and mixed nodes, as well as the gender bias of the mixed nodes. For example, a plant that had four male nodes, six mixed nodes, two of which were male biased and four of which were female biased, and six female nodes would be scored as female-biased. No attempt was made to take variation in flower number per node into account. Scoring thousands of progeny, each with thousands of flowers, made

quantitative assessment of gender expression of monoecious individuals, or even of mixed-gender nodes, impractical.

The difficulties in conducting crosses with wind-pollinated plants with minute flowers, coupled with pest management and watering problems, made for considerable variation in the number of progeny produced and grown to maturity per cross. In all cases of low numbers of progeny, whether few maternal families or few progeny within a maternal family, differences in sample size reflected difficulties in setting up the cross and/or culturing the progeny, not differences likely to be due to genetic factors or anything else inherent in the particular cross being performed. Any maternal family with fewer than 10 individuals was excluded from our analyses.

Analyzing sex ratios becomes complicated when more than two sex forms are involved. In dioecious and gynodioecious species, sex ratios may be defined as percentage of either females (Kohn 1989; Korpelainen 2002) or males (Taylor 1999; Glawe & de Jong 2005). With three sex forms, the relevant ratio depends on the comparison of interest. All three sex forms can be included in the sex ratio, or the sex ratio can be based on any two sex forms (ignoring the third), or the ratio of either males or females to all others might be the most appropriate (McArthur & Freeman 1982; Quinn & Engle 1986; Dorken & Barrett 2004). Sex ratios in our analyses are based on males and females (ignoring monoecious individuals) in some cases and on males, females, and monoecious individuals (hermaphrodites; M:F:H) in others.

Results:

All crosses were fertile, and in only a few cases (all intersubspecific crosses) did the F_1 progeny (particularly males) appear to be partially sterile. The results of Cross 7 are based on a single maternal family, and therefore must be interpreted with caution. Within monoecious progeny, there was quantitative variation in the proportions of male and female flowers (data not presented). Crosses between male and female *dioica* (Cross 1) show that although the primary sex ratio is unbiased (Fig. 1.a; $\chi^2_1 = 0.48$), there appears to be considerable sex ratio variation among maternal families; the effect is marginally significant (Fig. 2; $\chi^2_6 = 10.70$; $P<0.10$). A small percentage of the progeny were monoecious, and the fraction of monoecious progeny appeared to vary among families as well. The substantial among-family variation suggests that sex determination is influenced by many genes in this species.

Progeny segregation from male and female parents differed according to the origin of the parents. Figure 3 shows the results of a cross in which the male and female parents were the offspring of a selfed monoecious *dioica* (Fig. 3.a) or in which the male parent was the offspring of a selfed monoecious *dioica* and the female parent was a *dioica* × *gracilis* hybrid (Fig. 3.b). Although both crosses resulted in a large percentage of monoecious progeny, the ratio of male to female progeny is very different between them, and both progeny ratios are very different from those obtained when male and female parents were themselves the offspring of male and female parents (Cross 1; Fig. 1.a, Fig. 2).

Crosses between female *dioica* and either monoecious *dioica* (Cross 3; Fig. 1.c) or *gracilis* (Cross 5; Fig. 1.e) and between male *dioica* and either monoecious *dioica* (Cross 4; Fig. 1.d) or *gracilis* (Cross 6; Fig. 1.f), provide evidence that the genetic basis

of monoecy is not the same in the two subspecies. Results from a 2 × 3 contingency table analysis indicate that when the unisexual parent was held constant, regardless of whether it was male or female *dioica*, progeny ratios were very different when the monoecious parent was *gracilis* from those when the monoecious parent was *dioica* (Cross 3 M:F:H 101:280:210 vs. Cross 5 M:F:H 61:128:34, P=1.189e-08, and Cross 4 M:F:H 15:1:9 vs. Cross 6 M:F:H 33:231:34, P=7.968e-14), a strong indication that monoecy in *gracilis* has a different genetic basis from that in *dioica*.

Results from three pairs of crosses (Table 2; Fig. 1.a-e, g) show that monoecious individuals of *U. dioica* are not inconstant females. In each pair of crosses, the male parent was the same and the female parent was either female *dioica* or monoecious *dioica*. In all three pairs, progeny ratios when the female parent was monoecious *dioica* were strongly male biased and significantly different from progeny ratios when the female parent was female *dioica* (Cross 1 vs. Cross 4 M:F:H 196:214:62 vs. 15:1:9, *P*=6.98e-06; Cross 2 vs. Cross 3 M:F:H 90:17:78 vs. 101:280:200, *P*=2.2e-26; Cross 5 vs. Cross 7 M:F:H 61:121:34 vs. 30:8:20, *P*=1.99e-09), suggesting that sex determination is fundamentally different in female and monoecious *dioica*.

Results from the comparable pairs of crosses (Table 3, Fig. 1.a-d, f, h), in which the female parent was the same and the male parent was either male *dioica* or monoecious *dioica* give a less consistent, but perhaps more interesting, picture of the gender identity of monoecious *dioica*. When the female parent was female *dioica*, progeny ratios of monoeious *dioica* as male parent were strongly female-biased and significantly different from ratios of male *dioica* as male parent (Cross 1 vs. Cross 3 M:F:H 229:214:62 vs. 101:280:210, *P*=2.2e-16), suggesting that monoecious *dioica* are

genetically different from male *dioica*. Progeny ratios were also female biased when *gracilis* was the female parent, regardless of whether the male parent was male or monoecious *dioicia*, with marginally significant greater female bias when the male parent was male *dioica* (Cross 6 vs. Cross 8 M:F:H 33:231:34 vs. 13:33:6, P=0.029). In striking contrast, when the female parent was monoecious *dioica*, progeny ratios were strongly male-biased (especially when considering just males and females) and similar, regardless of whether the male parent was male or monoecious *dioica* (Cross 2 vs. Cross 4 M:F:H 93:17:78 vs. 15:1:9, P=0.6). The latter comparison suggests that monoecious *dioica* are genetically more similar to males than to females. In summary, these crosses provide evidence that monoecious *dioica* are genetically different from female *dioica*, but that some monoecious *dioica* behave as genetic males, at least to some extent. The very small fraction of female progeny (<10%) obtained from selfed monoecious *dioica* (Cross 2, Fig. 1.b) provides further evidence that some monoecious individuals are best interpreted as inconstant males.

One final comparison that sheds light on the gender of monoecious individuals is that of Crosses 3 and 4, between unisexual and monoecious *dioica* (Fig. 1.c, d). Not only are progeny ratios significantly different between male and female *dioica* as the unisexual parent, but in each case the ratio bias is in the direction of the sex of the unisexual parent. In other words, female crossed with monoecious yielded female-biased progeny and male crossed with monoecious yielded male-biased progeny (Cross 3 vs. Cross 4 M:F:H 101:280:200 vs. 15:1:9, P=8.4e-08). This comparison argues for monoecious individuals being neither inconstant males nor inconstant females, but rather a genetically distinct gender class.

Finally, several of our results demonstrate a maternal (presumably cytoplasmic) influence on sex determination. Not only did we find an excess of female progeny in many crosses, we also found that progeny ratios differed between reciprocal crosses. We found significant female biases in the progeny of crosses of female with monoecious *dioica* (Fig. 1.c), of *gracilis* with both male and female *dioica* (Fig. 1.e, f), and of *gracilis* with monoecious *dioica* (as male parent) (Fig. 1.h). Similarly, reciprocal crosses between *gracilis* and monoecious *dioica* yielded asymmetrical progeny segregation, strongly male biased in one cross and strongly female biased in the other (Crosses 7 and 8, Fig. 1.g, h; Cross 7 M:F 31:8; Cross 8 M:F 13:33 – Fisher's exact test: P=3.2e-6).

Discussion:

In his pioneering research on *Urtica dioica* genetics, Zuk (1970) concluded that this species has a "rather primitive" mechanism of sex determination, with sex-determining loci distributed over several chromosomes. Of his crossing results between hermaphrodites and between males or females and hermaphrodites he reported, "In the progeny...no regularity is found in the segregation of sex-determining factors, the proportion of ♂ and ♀ plants being quite fortuitous." As with Zuk's results, the complex inheritance pattern revealed in our crosses precludes construction of a simple genetic model for sex determination in *Urtica dioica*. Nevertheless, several important insights emerge from these results. First, more than one gene is involved in sex determination in this species. We cannot suggest how many, but the results are not consistent with single-locus sex determination, such as that found by Wolf (2001) for *Datisca*, under which 1:1 sex ratios from all crosses would be expected. Moreover, progeny sex ratios differ

among maternal families when crossing male and female *dioica* (Fig. 2, $P<0.10$), suggesting that genetic variation among maternal lines influences progeny sex ratios. De Jong and colleagues (de Jong & Klinkhamer 2002; de Jong et al. 2005) have obtained similar results. More maternal families need to be tested to determine whether the observed variation is continuous.

Second, there are apparently several different genetic mechanisms for producing males and females, as suggested by different progeny segregations from male and female parents of different ancestry. Such different progeny segregations also provide further evidence that sex determination in this species is under the control of multiple genes. Male and female *dioica* yielded different progeny segregations when crossed, depending on whether they were the offspring of male and female parents (Fig. 1.a) or of a selfed hermaphrodite parent (Fig. 3.a). A male *dioica* (from male and female parents) crossed with a female *dioica-gracilis* hybrid produced still a different progeny segregation (Fig. 3.b).

Third, monoecy in subsp. *dioica* clearly has a different genetic basis than monoecy in subsp. *gracilis*, a surprising result, given that they are so closely related. Monoecy is thought to be the ancestral condition in most lineages in which both monoecy and dioecy occur (Renner & Ricklefs 1995; Webb 1999). If dioecy evolved from monoecy in *U. dioica*, as expected, the genetic basis of monoecy in *dioica* would be expected to resemble that in *gracilis*, as both would reflect the ancestral (monoecious) condition. The different progeny ratios obtained from *gracilis* and monoecious *dioica* in otherwise equivalent crosses indicates a different genetic basis for monoecy in the two subspecies.

Fourth, the occasional monoecious individuals found in natural populations of *dioica* do not represent inconstant females (sensu Lloyd 1980), but some appear to represent inconstant males. When female and monoecious *dioica* were crossed with *gracilis* (as the male parent, Crosses 5 and 7), the progeny sex ratios are very different (Fig. 1.e, g), indicating that monoecious *dioica* are genetically different in their sex determination from female *dioica* (i.e., monoecious individuals are not simply females that are producing some flowers of the "wrong" sex).

In contrast, we found conflicting results on the question of whether monoecious *dioica* represent inconstant males. In some crosses, monoecious *dioica* behaved like males, or at least more similarly to males than to females, as can be seen in a comparison of Crosses 2 and 4 (Fig. 1.b, d; Table 3) and in the small fraction of female progeny from selfed female *dioica* in Cross 2 (Fig. 1.b). These results indicate that some monoecious individuals of subsp. *dioica* may represent inconstant males, consistent with the findings of de Jong and colleagues (2005), based on the direction of labile sex expression in greenhouse experiments. Pollard's (1981) limited sample of 20 progeny from two maternal families of selfed monoecious *dioica* showed a male bias in both families, with nearly equal proportions of female and monoecious progeny.

Other results, however, contradict the interpretation of monoecious *dioica* as inconstant males. Comparisons of Crosses 1 and 3 (Fig. 1.a, c; Table 3) and Crosses 3 and 4 (Fig. 1.c, d) indicate that monoecious *dioica* are distinctly different from males. The question of whether or not monoecious *dioica* represent inconstant males has important implications for the evolution of dioecy in this species, due to different theoretical expectations for the paradioecy vs. the gynodioecy pathway. The paradioecy

pathway predicts a series of mutations reallocating male and female function in monoecious individuals, in which case monoecious individuals would be equally likely to be males or females, whereas the gynodioecy pathway predicts inconstant males only (Lloyd 1980; Charlesworth & Guttman 1999; Dorken & Barrett 2004). It is possible that there are two genetically different types of monoecious individuals in *U. dioica*, those that are inconstant males (de Jong et al. 2005), as in *Sagittaria* (Dorken & Barrett 2004), and those that are genetically distinct in their sex determination from either males or females.

Finally, several of our results indicate a maternal (presumably cytoplasmic) influence on sex determination, although further crosses would be necessary to test the hypothesis of cytonuclear sex determination. When individuals with cytonuclear sex determination are crossed with individuals from another taxon, or even another population, and the cytoplasmic male sterility factors are decoupled from their restorers, two common types of progeny segregation occur: non-Mendelian ratios, particularly an excess of female progeny (Frankel & Galun 1977; Couvet et al. 1986; Belhassen et al. 1991) and asymmetrical progeny ratios in reciprocal crosses (Grun 1976; Kheyr-Pour 1980, 1981; Kaul 1988; Belhassen et al. 1991). Our results include examples of both, suggestive of a cytonuclear model of sex determination. While not all maternal effects on sex determination indicate a cytoplasmic basis for sex determination (Fishman & Willis 2006), the data presented here are consistent with predictions based on both theoretical and empirical results (Grun 1976; Frankel & Galun 1977; Kheyr-Pour 1980, 1981; Kaul 1988; Belhassen et al. 1991; Maurice et al. 1993; Maurice et al. 1994). In particular, our results are not consistent with the two-locus nuclear model that Dorken

and Barrett (2004) used to explain sex determination in *Sagittaria*. Evidence presented above that at least some monoecious individuals are inconstant males but never inconstant females suggests that females are male-sterile and that the cytoplasmic influence is therefore in the direction of female bias.

When we crossed *gracilis* with unisexual *dioica*, the progeny were strongly female biased, regardless of the direction of the cross (Crosses 5 and 6; Fig. 1.e, f). Two other crosses (3 and 8) also yielded significant female biases in the F_1 (Fig. 1.c, h). Female *dioica* parents produced female-biased progeny when crossed with a monoecious individual, whether that individual was *dioica* or *gracilis* (Crosses 3 and 5; Fig. 1.c, e). Several of the male and monoecious F_2 and backcross progeny from Cross 2 had small male flower buds that never opened and appeared to be sterile (data not presented). There is, therefore, evidence for a maternal influence in the direction of male sterility in *U. dioica*, although we cannot be certain that it arose prior to the evolution of dioecy.

Similarly, asymmetrical segregation in reciprocal crosses, such as Crosses 7 and 8 (Fig. 1.g, h), is critical for distinguishing between nuclear and cytonuclear sex determination (Kheyr-Pour 1980, 1981; Belhassen et al. 1991). Cross 7 resulted in strongly male-biased progeny and Cross 8 in strongly female-biased progeny, although these results must be interpreted with caution because Cross 7 results were based on a single maternal family. When male sterility is determined solely by nuclear genes, no differences between reciprocal crosses is expected (Grun 1976). The significant differences in progeny segregation between crosses indicates non-Mendelian heritance, and that the monoecious parents do not have the same cytoplasmic genes (Belhassen et al. 1991).

Our results have implications for the possible intermediate evolutionary stages between monoecy and dioecy in *U. dioica*. Webb (1999) reviewed two cases where dioecy appears to have evolved from monoecy via gynodioecy, but dioecy in lineages in which monoecy also occurs is generally assumed to have evolved via the "paradioecy" pathway (Lloyd 1980; Renner & Ricklefs 1995). Although the finding of multigenic control of sex determination is consistent with the paradioecy pathway, two other results from our study are not: differences in the genetic basis of monoecy in the two subspecies and possible inconstant males but no inconstant females. The finding of a maternal influence in sex determination is consistent with the gynodioecy pathway, as is the finding of female constancy (Charlesworth & Charlesworth 1978; Lloyd 1980). Clearly, evolutionary pathways cannot be inferred solely from presumed ancestral states. Based on our results, the gynodioecy pathway appears to have been a more likely route to dioecy than the paradioecy pathway in this species (Charlesworth & Charlesworth 1978; Lloyd 1980; Dorken & Barrett 2004). Although gynodioecy has been associated with the evolution of dioecy from monoecy in a few species (Webb 1999; Sarkissian et al. 2001; Dorken & Barrett 2004), cytonuclear sex determination has not previously been associated with species in which monoecy occurs. In *U. dioica*, dioecy may have evolved through an intermediate stage of cytoplasmically determined gynodioecy. Dorken and Barrett (2004) presented evidence that dioecy evolved from monoecy via gynodioecy in *Sagittaria latifolia*, but their results were consistent with purely nuclear sex determination. The genetic basis of sex determination in *U. dioica* and other species in which a monoecy-gynodioecy-dioecy pathway appears to have been likely should continue to be a fruitful area of research.

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Figure 1. Percentage of ♂, ♀, and monoecious progeny from the eight types of crosses (see Table I). Note scale differences in Y-axes. *P* values are for χ^2 tests on ♂ and ♀ progeny. a. *U. dioica* subsp. *dioica* ♀ × ♂ (Cross 1). N=505, seven maternal families, *P*=0.48. b. Selfed monoecious *U. dioica* subsp. *dioica* (Cross 2). N=188, two maternal families, *P*<0.0001. c. ♀ × monoecious *U. dioica* subsp. *dioica* (Cross 3). N=595, eleven maternal families, *P*<0.0001. d. Monoecious × ♂ *U. dioica* subsp. *dioica* (Cross 4). N=37, four maternal families, *P*<0.0001. e. ♀ *U. dioica* subsp. *dioica* × subsp. *gracilis* (monoecious) (Cross 5). N=238, six maternal families, *P*<0.0001. f. *U. dioica* subsp. *gracilis* (monoecious) × ♂ subsp. *dioica* (Cross 6). N=298, three maternal families, *P*<0.0001. g. Monoecious *U. dioica* subsp. *dioica* (as maternal parent) × subsp. *gracilis* (monoecious) (Cross 7). N=58, one maternal family, *P*=0.0002. h. *U. dioica* subsp. *gracilis* (monoecious, as maternal parent) × monoecious subsp. *dioica* (Cross 8). N=52, four maternal families, *P*=0.003.

Figure 2. Progeny ratios from Cross 1 (Fig. 1.a) broken down by maternal family, number of progeny in parentheses. Error bars indicate standard error.

Figure 3. Percentage of ♂, ♀, and monoecious progeny from crosses between male and female parents of different origins. *P* values are for χ^2 tests on ♂ and ♀ progeny. a. *U. dioica* subsp. *dioica* ♀ × ♂ (Cross 2 F_2). N=151, five maternal families, *P*<0.001. b. *U. dioica* subsp. *dioica* ♀ × ♂. ♂ parent from Cross 2 F_1, ♀ parent from Cross 6 F_1. N=48, one maternal family. *P*=0.4.

Table 1. The eight categories of crosses conducted using plants of *Urtica dioica* subsp. *dioica* and *U. dioica* subsp. *gracilis*. (All plants of subsp. *gracilis* were monoecious; some plants of subsp. *dioica* were unisexual, others were monoecious, as indicated.)

cross number	female parent	male parent
1	♀ *dioica*	♂ *dioica*
2	monoecious *dioica*	monoecious *dioica*
3	♀ *dioica*	monoecious *dioica*
4	monoecious *dioica*	♂ *dioica*
5	♀ *dioica*	*gracilis*
6	*gracilis*	♂ *dioica*
7	monoecious *dioica*	*gracilis*
8	*gracilis*	monoecious *dioica*

Table 2. Are monoecious *dioica* genetically female-like? *P* values are from 2 × 3 contingency tests comparing M:F:H progeny ratios.

Male Parent	**Female Parent**			
	Female *dioica*		**Monoecious *dioica***	
Male *dioica*	Cross 1	**vs.**	Cross 4	*P*=7.0e-06
Monoecious *dioica*	Cross 3	**vs.**	Cross 2	*P*=2.2e-26
gracilis (monoecious)	Cross 5	**vs.**	Cross 7	*P*=2.0e-09

Table 3. Are monoecious *dioica* genetically male-like? *P* values are from 2 × 3 contingency tests comparing M:F:H progeny ratios.

Female Parent	**Male Parent**			
	Male *dioica*		**Monoecious *dioica***	
Female *dioica*	Cross 1	**vs.**	Cross 3	*P*=2.2e-16
Monoecious *dioica*	Cross 4	**vs.**	Cross 2	*P*=0.6 (both male biased)
gracilis (monoecious)	Cross 6	**vs.**	Cross 8	*P*=0.03 (both female biased)

Results from all crosses. Number of progeny in each family given in parentheses. Monoecious progeny are divided into male biased/female biased/unbiased. χ^2_{het} tests the null hypothesis that progeny proportions are equal across maternal families. Statistics in the "females" column refer to a heterogeneity test of male:female proportions. Statistics in the "monoecious" column refer to a heterogeneity test of (male+female):monoecious proportions. $\chi^2_{1:1}$ tests the null hypothesis that the males and females occur in equal proportions in progeny. We report it only for F_1 crosses, because expectations for backcrosses are not clear in the absence of a genetic model. ($^+$ P < 0.10; * P < 0.05; ^{a} P < 0.01; ^{b} P < 10^{-4})

	Maternal Family (n)	males	females	monoecious
Cross 1:				
♀ × ♂ ***dioica***				
F_1	2510 (93)	51	37	4/1/0
	2511 (74)	30	29	5/9/1
	2512 (78)	38	28	4/8/0
	2513 (83)	35	43	2/3/0
	2514 (58)	35	21^+	1/1/0
	2515 (32)	14	16	1/1/0
	2516 (87)	26	40	10/9/2
	χ^2_{het}		10.68^+	28.9^b
	$\chi^2_{1:1}$		0.51	
Cross 2:				
selfed monoecious *dioica*				
F_1	2499 (89)	53	3	16/4/13
	2518 (99)	40	14	17/19/9
	χ^2_{het}		8.90^a	1.35
	$\chi^2_{1:1}$		52.5^b	
Cross 3:				
♀ × **monoecious *dioica***				
F_1	310 (32)	17	15	0
	139 (17)	5	9	3/0/0
	142 (15)	4	7	2/2/0
	145 (13)	6	4	0/2/1
	195/205 (114)	17	52	28/12/5
	201 (42)	9	18	9/4/2
	202 (96)	12	47	28/7/2
	203 (130)	26	66	27/8/3
	204 (20)	5	9	4/2/0
	207 (102)	0	53	11/21/17
	χ^2_{het}		40.3^b	36.3^b
	$\chi^2_{1:1}$		84.1^b	
Cross 4:				
monoecious × ♂ ***dioica***				
F_1	144 (15)	11	0	4/0/0
	246 (10)	4	1	2/3/0
	χ^2_{het}		2.34	1.42

	Maternal Family (n)	males	females	monoecious
	$\chi^2_{1:1}$		12.3[a]	
Cross 5:				
♀ ***dioica*** × ***gracilis*** (monoecious)				
F_1	080 (11)	5	6	0
	147 (105)	32	60[a]	11/1/1
	146 (107)	24	62[a]	10/9/2
	χ^2_{het}		1.89	4.23
	$\chi^2_{1:1}$		23.8[b]	
Cross 6:				
gracilis (monoecious) × ♂ ***dioica***				
F_1	2483 (100)	8	80	1/7/4
	2484 (98)	9	89	0
	2485 (100)	16	62	2/15/5
	χ^2_{het}		6.50*	23.8[b]
	$\chi^2_{1:1}$		148.5[b]	
Cross 7:				
monoecious *dioica* × *gracilis* (monoecious)				
F_1	045 (59)	31	8	9/11/0
	$\chi^2_{1:1}$		13.6[a]	
Cross 8:				
gracilis (monoecious) × **monoecious *dioica***				
F_1	104 (10)	2	8	0
	105 (18)	1	15	1/1/0
	153 (11)	4	4	2/0/1
	251 (13)	6	6	1/0/0
	χ^2_{het}		8.01*	2.12
	$\chi^2_{1:1}$		5.44[a]	