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Inheritance of progeny sex ratio in *Urtica dioica*

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Seed samples collected from female plants in the field showed considerable between-family variation in the progeny sex ratio (fraction of males) in the dioecious plant *Urtica dioica*. To investigate the inheritance pattern of the sex ratio trait, crosses were performed between individual male and female plants from different sex ratio families. Our results suggest that, at least for the families studied here, maternal plants strongly contribute to the variation in the primary sex ratio whereas paternal plants seem to have no effect on the sex ratio of the progeny. Furthermore, progeny sex ratios from reciprocal crosses were significantly different and resembled the sex ratios produced by their maternal parents. Apparently, the female parent incurs substantial influence on the sex ratio of its progeny. We discuss the possible mechanisms underlying maternal control.

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The evolution of sex ratios has long been a focus of scientific interest. Several theoretical studies have suggested that, under control of nuclear genes, the primary sex ratio of a sexually dimorphic species in a well-mixed population can be expected to be close to 1:1 in most cases (Fisher 1930, Shaw and Mohler 1953, Charnov 1975). In accordance with this theoretical prediction, several investigations into the sex ratio of various dioecious (separate sexes) plant species showed a 1:1 ratio in the progeny (e.g., *Rumex acetosa* and *R. acetosella*, Putwain and Harper 1972; *Actinidia deliciosa*, Testolin et al. 1995). In contrast, primary sex ratios different from 1:1 were found occasionally in popu-

lations and in families within populations of dioecious plants species (e.g., *Distichlis spicata*, Eppley et al. 1998; *Silene latifolia*, Taylor 1999; *Hippophae rhamnoides* and *Salix repens*, de Jong and van der Meijden 2004). A small bias in the primary sex ratio can still be expected with nuclear control of sex ratio when the population is not well-mixed and the distances over which pollen and seeds disperse are limited (de Jong et al. 2002). However, large biases and extensive genetic variation in sex ratios may well be due to 'selfish' genetic elements (sex ratio distorters) that are predominantly transmitted through either male or female parent (Cosmides and Tooby 1981). Uniparental inheritance causes such 'selfish' elements to conflict with nuclear genes. This conflict may be a source of sex ratio variation.

In numerous cases the existence of sex-ratio distorters has been demonstrated in animals. Examples are maternally inherited microorganisms (O'Neil et al. 1997), supernumerary 'B' chromosomes (Werren and Beukeboom 1998), and sex chromosome meiotic drive (Jaenike 2001). Feminizing *Wolbachia* bacteria have been found in various insect groups and alter their host's pattern of sex determination: individuals that would develop into males develop as females (Weeks et al. 2002). In the parasitic wasp *Nasonia vitripennis*, a paternally transmitted 'B' chromosome (paternal sex ratio chromosome) converts diploid female progeny into haploid male progeny, thereby destroying the paternal genome (Nur et al. 1988). A driving X-chromosome has been described in a number of *Drosophila* species: the selfish element destroys Y-bearing gametes during meiosis, resulting in female-biased sex ratios (Lyttle 1993).

In plants, the problem of intragenomic conflict has been studied intensively in a number of gynodioecious species. With gynodioecy, the population consists of individuals bearing female flowers only (i.e. they are male-sterile) and others that produce perfect flowers. In species like *Thymus vulgaris*, *Plantago lanceolata* and *P. coronopus*, male sterility is caused by a cytoplasmic factor associated with the mitochondrial genome (Thompson et al. 1998, Kuiper and Bos 1992). Since the cytoplasmic factor is only transmitted through the maternal parent (seed), cytoplasmic genes that increase the number of seeds at the cost of pollen production, will be selected for. Nuclear genes, however, that can restore the ability to produce mature pollen will also be selected for in female biased populations. Also in monoecious

species such as *Zea mays*, cytoplasmic male sterility and numerous nuclear restorer genes have been reported to occur (Gabay-Laughnan et al. 2004).

While the phenomenon of intragenomic conflict has been intensively studied in animals and gynodioecious plant species, yet it is relatively unexplored in dioecious plant species (but see Taylor 1993, 1994). Among dioecious plants, *S. latifolia* is the most widely documented example of sex ratio variation; the primary sex ratio is generally female biased. Taylor (1994) indicated that the female-biased sex ratio is influenced by a Y-linked sex ratio modifier which is polymorphic in natural populations. These modifying genes were interpreted to increase the production of males in female-biased populations and thus may be restorers. To test this 'restorer hypothesis', Taylor (1993) performed crosses between the sister species *S. latifolia* and *S. dioica* to break up the association between restorer genes and genes that bias sex ratio (distorter genes): if specific restorer alleles are present, the sex ratio bias is expected to be more severe in crosses between species than within species since the genes that cause the bias are no longer suppressed. The outcome of Taylor's (1993) experiment was consistent with the hypothesis: crosses with females of the sister species yielded a more severe female-bias in the progeny than did crosses within one species.

Another mechanism that has been investigated in dioecious species with respect to biased progeny sex ratios is certation. Certation refers to competition (usually more intense the larger the stigmatic pollen load) between X- and Y-bearing pollen due to slower pollen tube growth of Y-bearing pollen. With low pollen loads, primary sex ratios are close to 1:1, whereas high pollen loads result in female-biased sex ratios.

Working on the genus *Rumex*, various research groups found evidence for certation to play a role in causing biased sex ratios (Rychlewski and Zarzycki 1975, Conn and Blum 1981, Stehlik and Barrett 2005), while for the genus *Silene* the importance of certation for causing biased sex ratios is still controversial (Carroll and Mulcahy 1993).

Over the last years, we have studied the sex ratio trait in the dioecious plant *Urtica dioica*. We found primary sex ratios (fraction of males) to be significant different in families within a single popula-

tion, ranging from 0.05 to 0.76 (de Jong et al. 2005). Subsequent experiments indicated that the sex ratio variation was neither a consequence of parental nutrient condition nor environmental sex determination (Glawe and de Jong 2005). Studies into the genetic sex determination mechanism suggested a single locus to have a major effect on sex determination (with males representing the heterogametic sex) (Glawe and de Jong, Chapter 5). With a simple sex determination mechanism such as an XX/XY system, the sex ratio bias in *U. dioica* may be caused by additional genes that manipulate seed sex ratio. However, a polygenic sex determination mechanism cannot be completely ruled out (Glawe and de Jong, Chapter 5).

The objective of this study was to investigate the inheritance pattern of the seed sex ratio variation in *U. dioica* by performing crosses between individual plants from different sex ratio families. This will provide information whether the sex ratio is inherited biparentally (i.e. through both maternal and paternal parent) or uniparentally (i.e. through one of the parents). Uniparental sex ratio inheritance strongly suggests the presence of sex ratio distorters and, at the same time, narrows the field of potential mechanisms (i.e. maternal vs. paternal) involved in segregation distortion.

MATERIALS AND METHODS

Study organism

Urtica dioica is a wind-pollinated dioecious species. Occasionally, monoecious individuals (male and female flowers on the same plant) have been found (Kay and Stevens 1986, de Jong et al. 2005). Pollen dispersal is aided by an explosive release of pollen, which is caused by rapid stretching of the stamens at anthesis. Counts of pollen revealed that approximately 6,000 grains can be produced by a single male flower; the number of flowers per node (bearing four inflorescences at each) being approximately 1,300 flowers (Strasburger 1910). The fruits are single-seeded. Depending on the habitat, *U. dioica* subsp. *gracilis* from Canada was found to produce between 500 and 20,000 seeds per shoot.

Early reports (Meurman 1925) on the existence of heteromorphic sex chromosomes in *U. dioica* could not be confirmed by cytological investigation of mitotic chromosomes (Glawe et al., Chapter 6). The old literature (Strasburger 1910) stated male heterogamy and

female homogamy, which is a common situation in the majority of dioecious plants surveyed. We performed a series of genetic crosses (Westergaard 1958) to study the genetic basis of sex determination in our laboratory. The findings were consistent with males being the heterogametic sex (Glawe and de Jong, Chapter 5), but at the same time several crosses yielded such unexpected results that it is possible that sex determination is more complex. *U. dioica* is allotetraploid. Genetic mapping of polymorphic markers (Glawe et al., Chapter 6) and allozyme data from four loci (Mutikainen and Koskela 2002) indicate disomic inheritance.

Plant origin and growth procedure

The plant material originated from seeds collected from individual open-pollinated females at the field site in Meijendel (near The Hague, The Netherlands) (de Jong et al. 2005). Individuals from each seed batch (family) thus were at least half-sibs: all individuals from one family had the same mother but not necessarily the same father. By the time the study on *U. dioica* was initiated, cuttings were obtained from male and female plants from the different maternal families and cultured in vitro (MS 0 medium). Since then the plants have been used repeatedly in various experiments. Male and female clones used in the following experiments were transferred to 1.3 L pots containing a mixture of 50/50 dune sand/peat and grown in climate chambers under standard conditions: 20°C during 16 h light and 15°C during 8 h dark with 70% relative humidity, and with 180-200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD at plant growing level. Plants were watered accordingly but received no additional nutrients. Seeds obtained from the crosses were germinated under laboratory conditions (at 20°C during 16 h light and 15°C during 8 h dark) on moist filter paper in Petri dishes. After approximately 10 days, seedlings were transplanted to 1.3 L pots containing a mixture of 50/50 dune sand/peat and grown in climate chambers under standard conditions. At maturity, all plants were sexed. SSR was calculated per cross as the proportion of males to total progeny.

Crossing experiment

The inheritance of sex ratio was investigated using a reciprocal crossing design. Male and female individuals in the crosses were

selected based on the sex ratios produced by the open-pollinated females (families: L14, M24, H31) in the field. For example, in one cross a female from a low sex ratio family (L14) was pollinated by a male from a high sex ratio family (H31), while in another a female from a high sex ratio family (H31) was crossed to a male from a low sex ratio family (L14). Since maternal plants in the crosses appeared to have a strong effect on the sex ratio of the progeny (see Results), females from three additional families (L17, M16, H18) were also crossed to males from L14, M24 and H31 to obtain a rough estimate of the sex ratio heritability from offspring-mother regression.

The families L14, L17, M16, M24, H18, and H31 were selected from a larger sample used by de Jong et al. (2005). They estimated the primary sex ratio (SR) per family under standard conditions: L14 (SR=0.14), L17 (SR=0.04), M16 (SR=0.44), M24 (SR=0.50), H18 (SR=0.72), and H31 (SR=0.64); the between-family sex ratio being statistically significant ($G_{\text{het}}=80.375$, $df=5$, $P<0.0001$). The following types of crosses were performed (see also Table 7.1):

- (1) L x L: crosses in which partners were taken from families with female-biased (low) sex ratios ($0 < \text{SR} < 0.5$; SR significant different from 1:1 ratio)
- (2) M x M: crosses in which partners were taken from families with non-biased (medium) sex ratios (SR=0.5; SR not significant different from 1:1 ratio)
- (3) H x H: crosses in which partners were taken from families with male-biased (high) sex ratios ($0.5 < \text{SR} < 1$; SR significant different from 1:1 ratio)
- (4) L x M: crosses between males from a female-biased family and females from a non-biased family and vice versa
- (5) L x H: crosses between males from a female-biased family and females from a male-biased family and vice versa
- (6) M x H: crosses between males from a non-biased family and females from a male-biased family and vice versa.

Altogether, six maternal (one female per family) and three paternal (one male from family L14, M24, and H31, respectively) parents were used in the crossing experiment. Because female plants were repeatedly crossed to different males, cloned material was used for both sexes. As soon as plants reached maturity, male and female individuals were placed pair wise in pollination chambers according to the

crossing scheme (Table 7.1). Each cross was performed twice in the sense that two females from one family were crossed each with the same male plant. Because the progeny sex ratios obtained from each of the two crosses were very similar (χ^2 -test, $0.367 \leq P \leq 0.873$), sex ratios were pooled over the two 'replicate' crosses.

The sex ratios that were obtained from each reciprocal cross were checked whether they would significantly deviate from each other by calculating the confidence intervals of the sex ratios. To check for possible maternal or paternal effects on sex ratio, offspring-father and offspring-mother regressions were separately calculated (narrow-sense heritabilities can be estimated as twice the slope of the regression equation) (Falconer 1996; linear regression analysis).

Pollen fertility

To examine whether differential pollen fertility between males from varying sex ratio families was associated with a sex ratio bias (Taylor and Ingvarsson 2003), a simple staining technique was used to estimate pollen fertility/sterility. The male individuals were selected from 3 families exhibiting 1) a female-biased sex ratio (L14), 2) a male-biased sex ratio (H31), and 3) an equal sex ratio (M24). Per family, pollen grains from seven male plants, each including one male from the crossing experiment, were tested individually for viability using cotton blue in lactophenol (Dickison 1974). Pollen grains (a mixture of 200 to 300 flowers per individual) were allowed to stain for approximately 20 min; the ones staining darkly were presumed as being fertile whereas pollen grains that did not stain or stained faintly were presumed as being sterile. The number of pollen grains investigated varied between 800 to 1,600 grains per individual.

Seed-ovule ratio

The sex ratio variation among progenies observed in the crossing experiment prompted us to compare the seed-ovule ratio of females producing a strong bias in the progeny to that of females which produce an equal sex ratio when crossed to the same male parent. For instance, females may influence the progeny sex ratio by varying the degree of pollinated ovules or viable seeds. We therefore selected clones from all female genotypes that were used in the crossing experiment and fertilized them by a single male (H31). Male H31,

which was also used in the crossing experiment, produced the highest number of viable pollen grains. The selected male and female clones were grown in pots under standard conditions until the flowering stage. Two weeks after female plants had initiated flowering (flowers situated on the same inflorescence do not necessarily emerge at the same time), the number of flowers (one flower = one ovule) was counted. Because *U. dioica* plants can produce thousands of flowers, only the ones on the first four flowering nodes were considered. The females were then placed for three more weeks in a pollination chamber, next to the two clones of the selected single male parent. This was done to assure pollination, so that all ovules were fertilized. After fruit set, the seeds of each female parent were counted. To check if the seeds were fully developed and viable, per plant 200 seeds were put on moist filter paper in Petri dishes and germination rate was estimated.

RESULTS

Crossing experiment

In 9 out of the 15 sib ships, the sex ratio showed significant deviations from a 1:1 ratio (binomial test, $P < 0.05$); offspring from 6 crosses were female-biased and 3 were male-biased (Table 7.1). The overall mean sex ratio in the progenies was 0.46 ± 0.05 (SE). Females originating from female-biased families (L14, L17) also produced low sex ratios in their progenies while, in turn, females from male-biased families (H18, H31) generally yielded high progeny sex ratios (Table 7.1).

Sex ratios from reciprocal crosses between families were significantly different in two out of three crosses (Table 7.2). When a chi-square test was applied, progeny sex ratios from all three crosses were significantly different ($P < 0.05$). These results clearly demonstrate that sex ratio of the progeny depends on which plant is used as the maternal and which as the paternal parent. Interestingly, the sex ratios produced by the females generally resemble the sex ratios that were produced by their open-pollinated female parents. For example, when a female from a low sex ratio family was crossed to a male from a high sex ratio family, the progeny sex ratio was low, i.e. female-biased. Accordingly, when a female from a high sex ratio family was pollinated by a male from a low sex ratio family, the sex ratio of the progeny was higher than 0.5, i.e. was male-biased (Table 7.2b).

INHERITANCE OF THE SEX RATIO TRAIT

TABLE 7.1 – Seed sex ratios (and number of offspring) from crosses among parents from different sex ratio families with a low (L), medium (M), and high (H) fraction of males. Sex ratios were pooled over two replicate crosses. The sex ratios (SR) produced by families in the previous generation were: L14 (SR=0.14), L17 (SR=0.04), M16 (SR=0.44), M24 (SR=0.5), H18 (SR=0.72), and H31 (SR=0.64). Asterisks indicate progeny sex ratios significant different from a 1: 1 ratio (binominal test, $P < 0.05$).

Male parent	Female parent					
	L14	L17	M16	M24	H18	H31
L14		0.19* (102)	0.67* (120)	0.40* (116)	0.60 (106)	0.52 (131)
M24	0.25* (116)	0.31* (109)	0.48 (120)		0.57 (111)	0.61* (112)
H31	0.26* (106)	0.41 (121)	0.54 (122)	0.38* (127)	0.78* (121)	

TABLE 7.2 – Reciprocal crosses between a) a female from a female-biased family (L14; sex ratio: 0.14) and a male from a non-biased family (M24; sex ratio: 0.5), and vice versa, b) a female from a female-biased family and a male from a male-biased family (H31; sex ratio: 0.64), and vice versa, and c) a female from a non-biased family and a male from a male-biased family, and vice versa. Asterisks indicate progeny sex ratios obtained from reciprocal crosses that were significantly different. CI: confidence interval.

Parental combination		Number of offspring	SSR	SE	95% CI
Female	Male				
a)	L14 M24	116	0.25	0.0403	0.0793
	M24 L14	116	0.40	0.0457	0.0898
b)	L14 H31	106	0.26*	0.0428	0.0841
	H31 L14	131	0.52*	0.0438	0.0861
c)	M24 H31	127	0.38*	0.0432	0.0850
	H31 M24	112	0.61*	0.0463	0.0910

The slope of the regression on sex ratio among progeny on sex ratio of the maternal families was 0.522 ± 0.105 (SE) and was significant different from 0 (Student t-test, $t=4.983$, $P=0.0003$) (Figure 7.1). The relationship is similar if we consider the crosses with each of the three males separately (Figure 7.1). The proportion (R^2) of the total variation in sex ratio that can be accounted for by maternal effects is 0.66. In contrast, the slope of the regression on sex ratio among paternal families was not significantly different from 0 (Student t-test, $t=0.970$, $P=0.925$) (Figure 7.2). Also with multiple regression analysis, the male parent did not contribute significantly to explaining the sex ratio variation in the offspring (Student t-test, $t=0.655$, $P=0.525$).

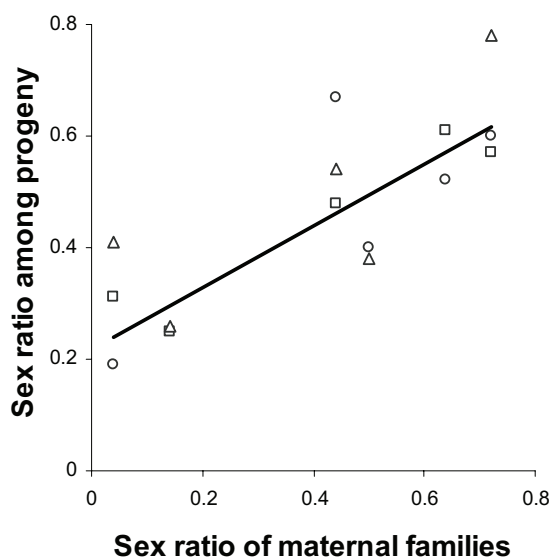


FIGURE 7.1 – Regression line of sex ratio among progeny on sex ratio of the maternal families ($y = 0.5222x + 0.2502$). Each symbol denotes a different male type that was used in the crosses as the paternal parent. Circles: L14; squares: M24; triangles: H31.

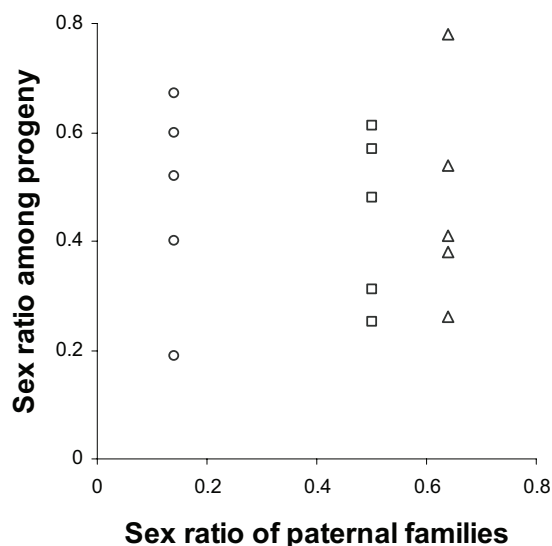


FIGURE 7.2 – Regression line of sex ratio among progeny on sex ratio among paternal families ($y = -0.0208x + 0.4736$). Each symbol denotes a different male type that was used in the crosses as the paternal parent. Circles: L14; squares: M24; triangles: H31.

Pollen fertility

The percentage of viable pollen per male plant almost significantly differed between the families (ANOVA, $F=3.432$, $df=2$, $P=0.0546$). In two males of family L14, half of the pollen produced was sterile (Table 7.3). Pollen fertility of all males from families producing an equal (M24) or male-biased (M31) SSR generally was high (Table 7.3).

Seed-ovule ratio

The seed-ovule ratio of the six pollinated females from different families was on average 0.95 ± 0.01 (SE) (Table 7.4). The germination rates indicate that seeds produced from all all crosses were highly viable (Table 7.4).

DISCUSSION

Male versus female parent

The results of our analysis indicate that sex ratio variation among families in the Meijndel population of *U. dioica* is strongly affected by the maternal parent (Figure 7.1). This finding is supported by the outcomes of the reciprocal crosses in which sex ratios were signifi-

TABLE 7.3 – Pollen staining results from three different seed sex ratio (SSR) families of *Urtica dioica*. Per family, seven male individuals (the first ones being used in the crossing experiment) were tested for viability using lactophenol cotton blue.

Family	SSR	% viable grains for each male genotype							Average % viable grains
		1	2	3	4	5	6	7	
H31	0.64	92.4	75.0	96.3	82.0	96.0	87.9	96.1	89.4
M24	0.50	86.4	89.3	99.7	99.8	95.3	82.8	90.3	91.9
L14	0.14	70.9	91.7	51.8	53.9	88.9	84.6	93.1	76.4

TABLE 7.4 – Seed-ovule ratios and germination rates from *U. dioica* females, originating from six different seed sex ratio (SSR) families, after they were crossed to the same male parent (H31).

Family	SSR	Number of ovules	Number of seeds	Seed-ovule ratio	Germination rate (%)
M18	0.72	841	815	0.97	91.9
M31	0.64	584	534	0.94	98.1
M24	0.50	694	638	0.92	96.9
M16	0.44	787	772	0.98	96.6
M14	0.14	862	846	0.97	93.3
M17	0.04	585	536	0.92	98.1

cantly different and generally resembled the sex ratios produced by their open-pollinated maternal parents. In contrast, it seems that paternal parents contribute little to the sex ratio variation of their progenies (Figure 7.2 and Table 7.2). However, we have to temper our conclusions because our data provide only a rough estimate. Firstly, due to multiple paternity the effect of a single paternal parent on sex ratio of its progeny can be very small, depending on the number of fathers that sired offspring on the same female plant in the field. By choosing a single male offspring per female for the crosses, we selected one male parent from the many that were possibly siring seeds on the open-pollinated female. Therefore, the male parent we selected may not be representative for the sex ratio of the entire progeny sired in the field. Secondly, an increase in the number of families especially for the male parents would have improved the precision of the estimates of heritability. Our conclusion is based on three males only and a more extensive sampling of plants from the Meijendel population would have perhaps revealed males that can exert influence on the sex ratio of their progeny. Thirdly, maternal and paternal parents used in the crossing experiment do not present a random selection of genotypes that occur at our field site. Consequently these results should be interpreted cautiously.

Nevertheless, the three males we examined here in great detail showed no effect on the sex ratio of their offspring (Figure 7.2). Between-family sex ratio variation is large in *U. dioica* and ranged from 0.05 to 0.76 in a collection of seeds sampled from 33 maternal parents (de Jong et al. 2005). So it is unlikely that the males we sampled were all from parental combinations producing the same sex ratio. In addition we found no difference in progeny sex ratio between the offspring of the two sisters that we used in the crossings. Since these sisters originate from seeds that could well have been sired by different fathers in the field (i.e. they could well be half-sibs), this also indicates that the father is less important for the sex ratio of the progeny.

Investigation into the viability of pollen grains from *U. dioica* males from different sex ratio families revealed that pollen fertility was comparatively low in several male plants selected from family L14 which exhibits a strongly female-biased sex ratio. Testing pollen fertility from male plants of *S. latifolia* producing different sex ratios in their progeny, Taylor and Ingvarsson (2003) found a significant rela-

tionship between the fraction of fertile pollen and the SSR they produced. Particularly males originating from female-biased families aborted almost half of their pollen. They interpreted that the severe female-biased sex ratios produced by 'sex ratio' males may be due to abortion of Y-bearing pollen, a mechanism which is similar to drive systems found in animals. Whether there is a relationship in *U. dioica* between the proportion of viable pollen produced by males and the sex ratio in the progeny is not known. However, this can be tested in crosses between males producing high numbers of sterile pollen and females that are known to produce equal sex ratios: if Y-bearing pollen is aborted, the sex ratio will bias toward more females in the progeny.

With the reserve that males in the population should be sampled more extensively, we suggest that it is mainly the female parent that affects the gender of its offspring and we now focus on possible mechanisms behind sex ratio bias.

Mechanisms of sex ratio control through the female parent

What are the mechanisms that could account for maternal control? Werren et al. (2002) theoretically showed that, if sex ratio among offspring in a family affects maternal fitness or the fitness of male or female progeny in that family, maternal-zygotic conflict can occur. However in *U. dioica*, sex ratio variation could not be interpreted as being a consequence of maternal condition (Glawe and de Jong 2005). Also, there could be no cost associated with rearing male vs. female offspring (Glawe and de Jong 2005). This is consistent with the present study where the seed-ovule ratios among maternal plants from different sex ratio families were all high and did not differ from each other. This eliminates embryo abortion, a post-zygotic mechanism, which is known to lead to the investment of resources only in the offspring with the highest potential fitness (reviewed by Korbecka et al. 2002).

Two further mechanisms are possible. Firstly, the sex ratio bias may arise in the pre-zygotic stage. Interactions between the tissues of the pistil and pollen grains play a central role in regulating the fertilization, including pollen germination, pollen tube growth, and pollen tube guidance (Boavida et al. 2005). Particularly the female parent has been shown to have considerable control over pollen performance (Sanders and Lord 1989). In *U. dioica*, the maternal parent could possibly exert influence on the relative growth of X- vs. Y-bearing pollen

tubes in the style, resulting in variation in progeny sex ratios among maternal families. This mechanism also was suggested by Taylor (1994) for *S. latifolia*. Although in this species, the male parent has been demonstrated to have the strongest effect on progeny sex ratio, Taylor found in his crosses an interaction between paternal and maternal parents. In *U. dioica*, females from female-biased families generally were observed to predominantly produce female offspring, while maternal plants from male-biased families were found to mainly give male-biased sex ratios (Table 7.2). Therefore we have to assume that certain females may favour the performance of X-bearing pollen and others, in turn, would support the growth of Y-bearing pollen tubes. As with certation, the degree of bias that the mother can impose should depend on pollination intensity. With abundant pollen the fastest pollen grain wins, with poor pollination even a slow pollen grain that is inhibited by maternal tissue may finally be successful.

Secondly, sex determination may be caused by many genes, including cytoplasmic factors. The classical view of sex determination in plants is that the female is the homogametic sex and the male is the heterogametic sex (Westergaard 1958). Alternatively, there could be polygenic sex determination in plant species like *U. dioica* that lack heteromorphic sex chromosomes. Bull (1983) suggested that although gender is a discrete character (male or female), it could also be based on an underlying continuous scale. With a low value on this scale the individual develops as a female, with a value above a certain threshold the individual develops as a male. Bull et al. (1982) applied this idea to environmental sex determination in sea turtles. In this case gender depends on whether temperature during early development is above or below a threshold and this threshold may be different for different genotypes. Although sex determination in *U. dioica* could not be changed by varying environmental factors (Glawe and de Jong 2005) and therefore appears to be solely genetically based, the idea of an underlying continuous scale for gender could still apply. Several of Bull's (1983) criteria for polygenic sex determination hold for *U. dioica*. Between-family sex ratio variance is large and in crosses we found a fast response of sex ratio to selection (de Jong et al. 2005 and this Chapter). However, polygenic inheritance through nuclear genes would imply an equal opportunity for maternal and paternal parent to contribute equally to the sex ratio of their off-

spring and this is not what we find here. The model of polygenic sex determination can only apply to *U. dioica*, when there is also an extra factor in the female parent that affects sex determination. For instance, during embryo development the mother could place hormones in the seed to feminize it. Genetic variation in how much hormone is added to the seed would be inherited from mother to daughters and sons, but would only be expressed in the daughters (the sons would pass copies of this hormone gene on but the gene would only be expressed when present in tissue of a maternal plant). Alternatively, cytoplasmic genes could have a major effect on femaleness measured on the underlying scale and this would result in an uneven contribution of male and female parents to the sex ratio of their progeny. In this novel view the gender of an organism would be determined by its cytoplasm and by the nuclear genes of both parents. The maternal parent then would have a major effect on progeny sex ratio and the father would have a minor effect.

While the opportunities for the maternal parent to recognize 'male' and 'female' pollen and to select between these, would be greatest with a clear distinction between the two types of pollen, polygenic sex determination involves effects of many genes and their interactions. One can distinguish between the two alternative explanations for the prominent role of the mother for sex ratio of the progeny, by genetic mapping of sex-linked markers. When a marker can be found that is completely linked to sex, the first explanation for maternal control is appropriate. However, when gender is not associated with a single genetic marker, but rather is partly associated with many markers on different chromosomes, the second explanation comes into play. This work is currently under way for *U. dioica* (see Chapter 6).

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