Sex ratio variation and sex determination in Urtica dioica

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Genetic mapping of sex-linked markers in *Urtica dioica*

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Genetic crosses that we performed in previous experiments (Chapter 5) were largely consistent with males being the heterogametic sex and females being the homogametic sex in *U. dioica*. Based on these crosses, a single locus was suggested to have a major effect on sex determination. However, the crosses also yielded numerous unpredicted sexual phenotypes, raising the question whether more genes on other loci are involved in sex determination. The aim of this study was to investigate this issue by using (1) molecular marker analysis and (2) karyological analysis. For the random amplified DNA fingerprinting (RAF) analysis, progeny from a cross of a single female with a single male were used. A total of 63 polymorphic markers from 14 primers were obtained, of which seven markers were found to be significantly associated with sex. Of the markers linked with sex, three were detected for which the male parent was heterozygous, and four for which both parents were heterozygous. No sex-linked marker was found segregating in female plants only. Two of the sex-linked markers could be placed on a linkage map, but in different linkage groups. Cytological investigations of mitotic chromosomes showed no evidence for the existence of morphologically distinct sex chromosomes. A major sex determination locus for which the male is heterozygous is possible but has not been detected yet.

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Dioecy, or individuals of a species being either male or female, is the most common sex type in animals. In the plant kingdom, however, most flowering species are hermaphroditic, i.e. they develop bisexual flowers containing both functional male and female organs. Only 6% of the angiosperm species are dioecious, with separate individuals producing male and female flowers (Renner and Ricklefs 1995). Some dioecious plants are used as crops, and often one sex is preferred, such as the male sex in *Cannabis sativa* (fibre quality and quantity) and *Asparagus officinalis* (increased vigour) or the female sex in *Spinacia oleracea* (retarded shooting) and *Humulus lupulus* (unpollinated flowers for brewing). Comparative evidence suggests that dioecy has evolved independently from hermaphroditism in different plant families and plant genera, thereby leading to the great diversity of sex determination mechanisms in plants (Ainsworth 1998, Charlesworth 2002). The sexual phenotype in dioecious plants can be controlled by (1) a single locus (*Ecballium elaterium*), (2) a number of unlinked loci on different chromosomes (*Mercurialis annua*), (3) loci on homomorphic sex chromosomes (e.g. *Actinidia deliciosa* and *Dioscorea tokoro*), or (4) loci on heteromorphic sex chromosomes (e.g. *Humulus*, *Silene* and *Rumex* spp.) (Grant 1999, Matsunaga and Kawano 2001).

In his 1958 review, Westergaard already listed three important steps for the genetic analysis of the sexuality of a given species: (1) establishment of the heterogametic sex, (2) localization of sex-deciding genes, and (3) study of individual gene action by breaking up gene complexes. He also realized that the genetics of sex determination is more complicated than would appear from the simple scheme of Mendelian inheritance in which gender is determined by the heterogametic sex. He stated that, in order to understand the mode of sex inheritance, sex expression rather should be viewed as a quantitative phenomenon with maleness and femaleness expressed to varying degrees.

There are several methods to identify the heterogametic sex. Sex chromosomes have been reported in several dioecious plant species. The most obvious way thus seems to demonstrate the existence of heteromorphic chromosomes. Unfortunately, the large number and small size of the chromosomes make it difficult testing for the presence of heteromorphic chromosomes in some species (e.g. *Salix*, *Dioscorea*). Also, in species carrying sex loci on homomorphic chro-

mosomes, the heterogametic sex cannot be established by cytological investigations. Another way, and favoured by most recent studies, is to establish the heterogametic sex through the identification of genetic markers which are tightly linked to sex. Sex-linked markers suggesting that a single locus governs sex determination have been found, for example, in *Silene latifolia* (Mulcahy et al. 1992), *Actinidia chinensis* (Harvey et al. 1997), *H. lupulus* (Polley et al. 1997), *Cannabis sativa* (Mandolino et al. 1999), and *Salix viminalis* (Semerikov et al. 2003).

In the clonal herb *Urtica dioica*, both progeny sex ratio (fraction of males, de Jong et al. 2005) and sex ratio of flowering plants in the field (Glawe et al., Chapter 3) have been shown to vary dramatically, making it an interesting object to study the mechanism behind sex ratio variation. Another intriguing feature of *U. dioica* is the occurrence of low proportions of monoecious plants beside male and female individuals in natural populations (Kay and Stevens 1986; Glawe et al., Chapter 3) as well as in controlled crosses between males and females (Glawe and de Jong, Chapter 5).

At the present time, the general accepted theory is that determination of sex in dioecious plants species can be controlled by both environmental and genetic factors (Ainsworth et al. 1998). Because sex ratio variation in *U. dioica* may be attributed to environmental sex determination (ESD), extensive experiments were performed to investigate whether progeny sex ratio and sex expression of male and female plants can be influenced by varying environmental factors, such as soil fertility and soil moisture, temperature and light intensity (Glawe and de Jong 2005). However, since the progeny sex ratio was not affected by varying environmental conditions and sex expression in male and female plants was stable, the observed sex ratio variation in *U. dioica* is assumed to be solely genetically based. Little is know about the genetic mechanism of sex determination in *U. dioica*. The older literature (Strasburger 1910) stated male heterogamy. However, the presence of heteromorphic sex chromosomes has not been convincingly demonstrated (Meurman 1925). A recent study, based on a series of experimental crosses among male, female and monoecious *U. dioica*, was consistent with male heterogamy and suggested a single locus that has a major effect on sex determination (Glawe and de Jong, Chapter 5). With a simple sex determination mechanism such as an XX/XY system, sex ratio bias in *U. dioica* may be caused by addition-

al genes which distort segregation. Alternatively, a quantitative genetic model may be more appropriate. With polygenic sex determination the gender of the offspring will be determined by the sum of the genetic effects of sex alleles over different loci. Such a system can easily generate sex ratios deviating from 1:1.

The main goal of the present study was to identify and map sex linked markers and, more specifically, to test the hypothesis that male *U. dioica* plants represent the heterogametic sex. In this case we expect a single marker or a group of linked markers segregating with sex. Furthermore, this paper presents a karyological study of both male and female individuals of *U. dioica* to screen for heteromorphic sex chromosomes.

MATERIALS AND METHODS

Genome size

Urtica dioica is allo-tetraploid (2n=4x=52, IPCN data base; Sitte et al. 1998). Allozyme data on four loci suggested that inheritance in this species follows typical Mendelian patterns (Mutikainen and Koskela 2002). The genome size of *U. dioica* is 1C=1.58 pg DNA (Mowforth 1986). Converting picogram of DNA into base pairs shows that the genome of *U. dioica* is 1540 Mb. On average, each chromosome contains therefore 29.6 Mb. In order to obtain a rough estimate of the average chromosome size in map units, we compared *U. dioica* with *Solanum lycopersicum*. The genome size of *S. lycopersicum* is 1C=1.00 pg DNA. Mapping studies indicated that on average 1 cM is 510 kb (see Lynch and Walsh 1998). Using the data known from *S. lycopersicum*, the average chromosome size in *U. dioica* would be 58 cM. However, since the conversion of Mega base into centi Morgan is variable between species, the average chromosome size may deviate from this estimate.

Plant material

For this study, we selected plant material from a single open-pollinated female (M31). Offspring of this plant was already used in a series of crosses to investigate inheritance of sex (Glawe and de Jong, Chapter 5). The progeny, consisting of 38 female and 40 male plants of the cross of a single female with a single male from M31, were

used for marker analysis. The progeny was grown in a climate chamber under standard conditions: 20°C during 16 h light and 15°C during 8 h dark with 70% relative humidity, and with 180-200 µmol m^{-2} s^{-1} PPFD at plant growing level. Gender was determined several times during flowering.

RAFs (Random Amplified DNA fingerprinting)

RAF is a modified DNA amplification fingerprinting technique that represents a culmination of the DNA marker technologies (RAPD, DAF, AFLP) based on arbitrarily-primed PCR (Waldron et al. 2002). RAF is a dominant marker technique and compares with AFLP for efficiency and reliability on many plant genomes (Waldron et al. 2002). The advantages of RAF over AFLP include: (1) no requirement for enzymatic template preparation, (2) one instead of two PCR's, and (3) lower costs. Preliminary investigations indicated that this method is working well and reliably detects polymorphic markers in *U. dioica*. For example, we tested whether the RAF technique applied to *Urtica* would give fully reproducible results. Also, for each gel an independent PCR reaction was performed for both parents and run together with all offspring. DNA was extracted from both parental and progeny leaf material (2x2 cm^2) using the Dneasy tissue kit (Qiagen).

Each RAF reaction contained 1x Stoffel Buffer, 5 mM $MgCl_2$, 20 µM dNTPs, 0.1mg/ml BSA, 1.5 units of DNA polymerase Stoffel Fragment (Applied Biosystems), 5 µM of a single oligonucleotide primer of 10 or 11 nucleotides and 1 µl of diluted template DNA (on average 15 ng/µl) in a total volume of 10 µl. The primers contained a FAM (6-carboxy-fluorescein)-tagged oligonucleotide. The 14 primers used here and their annealing temperatures are listed in Table 6.1. The PCR was performed using the following protocol: an initial denaturation step of 94°C for 5 min, followed by 30 amplification cycles of 30 s denaturation at 94°C, and annealing for 5 min with 60 s at 51°C, 50°C, 49°C, 48°C, 47°C, respectively. This was followed by an extension step of 45 s at 72°C. There was a final extension of 5 minutes at 72°C. The size of RAF fragments in base pairs (bp) was determined by using the size standard ROX 1000 (Applied Biosystems). The DNA fragments were separated on a 5% polyacrylamide gel on an ABI 377 automated sequencer (Applied Biosystems).

TABLE 6.1 – Primers, sequences, annealing temperature in the PCR program, and number of markers detected by each single primer.

Primer code	Sequence 5'-3'	Annealing temperature (°C)	Polymorphic markers
RP 2	ATGAAGGGGTT	51	4
RP 3	TGCTGGCTCCC	51	3
RP 4	TGCTGGTTCCC	53	1
RP 6	TGCTGGTTTCC	51	2
RP 10	ATAGCAAGCG	52	5
RP 12	ATGCAGTAGCC	51	8
RP 13	TACGTCATCGC	51	0
RP 23	TAGGCAAGTGG	51	1
RP 27	CGTTATGGTGT	51	9
RP 37	AGCTTAGGCT	51	6
RP 38	TACTGTGTCC	51	1
RP 40	CCTCAACAGT	51	13
RP 51	TCAGAGGATG	53	3
RP 55	TAAGATGCCC	53	7

Marker analysis

All gels were extracted and aligned in GeneScan. Next, the aligned gels were analyzed in Genographer. Polymorphic markers from the RAF profiles were detected by eye in Genographer. All markers between 100 and 950 bp, showing clear differences between presence and absence of bands were chosen. The markers were tested if they were inherited following disomic Mendelian rules using a χ^2-test. If only one parent was heterozygous then a 1:1 ratio of bands (heterozygous) to no bands (homozygous recessive) was expected in the progeny. For a single marker, the χ^2 was calculated as follows:

$$\chi^2 = \left[\frac{(B - 0.5T)^2}{0.5T}\right] + \left[\frac{(NB - 0.5T)^2}{0.5T}\right],$$

with B as the total number of individuals where a band was present, NB as the total number of individuals where a band was absent, and T as the total number of offspring.

When both parents are heterozygous, the ratio of homozygous dominant, heterozygous and homozygous recessive in the progeny is 1:2:1. Because homozygous dominant and heterozygous results in a band, the ratio of individuals with a band to individuals with no band is 3:1. The formula for this situation is:

$$\chi^2 = \left[\frac{(B - 0.75T)^2}{0.75T}\right] + \left[\frac{(NB - 0.25T)^2}{0.25T}\right].$$

The association between sex and single markers was tested using a χ^2 test (χ^2_S) (Sokal and Rohlf 1998; without Yates adjustment for continuity).

The mapping program Joinmap 3.0 (Van Ooijen and Voorrips 2001) has been used for the calculation of recombination values and the construction of linkage groups. The groups with a LOD score above 3 were selected. Kosambi's mapping function was used to convert the percentage of recombination into map distances (cM).

Discriminant analysis (SPSS 10.0) with sex as grouping variable and sex-linked markers as independent variables was performed stepwise to assess how accurately sex of male and female *U. dioica* plants can be predicted.

Poisson distributions were calculated separately for both male and female parent to evaluate the chance of detecting different numbers of polymorphic markers per chromosome. This allows us to estimate the percentage of chromosomes that are not covered by the markers found. Next, a χ^2-test was performed to compare the observed markers per linkage group with the expected data.

Cytological analysis

For chromosomal analysis, mitotic studies were made on young root tips of male and female plants obtained from seeds collected at our field site in Meijendel. The root tips were pre-treated in a 1% aqueous solution of hydroxyquinoline at 4°C for 6 h and fixed in acetic acid:ethanol (1:3). Chromosome spread preparations were prepared according to the techniques described in Pijnacker and Ferverda (1984). Chromosomes were stained with DAPI (4', 6 diamidinopropyl-indole) and studied under a fluorescence microscope. Images were captured and processed digitally in Adobe Photoshop and Corel Draw.

RESULTS

Molecular markers

A total of 63 polymorphic markers were identified with 14 RAF primers (Table 6.2). Of the total number of markers detected, 20 were heterozygous in the paternal parent, 23 markers were heterozygous in the maternal parent, and 20 markers were found to be het-

TABLE 6.2 – Polymorpic markers, parental genotypes, expected marker segregation ratios and χ^2-square tests (χ^2_M: testing Mendelian inheritance, and χ^2_S: testing the association with sex). Markers (grouped and ungrouped) are represented containing the primer code letter and the size of the mapped fragment (bp). The genotype of the parental plants are nn x np when markers segregated in the female parent, lm x ll when markers segregated in the male parent, and hk x hk when markers segregate in both parents. *P<0.05, **P<0.01, ***P<0.001

Marker	Genotype [Male x female]	Expected Ratio	χ^2_M	χ^2_S
Mapped (see Figure 6.1)				
Group 1				
RP12-547	nn x np	1: 1	2.922	0.264
RP12-552	nn x np	1: 1	2.195	0.083
Group 2				
RP51-457	hk x hk	1: 3	2.396	4.739*
RP51-717	hk x hk	1: 3	0.378	1.833
Group 3				
RP27-548	nn x np	1: 1	3.959*	0.077
RP40-384	nn x np	1: 1	2.513	0.422
RP12-474	nn x np	1: 1	2.195	0.030
Group 4				
RP2-877	hk x hk	1: 3	0.427	0.889
RP55-881	hk x hk	1: 3	1.640	3.331
Group 5				
RP55-662	nn x np	1: 1	2.297	0.377
RP55-642	hk x hk	1: 3	1.640	0.017
Group 6				
RP6-316	hk x hk	1: 3	1.423	0.600
RP10-502	hk x hk	1: 3	0.524	0.878
Group 7				
RP40-438	lm x ll	1: 1	0.821	0.229
RP2-669	hk x hk	1: 3	0.154	2.000
Group 8				
RP3-471	lm x ll	1: 1	0.001	0.205
RP12-465	lm x ll	1: 1	0.117	0.311
Group 9				
RP27-634	lm x ll	1: 1	0.486	0.046
RP37-551	lm x ll	1: 1	0.001	10.058**
Unmapped				
RP2-680	hk x hk	1: 3	0.838	1.928
RP2-688	nn x np	1: 1	0.821	0.001
RP3-522	hk x hk	1: 3	7.538**	0.074
RP3-560	lm x ll	1: 1	1.846	0.179
RP4-333	lm x ll	1: 1	0.821	1.804
RP6-279	nn x np	1: 1	1.836	0.200
RP10-410	nn x np	1: 1	0.117	2.912
RP10-495	lm x ll	1: 1	0.636	0.111
RP10-632	hk x hk	1: 3	0.974	0.452
RP10-640	nn x np	1: 1	15.909***	2.964

TABLE 6.2 – Continued

Marker	Genotype [Male x female]	Expected Ratio	χ^2_M	χ^2_S
[*Unmapped*]				
RP12-378	lm x ll	1: 1	0.117	0.311
RP12-414	hk x hk	1: 3	16.108***	4.561*
RP12-491	nn x np	1: 1	0.325	0.352
RP12-512	hk x hk	1: 3	0.732	0.150
RP23-217	hk x hk	1: 3	0.001	0.148
RP27-277	lm x ll	1: 1	2.579	0.054
RP27-367	hk x hk	1: 3	8.491**	2.235
RP27-410	hk x hk	1: 3	0.281	6.138*
RP27-489	hk x hk	1: 3	11.860***	0.724
RP27-572	nn x np	1: 1	6.368*	1.436
RP27-622	hk x hk	1: 3	0.001	5.684*
RP27-772	lm x ll	1: 1	0.211	0.001
RP37-470	lm x ll	1: 1	0.001	0.842
RP37-489	nn x np	1: 1	0.117	2.912
RP37-498	nn x np	1: 1	1.571	1.563
RP37-685	lm x ll	1: 1	14.821***	1.872
RP37-808	lm x ll	1: 1	8.667**	0.411
RP38-370	lm x ll	1: 1	2.513	6.627*
RP40-233	lm x ll	1: 1	4.154*	4.168*
RP40-321	nn x np	1: 1	0.051	2.535
RP40-326	nn x np	1: 1	0.051	0.470
RP40-346	lm x ll	1: 1	6.205*	1.241
RP40-436	nn x np	1: 1	0.821	0.187
RP40-493	hk x hk	1: 3	9.043**	2.007
RP40-509	nn x np	1: 1	0.205	0.195
RP40-590	lm x ll	1: 1	1.846	0.907
RP40-600	hk x hk	1: 3	14.376***	0.272
RP40-814	nn x np	1: 1	0.051	2.535
RP40-868	nn x np	1: 1	0.051	0.470
RP51-175	nn x np	1: 1	0.027	1.083
RP55-306	lm x ll	1: 1	2.297	0.032
RP55-351	nn x np	1: 1	0.135	0.135
RP55-532	nn x np	1: 1	3.054	1.733
RP55-632	hk x hk	1: 3	9.964**	1.501

erozygous in both male and female parent. Altogether, 14 loci (i.e. 22.2%) deviated from disomic Mendelian inheritance. This applied particularly to loci heterozygous in both parents (Table 6.2). Nineteen loci which all, except one, were inherited according to disomic Mendelian inheritance, mapped to 9 linkage groups (Figure 6.1). Each linkage group contains 2 or 3 markers, with a total map length of 182

cM (average = 20.2 cM per linkage group). Linkage group 2 showed association with one sex marker heterozygous in both parents and linkage group 9 showed association with another sex marker heterozygous in the male parent (Figure 6.1).

None of the primers amplified markers that were perfectly associated with sex, but seven markers showed significant sex linkage (χ^2-test, $P<0.05$, Table 6.2). Of the seven markers associated with sex, three were detected of which the male parent was heterozygous, and four of which both parents were heterozygous. In a stepwise discriminant analysis of *U. dioica* individuals that where either male or female the canonical discriminant function classified gender correctly in 56 (= 72%) of the 78 plants (binomial test, $P<0.0001$) using four of the seven markers that were found to be significantly associated

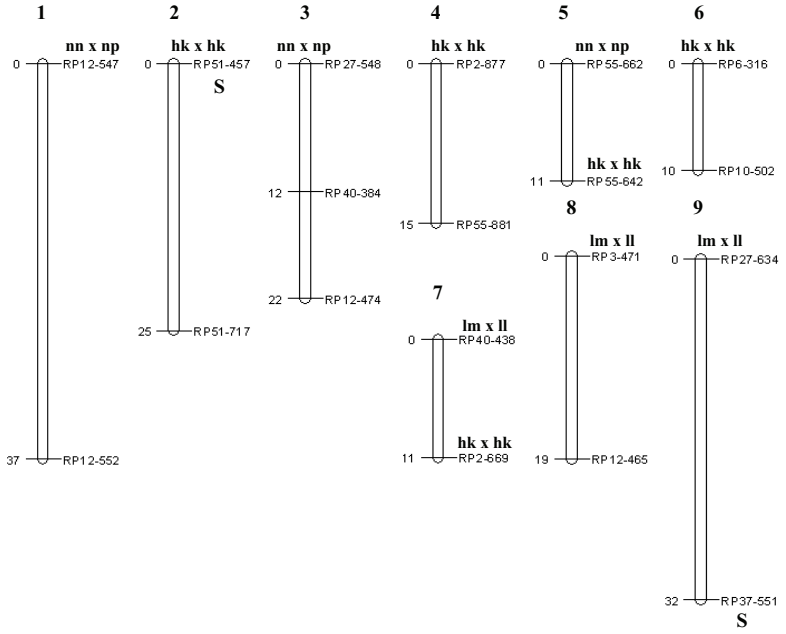

FIGURE 6.1 – Genetic linkage map of RAF markers in *U. dioica*, with a marker linked to sex (S) on linkage group 2 and 9, respectively. Markers are indicated on the right of each linkage group, and the genetic distance between each marker (Kosabi cM) is on the left. Markers are represented by the primer code letter and the size of the mapped fragment (bp). All markers, except RP27-548 (linkage group 3), were inherited following disomic Mendelian rules. Nn x np: female parent is heterozygous; lm x ll: male parent is heterozygous; hk x hk: both parents are heterozygous.

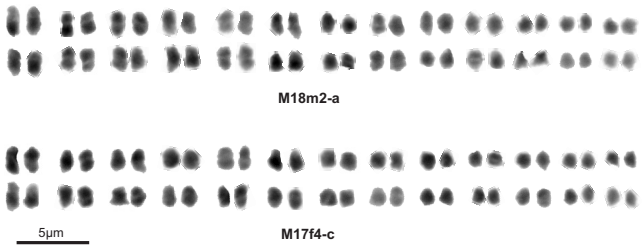

FIGURE 6.2 – Karyogram of mitotic chromosomes of *U. dioica* from root tip preparations stained with DAPI (4', 6 diamidino-propyl-indole). M18m2-a: male, M17f4-c: female.

with sex. Of the seven markers significantly linked to sex, only two could be mapped (Figure 6.1).

In summary, 43 polymorphic markers were found to segregate in the female parent and 40 markers could be detected that segregate in the male parent (Table 6.2). With 43 or 40 markers on 26 chromosomes, the chance that a chromosome contains no marker at all is 18% in females and 22% in males (Poisson distribution). Furthermore, a χ^2-test comparing the observed and expected number of markers per chromosome showed that, in both males and females, too many markers were found that could not be linked in a group ($P<0.0001$ for both males and females). This indicates that, on average, chromosome size in *U. dioica* is too large to be covered by a single marker.

Mitotic chromosomes

Our findings agree with the chromosome numbers given for this species by several investigators (ICPN data base) who reported 2n=4x=52 in dioecious *U. dioica*. Figure 6.2 shows a karyogram of metaphase chromosomes of root tip mitosis from a selected male (M18m2-a) and female (M17f4-c) plant each. The apparent size of chromosomes varies between 1.5 µm and 3 µm. Chromosome analysis showed no obvious evidence for morphological distinct sex chromosomes (Figure 6.2). Chromosomes are small to medium small making it difficult to identify heteromorphic sex chromosomes by this method.

DISCUSSION

Although 22% of the markers were observed to deviate from disomic inheritance, this does not mean *per se* that inheritance in *U. dioica*

does not follow Mendelian rules. Firstly, allozyme data of *U. dioica* based on four loci indicated disomic inheritance (Mutikainen and Koskela 2002). Secondly, a literature review on genetic maps from intra-specific crosses of a great number of wild as well as cultivated diploid plant species indicated that non-Mendelian segregation can be high and varied between zero and 40% (Korbecka et al. 2002). Korbecka et al. (2002) listed several biological mechanisms, such as meiotic drive, cytoplasmic inheritance and gametophytic selection that have been invoked to explain non-Mendelian segregation.

Seven RAF fragments associated with sex in *U. dioica* were found with 63 markers. With the sex-linked markers, sex expression in 72% of the progeny could be predicted correctly. We could not distinguish between sex-specific morphological chromosomes. However, the absence of heteromorphic sex chromosomes may not be very surprising, as these are apparently rare in flowering plants (Parker 1990).

So far, our results do not conflict with the findings obtained from an earlier study in which the inheritance of sex was investigated by performing crosses among male, female and monoecious *U. dioica* plants, and which indicated male heterogamy. Also, data from the crossing experiments suggested a single locus that has a major effect on sex determination. Because crosses between males and females resulted in variable progeny sex ratios that neither could be explained by a single sex determination locus nor could be attributed to be a result of sex-by-environment interactions or sex differential germination and mortality, we suspected additional genes on other loci that distort segregation. Recent research (see Chapter 7) indicated that variation in the sex ratio of the progeny is inherited through the female parent, i.e. the progeny sex ratios produced by the females were quite similar to the sex ratios produced by their maternal parent.

The molecular data obtained from this study did not indicate a major sex determination locus and the linkage map comprises 9 groups with 2 or 3 markers each. However, the mapping of sex-linked markers is related to the chromosome number (2n=52) and the total size of the genome (2C=3.15 pg). In our molecular analysis, about 20% of the chromosomes in males and females did not contain any marker. Moreover, too many markers could not be placed in a linkage group because the average chromosome size in *U. dioica* appears to be larger than can be covered by a single marker. According to the dis-

criminant analysis, the gender in 72% of the plants could be predicted correctly using only four out of seven markers that were found to be significantly associated with sex. Naturally, the chance to foretell the gender correctly is 50%. So if we count the other half as 100%, we only can accurately predict sex expression in 44% of the plants. Therefore, further screening including more primers and individuals still can reveal a major sex determination locus. A single sex determination locus consisting of several closely linked fragments, has been described for *Salix viminalis* (Semerikov et al. 2003). As in *U. dioica*, biased sex ratios also have been frequently observed in *S. viminalis* (Alström-Rapaport et al. 1997). Interestingly, Semerikov et al. (2003) found a close association between sex ratio bias and segregation distortion at the sex determination locus in this species.

Alternatively, multiple loci that are situated on several chromosomes can be involved in sex determination. So far, such a system has been found in one plant species only. In *M. annua*, three unlinked loci affect gender determination: A_1, B_1 and B_2 (Louis 1989). Males have at least one dominant allele at the A locus and one or both on the B loci. Female plants are homozygous recessive at any two of the three loci. By performing a set of genetic crosses with plants having two segregating alleles (dominant and recessive) at each of the three loci, Louis (1989) obtained segregations in the progeny sex ratios that could be explained by this three-gene mechanism. In *U. dioica*, however, the sex ratio variation in the progeny is more continuous as compared to *M. annua*, suggesting that therefore more than three unlinked loci are involved.

In summary, RAF analysis has led to the successful identification of several sex-linked markers which all may independently contribute, to a rather small proportion, to sex determination. Still, a single locus that has the major effect on sex determination may be present but has not been detected yet. Possibly, a significant proportion of non-Mendelian segregation could be linked to a mechanism (e.g. gametophytic selection) that would explain the variation in progeny sex ratios of *U. dioica* to some extent. The genetic model will continue to be revised as more information becomes available from additional markers and individuals. Most likely, the key of understanding the genetics of sex determination in *U. dioica* is to realize that sexuality in this species is a quantitative trait in which maleness and femaleness

are expressed to varying degrees involving the interaction of multiple genes on different loci.

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