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ORIGINAL ARTICLE

Meiotic recombination in *Turnera* (Turneraceae): extreme sexual difference in rates, but no evidence for recombination suppression associated with the distyly (*S*) locus

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To explore the rate of recombination resulting from male vs female meiosis, crosses were performed using distylous *Turnera subulata* as well as a cross involving the introgression of genes from *T. krapovickasii* into *T. subulata*. We assayed four loci on the chromosome bearing the *S*-locus as well as two loci on each of two other linkage groups. Substantial and consistent dimorphism in recombination rates was found with female meiosis resulting in as much as a ~6-fold increase relative to male. Aberrant single locus segregation ratios occurred for some loci, particularly when the male (pollen) parent was heterozygous and the cross involved introgressed genes. The extreme trend of greater recombination resulting from female meiosis was, however, maintained in crosses where no aberrant ratios occurred, indicating that the sex dimorphism in recombination is not the result of aberrant segregation. We also exploited this distylous species and tested whether there is recombination suppression around the *S*-locus because of an inversion or other chromosome rearrangement(s). We found no significant evidence for recombination suppression.
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Introduction

Rates of recombination had been shown to vary nearly a century ago when Thomas Morgan found no evidence of crossing-over in *Drosophila* males, as opposed to females (Morgan, 1912). Since then, data collected on numerous dioecious and hermaphroditic species have shown that recombination rates vary from female- to male-biased in both animals (e.g., humans, Broman *et al*., 1998; *Oncorhynchus mykiss*, Sakamoto *et al*., 2000; *Danio rerio*, Singer *et al*., 2002; *Monodelphis domestica*, Samollow *et al*., 2004; mouse, Shifman *et al*., 2006) and plants (e.g., *Pinus radiata*, Moran *et al*., 1983; *Arabidopsis thaliana*, Vizir and Korol, 1990; *Brassica oleracea*, Kearsey *et al*., 1996; *Pinus pinaster*, Plomion and O'Malley, 1996). Reviews of the literature and hypotheses for recombination rate differences may be found in Bell (1982), Trivers (1988), Burt *et al*. (1991), Lenormand (2003), Lenormand and Dutheil (2005) and Lorch (2005).

Another kind of heterogeneity in recombination rate involves chromosome regions containing mating system loci, which have been shown to exhibit recombination suppression in a wide diversity of organisms (e.g., Ferris and Goodenough, 1994; Lee *et al*., 1999; Gallegos *et al*., 2000; Ferris *et al*., 2002; Wang *et al*., 2003). In plants, it had perhaps been first proposed that recombination suppression might be associated with the distyly locus (*S*-locus) in *Primula* (de Winton and Haldane, 1935; Mather, 1950). Distyly is a genetic polymorphism where two floral morphs (long- vs short-styled), having reciprocal arrangements of styles and stamens and commonly a self-incompatibility system, occur (Darwin, 1877). De Winton and Haldane (1935) suggested that the *S*-locus might be located within a small inversion in *Primula sinensis* that suppresses recombination. They, however, found no evidence for such suppression.

In this paper we explore the rate of meiotic recombination in a hermaphroditic outcrossing distylous species, *Turnera subulata*, and in a cross involving introgression of genes from distylous *T. krapovickasii* into *T. subulata*. Models and analyses by Lenormand (2003) and Lenormand and Dutheil (2005) led to the prediction that there should be a lower rate of recombination resulting from male vs female meiosis in outcrossing species. We also designed a series of controlled crosses, using genetic markers flanking the *S*-locus, to investigate whether there is recombination suppression associated with the *S*-locus because of an inversion or other chromosome rearrangement(s) in this region.

Materials and methods

Recombination suppression

To detect recombination suppression under the hypothesis that the *S*-locus is located within an inversion or other chromosome rearrangement(s), two types of controlled crosses were made in 2005. For the first, a

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heterozygous (*Ss*) short-styled plant of *T. subulata* (termed Bry) was crossed reciprocally to a homozygous (*ss*) long-styled plant of *T. subulata* (termed S16L). Progeny were grown and assayed at two isozyme loci (*Pgd-c* and *Aco-1*) that lie on opposite sides of the *S*-locus (Athanasiou and Shore, 1997). Genotypes of the parental plants are provided (Table 1).

The second set of reciprocal crosses was between short-styled plants of *T. subulata* homozygous for the *S* allele and the long-styled plant used above (S16L). The short-styled plants (SS3314–1S, SS3314–8S, SS3314–9S, SS1433–24S, SS1433–28S) were constructed by exploiting the unusual self-compatible short-styled plant, Bry (Athanasiou and Shore, 1997), such that they were heterozygous at both isozyme loci (Figure 1, Table 1). All progeny were assayed for *Pgd-c* and *Aco-1*.

To minimize environmental effects on recombination, crosses were done at the same time using replicate rooted cuttings of all parental plants. Seeds were sown and progeny raised in the same glasshouse over the same period of time. A pilot study from a series of similar crosses was performed in 2004 using only the pollen donors (males) as the heterozygous parents.

Recombination resulting from male vs female meiosis

The crosses above (from 2005) also yield information on recombination resulting from female vs male meiosis between three loci (*Aco-1*, *S*-locus and *Pgd-c*). A second reciprocal cross was available to estimate differences in rates of recombination resulting from female vs male meiosis. We exploited a short-styled plant (MhBry-9S) carrying genes introgressed from *T. krapovickasii* into *T. subulata* (Tamari *et al*., 2005). The plant was crossed to S16L. We use this cross and its reciprocal (data from male meiosis were published in Athanasiou *et al*. (2003)), for the comparison of recombination resulting from female vs male meiosis. We assayed progeny at the *Aco-1*, *S*, *Pgd-c* and *TsPG* loci (Table 1). We also studied recombination among four random amplified polymorphic DNA (RAPD) loci that occur on two additional linkage groups.

Table 1 Genotypes of plants used in crosses

Plant	*Locus*			
	Aco-1	*S*	*Pgd-c*	*TsPG*
MhBry-9S	$Aco\text{-}1^S$	S	$Pgd\text{-}c^F$	$TsPG^B$
	$Aco\text{-}1^M$	S^H	$Pgd\text{-}c^S$	$TsPG^K$
S16L	$Aco\text{-}1^F$	s	$Pgd\text{-}c^S$	$TsPG^B$
	$Aco\text{-}1^F$	s	$Pgd\text{-}c^S$	$TsPG^B$
Bry	$Aco\text{-}1^S$	S	$Pgd\text{-}c^F$	
	$Aco\text{-}1^F$	s	$Pgd\text{-}c^S$	
SS3314-1S	$Aco\text{-}1^F$	S	$Pgd\text{-}c^F$	
	$Aco\text{-}1^S$	S	$Pgd\text{-}c^S$	
SS3314-8S	$Aco\text{-}1^F$	S	$Pgd\text{-}c^F$	
	$Aco\text{-}1^S$	S	$Pgd\text{-}c^S$	
SS3314-9S	$Aco\text{-}1^F$	S	$Pgd\text{-}c^F$	
	$Aco\text{-}1^S$	S	$Pgd\text{-}c^S$	
SS1433-24S	$Aco\text{-}1^F$	S	$Pgd\text{-}c^F$	
	$Aco\text{-}1^S$	S	$Pgd\text{-}c^S$	
SS1433-28S	$Aco\text{-}1^F$	S	$Pgd\text{-}c^F$	
	$Aco\text{-}1^S$	S	$Pgd\text{-}c^S$	

Genotypes at the *Aco-1*, *distyly* or *S*-locus, *Pgd-c* and *TsPG* loci are provided with the linkage phases indicated on separate lines for each plant. $Pgd\text{-}c^F$ and $Pgd\text{-}c^S$ are alleles encoding fast- and slow-migrating allozymes of cytosolic 6-phosphogluconate dehydrogenase, $Aco\text{-}1^F$, $Aco\text{-}1^M$ and $Aco\text{-}1^S$ are alleles encoding fast-, intermediate- and slow-migrating allozymes of aconitase-1, $TsPG^B$ and $TsPG^K$ are alleles encoding a short-style-specific polygalacturonase and S, S^H and s are alleles of the distyly locus.

Isozyme and RAPD analysis

Assays of progeny for the isozyme loci followed Shore and Barrett (1987). RAPD assays were performed using decamer primers of arbitrary sequences (Operon Technologies Inc., Alameda, CA, USA). Three primers (Opi04: 5′ CCGCCTAGTC; Oph06: 5′ ACGCATCGCA; Opk14: 5′ CCCGCTACAC) that produce markers located on two distinct linkage groups were used (Labonne and Shore, unpublished data). For the first primer, we mapped two polymorphic bands, whereas for each of the latter primers we mapped a single polymorphic band. Genomic DNA was extracted from all progeny following the protocol of Doyle and Doyle (1987) with minor modifications. RAPD-PCR was performed in a 12.5-μl reaction volume containing $\sim$50 ng of genomic DNA, 10 pmol

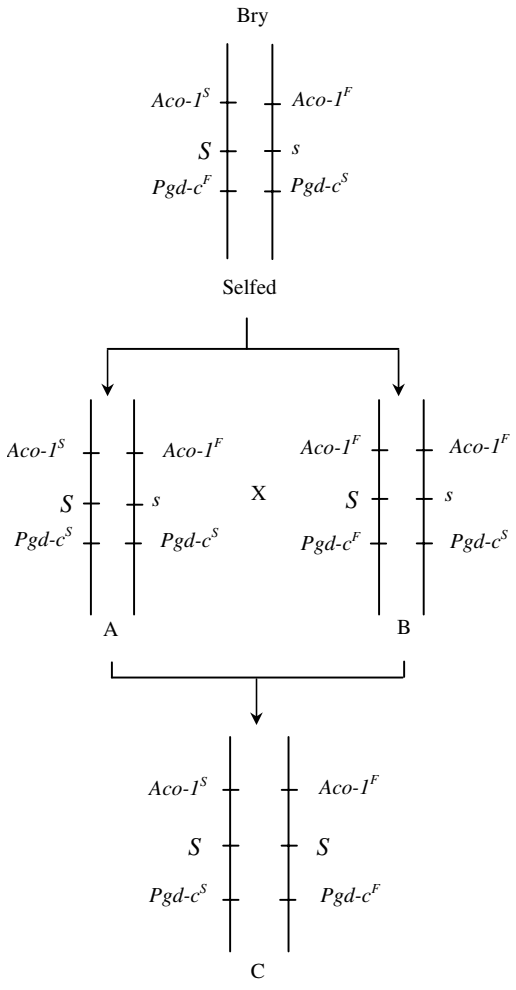

Figure 1 Crossing scheme to produce the SS-series of plants: Bry was selfed and short-styled progeny were screened to detect recombinants for one of the two isozyme loci. The *Pgd-c* recombinant (A) was then crossed to the *Aco-1* recombinant (B) to produce homozygous (*SS*) plants with desired genotype (C).

primer and JumpStart ReadyMix REDTaq DNA polymerase (Sigma-Aldrich, Oakville, ON, Canada). Amplifications were performed using a thermal cycler (Mastercycler Gradient, Eppendorf, Mississauga, ON, Canada) programmed for an initial denaturation of 2 min followed by 35 cycles of 94°C for 30 s, 37°C for 1 min, 72°C for 2 min and a final extension step of 72°C for 5 min. PCR products were run on 7.5% polyacrylamide gels, then immersed in silver nitrate solution (0.5 ml of 20% $AgNO_3$ in 50 ml distilled water). Following five washes in distilled water, stain was developed by soaking the gels for 5–8 min in ~30 ml of a solution containing 16 g NaOH/L and 0.4% formaldehyde. Gels were fixed in 8% acetic acid, scored and scanned. Ambiguous samples and recombinants were rerun to verify their genotype.

Statistical analyses

We used the method of maximum likelihood to estimate recombination frequencies and their standard errors. We used the G statistic (log likelihood ratio) to test for goodness of fit and/or heterogeneity among frequencies. To detect the possibility of recombination suppression, we also used Fisher's exact test (one-tailed test) as our alternative hypothesis proposes that the putative 'suppressed' crosses have a reduced recombination rate.

To investigate the ratio of recombination resulting from male vs female meiosis, we used the method of maximum likelihood to estimate recombination proportions (P_i) using a multipoint linkage analysis. We also included one to three 'ratio' parameters (R_i) to compare recombination resulting from male vs female meiosis. We contrasted models with different numbers of ratio parameters using the log likelihood ratio test. Maximum likelihood estimation was performed using R (R Development Core Team, 2004; Ricci, 2005) and MAXLIK (Reed, 1969).

We classified each backcross progeny as to whether it was non-recombinant or had recombination between the first and second loci, second and third, etc. or was a double recombinant. In the equation below, the observed numbers of various kinds of recombinant genotypes are represented as O's for females and M's for males with subscripts denoting whether the recombination occurred between first and second loci, second and third loci, etc. (e.g., M_2 is number of progeny from the male cross exhibiting recombination between the second and third loci). For double recombinants, two subscripts were used to indicate the locations of recombination (e.g., O_{13} is the number of progeny from the female cross exhibiting recombination between the first and second loci as well as between the third and fourth loci). O_{non} and M_{non} are the numbers of non-recombinants from female and male meiosis, respectively. The parameters, P_is, are the expected recombination proportions (or rates) between a pair of loci (P_{nf} is the expected proportion of non-recombinants from the female cross and P_{nm} is the expected proportion of non-recombinants from the male cross) and the R_is are the expected ratios of male to female recombination allowing a different R_i between each adjacent pair of loci or solving for a single R_i (setting $R_1 = R_2 = R_3$). As the loci used in this study had been mapped previously, we took the order as known (Athanasiou *et al*., 2003; Tamari and Shore, 2006). We assumed there was no crossover interference.

We provide the log likelihood expression (log L) used for estimation, for four linked loci of known order. The constant term (*const*) in the expression below is a function of sample size and counts of various progeny and is not needed for estimation.

$$\begin{aligned}\log L = \text{const} &+ \sum_{\substack{i,j,k=1\\ i\neq j\neq k}}^{3} O_i \log\left(P_i - P_iP_j - P_iP_k\right)\\ &+ \sum_{\substack{i,j=1\\ i\neq j}}^{3} O_{ij}\log\left(P_iP_j\right) + O_{\text{non}}\log\left(P_{\text{nf}}\right)\\ &+ \sum_{\substack{i,j,k=1\\ i\neq j\neq k}}^{3} M_i \log\left(R_iP_i - R_iR_jP_iP_j - R_iR_kP_iP_k\right)\\ &+ \sum_{\substack{i,j=1\\ i\neq j}}^{3} M_{ij}\log\left(R_iR_jP_iP_j\right) + M_{\text{non}}\log\left(P_{\text{nm}}\right)\end{aligned}$$

where

$$P_{\text{nf}} = 1 - \sum_{i=1}^{3} P_i + \sum_{\substack{i,j=1\\ i\neq j}}^{3} P_iP_j, \text{ and}$$

$$P_{\text{nm}} = 1 - \sum_{i=1}^{3} R_iP_i + \sum_{\substack{i,j=1\\ i\neq j}}^{3} R_iR_jP_iP_j$$

For crosses involving segregation of three linked loci, we used an analogous expression with just two recombination frequencies and two ratio parameters. For two linked loci, there is one recombination frequency and one ratio parameter.

Results

Recombination suppression

We test the hypothesis that the *S*-locus is located within an inversion or other chromosome rearrangement(s) that suppresses recombination in the vicinity of the *S*-locus. If the *S*-locus is located within an inversion, we expect reduced recombination for the cross of S16L × Bry compared with crosses involving the SS-series of plants. Recombination rates for crosses involving the SS-series plants in 2004, were not heterogeneous ($G_{\text{df}=4} = 9.18$, $P > 0.06$; Table 2). Recombination percentages for pooled data from the SS-series of crosses vs the cross of S16L × Bry were 5.35 ± 1.30 (cM) vs 3.91 ± 1.11 (cM), and were not statistically different (Fisher's exact test, $P = 0.26$; Table 2).

For the 2005 data, the frequency of recombinants obtained from male meiosis involving *SS* homozygotes was not heterogeneous ($G_{\text{df}=1} = 0.08$, $P > 0.7$). Pooled data for the *SS* homozygotes gave a recombination percentage of 3.47 ± 1.08 (cM) vs 2.43 ± 0.91 (cM) for the cross of S16L × Bry (Fisher's exact test, $P = 0.31$; Table 2). The data obtained from female meiosis involving the SS-series of plants were not significantly heterogeneous ($G_{\text{df}=1} = 3.82$, $P > 0.05$). A recombination percentage of

16.32 ± 2.18 (cM) obtained from the pooled data is not significantly different from 13.47 ± 1.98 (cM) for the cross of Bry × S16L (Fisher's exact test, $P = 0.20$; Table 2).

We used all the data to explore simultaneously whether there was evidence for suppression as the increased sample size ($N = 1767$) should provide the greatest power in detecting such an effect. We used maximum likelihood estimation to estimate three parameters: the recombination rate between the *Aco-1* and *Pgd-c* loci, a parameter accounting for the reduced recombination of the 'suppressed' crosses and a ratio parameter for the reduced rate of recombination resulting from male vs female meiosis (see below). Parameter estimates for this model gave a recombination percentage of 16.63 ± 2.04 (cM) resulting from female meiosis, a male to female recombination ratio of 0.26 ± 0.02 and a recombination suppression value of 0.79 ± 0.02 (a value of 1 would indicate no suppression). A model without the suppression parameter fits the data ($G_{df=2} = 2.18$, $P > 0.34$) and the log likelihood ratio test for the suppression parameter indicates it is not statistically significant ($G_{df=1} = 2.03$, $P > 0.15$). Across all the data, there is no evidence for recombination suppression associated with the *S*-locus.

Table 2 Comparison of recombination frequency (cM) between the *Aco-1* and *Pgd-c* loci for crosses involving S16L × SS-series of plants, and Bry × S16L

	N	*Non-Rec*	*Rec*	*Rec freq (s.e.)*	
Male recomb 2004					
S16L × SS1433-24S	72	70	2	2.78 (1.94)	
S16L × SS1433-28S	48	48	0	0.00 (2.04)	
S16L × SS3314-1S	71	67	4	5.63 (2.74)	$G_{het} = 9.18^{NS}$
S16L × SS3314-8S	72	65	7	9.72 (3.49)	
S16L × SS3314-9S	36	33	3	8.33 (4.61)	
POOLED	299	283	16	5.35 (1.30)	$P = 0.26$
S16L × Bry	307	295	12	3.91 (1.11)	
Male recomb 2005					
S16L × SS3314-9S	160	154	6	3.75 (1.50)	$G_{het} = 0.08^{NS}$
S16L × SS3314-1S	128	124	4	3.13 (1.54)	
POOLED	288	278	10	3.47 (1.08)	$P = 0.31$
S16L × Bry	288	281	7	2.43 (0.91)	
Female recomb 2005					
SS3314-9S × S16L	160	140	20	12.50 (2.61)	$G_{het} = 3.82^{NS}$
SS3314-1S × S16L	128	101	27	21.09 (3.61)	
POOLED	288	241	47	16.32 (2.18)	$P = 0.20$
Bry × S16L	297	257	40	13.47 (1.98)	

Abbreviations: NS, not significant; Rec, recombination.
G_{het} values compare whether the observed frequencies differ statistically. For 2004, five crosses were compared with 4 degrees of freedom. For 2005, two crosses were compared with 1 degree of freedom. The *P*-values were obtained from one-tailed Fisher's exact test comparing the pooled vs the S16L × Bry crosses (or its reciprocal).

Recombination resulting from male vs female meiosis

Crosses between S16L and Bry (from 2005) provide information on recombination resulting from male vs female meiosis. The female recombination rate is significantly greater for both pairs of loci (*Aco-1–S*, $G_{df=1} = 7.22$, $P < 0.006$; *S–Pgd-c*, $G_{df=1} = 25.97$, $P < 0.001$; Table 3).

Maximum likelihood estimation followed by a log likelihood ratio test revealed no significant difference between the ratio of recombination resulting from male vs female meiosis for the two pairs of loci ($G_{df=1} = 2.71^{NS}$). The model of two recombination rates and one ratio fits the data ($G_{df=3} = 2.93^{NS}$). The maximum likelihood estimate of the single estimated ratio of male:female recombination is 0.164 ± 0.066 or there is roughly a 6.1-fold increase in recombination resulting from female vs male meiosis (Table 3).

The reciprocal cross of S16L × MhBry-9S provides data on recombination rates for three pairs of loci (Table 3). The rate of recombination resulting from female meiosis was consistently and significantly greater for each locus pair ranging from about three- to sixfold greater (Table 3). Maximum likelihood estimation revealed that a single ratio is sufficient to account for the data ($G_{df=2} = 0.89^{NS}$). The model of three recombination frequencies and one ratio fits the data ($G_{df=8} = 14.58^{NS}$). The maximum likelihood estimate of the single ratio of male:female recombination is 0.219 ± 0.061, indicating a 4.6-fold increase for female relative to male recombination.

Table 3 Male and female recombination rates (cM) between three or four pairs of loci for two crosses

Cross	N	*Recombination frequency ± s.e. (cM)*			*Maximum likelihood*
		Aco-1-S	*S-Pgd-c*	*Pgd-c-TsPG*	*Estimate of M/F ratio*
S16L × Bry (M)	288	1.74 ± 0.77	0.69 ± 0.49	—	
Bry × S16L (F)	297	5.72 ± 1.35	9.09 ± 1.67	—	
G		7.22**	25.97***	—	
M/F		0.303 ± 0.152	0.076 ± 0.056	—	0.164 (0.066) $G_{df=1} = 2.71^{NS}$
S16L × MhBry-9S (M)	169	3.55 ± 1.42	1.78 ± 1.02	2.96 ± 1.30	
MhBry-9S × S16L (F)	239	11.72 ± 2.08	10.88 ± 2.01	15.90 ± 2.37	
G		9.55**	28.12***	20.39**	
M/F		0.303 ± 0.133	0.163 ± 0.098	0.186 ± 0.087	0.219 ± 0.061 $G_{df=2} = 0.89^{NS}$

Abbreviations: M, male; F, female; M/F, ratio of male:female recombination; N, sample size; NS, not significant.
The G statistic with 1 df comparing heterogeneity in recombination rates between male vs female is provided for each locus pair.
A maximum likelihood estimate of a single male:female ratio is provided for each cross as well as a G statistic assessing whether it accounts for the data.
**$P < 0.01$; ***$P < 0.001$.

Table 4 Male and female recombination rates ± s.e. (cM) between *Aco-1* and *Pgd-c*

Cross	*S16L × MhBry-9S*	*S16L × SS3314-9S*	*S16L × SS3314-1S*	*S16L × Bry*
M	5.33 ± 1.73 (169)	3.75 ± 1.50 (160)	3.13 ± 1.54 (128)	2.43 ± 0.91 (288)
F	20.92 ± 2.63 (239)	12.5 ± 2.62 (160)	21.09 ± 3.61 (128)	13.47 ± 1.98 (297)
M/F	0.25 ± 0.09	0.30 ± 0.14	0.15 ± 0.08	0.18 ± 0.07

Abbreviations: M, male; F, female; M/F, ratio of male:female recombination ± s.e.
Sample size for each cross is in parentheses.
A log likelihood ratio test indicates a single ratio fits the data, $G_{df=3} = 2.62^{NS}$.
The maximum likelihood estimate of the male:female recombination ratio = 0.209 ± 0.044.

Table 5 Male and female recombination rates ± s.e. (cM) for two pairs of RAPD loci

Cross	N	*Oph06a-Opi04a*	*Opk14a-Opi04b*
S16L × MhBry-9S (M)	169	0.00 ± 0.01	1.18 ± 0.83
MhBry-9S × S16L (F)	239	3.35 ± 1.16	7.53 ± 1.71
M/F		—	0.16 ± 0.12
G		8.7**	10.2**

Abbreviations: M/F, ratio of male:female recombination; N, sample size; RAPD, random amplified polymorphic DNA.
The *G* statistic with 1 df comparing heterogeneity in recombination rates between male vs female is provided for each locus pair.
**$P < 0.01$.

Table 6 Single-locus segregation ratios for test crosses and their reciprocals

Locus	*Female heterozygous G*		*Male heterozygous G*	
1. MhBry-9S × S16L and reciprocal[a]				
Aco-1	108 *FM*:131 *FS*	2.22^{NS}	40 *FM*:129 *FS*	49.3***
S-locus	118 H:121 S	0.04^{NS}	36 H:133 S	52.9***
Pgd-c	112 *SS*:127 *FS*	0.94^{NS}	35 *SS*:134 *FS*	61.8***
TsPG	118 *BK*:121 *BB*	0.04^{NS}	40 *BK*:129 *BB*	49.3***
Oph06a	89 *Aa*:150 *aa*	15.74***	46 *Aa*:123 *aa*	37.6***
Opi04a	89 *Aa*:150 *aa*	15.74***	46 *Aa*:123 *aa*	37.6***
Opi04b	119 *Aa*:120 *aa*	0.01^{NS}	83 *Aa*:86 *aa*	0.05^{NS}
Opk14a	109 *Aa*:130 *aa*	1.85^{NS}	81 *Aa*:86 *aa*	0.29^{NS}
2. SS3314-9S × S16L and SS3314-1S × S16L pooled and reciprocal (2005)				
Aco-1	144 *FF*:144 *FS*	0.00^{NS}	147*FF*:141 *FS*	0.13^{NS}
Pgd-c	139 *FS*:149 *SS*	0.35^{NS}	143 *FS*:145 *FS*	0.01^{NS}

Abbreviation: NS, not significant.
For *Aco-1* and *Pgd-c*, *F*, *M*, *S*, refer to alleles encoding fast-, intermediate- and slow-migrating allozymes. For the *S*-locus, S and H refer to short- and homostyled morphs. For the RAPD loci, *Aa* genotypes yield an amplified PCR band, whereas *aa* genotypes do not have the band.
The *G*-statistic for goodness of fit to a 1:1 ratio with 1 df is provided. The pooled data for the reciprocal crosses of SS3314-9S × S16L and SS3314-1S × S16L were not statistically heterogeneous for either of the loci.
[a]Segregation data for S16L × MhBry-9S for the first four loci were published in Athanasiou *et al*. (2003) and data for the *S*-locus for MhBry-9S × S16L were published in Tamari *et al*. (2005).
***$P < 0.001$.

For the isozyme loci, *Aco-1* and *Pgd-c*, data on recombination rates are available for four reciprocal crosses (Table 4). Maximum likelihood estimation revealed no evidence of heterogeneity in the ratio of male:female recombination among the crosses ($G_{df=3} = 2.62$, $P > 0.45$) and the model with one ratio fits the data ($G_{df=6} = 11.70$, $P > 0.06$). The maximum likelihood estimate of the male:female ratio is 0.209 ± 0.044 or there is 4.8 times more recombination resulting from female vs male meiosis for these loci.

To determine whether greater female than male recombination occurs for loci other than those linked to the *S*-locus, two pairs of loci on two different linkage groups were analyzed. For two RAPD loci, *Oph06a* and *Opi04a*, the recombination percentage for male was 0.0 ± 0.01 (cM) vs 3.35 ± 1.16 (cM) for female meiosis (Table 5, $G_{df=1} = 8.7$, $P < 0.003$). Similarly, for the locus pair *Opk14a–Opi04b*, recombination from male vs female meiosis varied from 1.18 ± 0.83 (cM) to 7.53 ± 1.71 (cM) (Table 5, $G_{df=1} = 10.2$, $P < 0.002$). The latter value indicates that recombination resulting from female meiosis is about six times greater than that for male and is comparable to estimates above.

Our maximum likelihood estimation assumes there is no crossover interference and this appears to be a reasonable assumption. For example, the model without interference fits the data for the cross Bry × S16L ($G_{df=1} = 0.14$, $P > 0.70$), and MhBry-9S × S16L ($G_{df=1} = 0.51$, $P > 0.47$) for estimates of recombination between *Aco-1* and the *S*-locus and the *S*-locus and *Pgd-c*.

Aberrant single-locus ratios

Aberrant ratios were observed for some loci, particularly those involving the cross S16L × MhBry-9S, where genes were introgressed from *T. krapovickasii* into *T. subulata*. For the latter cross, where the pollen parent is heterozygous, all but two of the eight loci show marked statistical departures from the expected 1:1 ratios (Table 6). For the reciprocal cross (MhBry-9S × S16L), only two of the eight loci show significant departures from the expected ratio. For crosses solely involving *T. subulata* (e.g., SS3314-9S × S16L, SS3314-1S × S16L and reciprocals, from 2005), there were no statistical departures from the expected (Table 6).

Discussion

Recombination resulting from male vs female meiosis

The degree of dimorphism in recombination rate in *Turnera* spp. ranged from 4.6- to 6.1-fold greater for female vs male meiosis for the crosses analyzed. To our knowledge, this represents one of, if not the most extreme, difference in recombination resulting from

female vs male meiosis in a hermaphroditic plant (Bell, 1982; Burt *et al*., 1991; Lenormand and Dutheil, 2005).

In *P. sinensis*, the only other distylous species to have been investigated (to our knowledge), de Winton and Haldane (1935) found no consistent difference in recombination rates. Some locus pairs showed greater recombination resulting from male vs female meiosis, whereas others showed the reverse or no difference at all (de Winton and Haldane, 1935). This is in sharp contrast to our data where the frequency of recombination resulting from female meiosis is consistently and considerably greater than male.

Sex differences in recombination rate have been shown to vary among pairs of loci within a species (e.g., de Vincente and Tanksley, 1991; Singer *et al*., 2002; Woram *et al*., 2004; Hansson *et al*., 2005). For example, linkage analysis in *Lycopersicon peruvianum* showed that although one region of the genome has a 1.6-fold difference in male vs female recombination, another has a difference of 10-fold (Ganal and Tanksley, 1996). Our analysis, however, revealed no significant variation in the ratio of recombination resulting from female vs male meiosis across all pairs of loci.

A number of studies have reported that during male meiosis, the recombination rate is lower in centromeric regions and greater towards the chromosome ends (Tanksley *et al*., 1992; Sakamoto *et al*., 2000; Singer *et al*., 2002; Woram *et al*., 2004; Shifman *et al*., 2006). Observations of diakinesis and first metaphase of meiosis in pollen mother cells (male meiosis) appear to reveal that chiasmata are restricted towards the chromosome ends in *T. subulata* and *T. scabra* (Shore, 1991; JS Shore, personal observations). It is therefore possible that the reduced recombination rate we have measured (i.e., for male vs female meiosis) is a function of the position of loci investigated. The study of a greater number of loci, especially those located towards the chromosome ends, might aid in clarifying whether the greater recombination in female meiosis is maintained throughout the genome.

According to models and analyses (involving differential gametophytic competition) of Lenormand (2003) and Lenormand and Dutheil (2005), outcrossers should commonly exhibit lower recombination resulting from male vs female meiosis, in contrast to selfing species. The recombination rates estimated in our study are in accordance with this prediction. Why distylous *P. sinensis* (de Winton and Haldane, 1935) does not show a comparably large difference in female vs male recombination is unclear. Taxa possessing both distylous (outcrossing) and homostylous (selfing) species might be ideally suited to testing this model of Lenormand (2003).

In a study of genome-wide sex differences in recombination for an interspecific cross of tomato, de Vincente and Tanksley (1991) explored whether gametic selection favoring alleles derived from the recurrent parent, might be responsible for the apparent lower recombination rate in male meiosis. Their hybrid cross does exhibit aberrant ratios at some loci. Although they could not eliminate gametic selection as a factor, there was no clear evidence that single locus gametic selection was responsible for differences in recombination rate (de Vincente and Tanksley, 1991). Aberrant segregation appears to be quite common in linkage studies, particularly those involving hybridization or introgression of genes between species (e.g., Graner *et al*., 1991; Jenczewski *et al*., 1997; Ky *et al*., 2000; Fishman *et al*., 2001; Villalta *et al*., 2005).

We observed a considerable number of aberrant segregations for a hybrid cross (S16L $\times$ MhBry-9S) but not for crosses involving parental plants that were purely *T. subulata* (Table 6; Athanasiou and Shore, 1997; Tamari and Shore, 2006). As both crosses show comparably reduced recombination resulting from male vs female meiosis, the difference in these rates cannot be attributed to aberrant segregation at single locus.

Recombination suppression

We tested the hypothesis that the *S*-locus is located within an inverted or rearranged chromosomal segment that suppresses recombination. Although the recombination rate around the *S*-locus obtained from $SS \times ss$ crosses (putatively 'unsuppressed cross') was greater than that for $Ss \times ss$ crosses (putatively 'suppressed cross'), they were not statistically different. De Winton and Haldane (1935) also did not find any statistical evidence for recombination suppression in distylous *P. sinensis*.

It is possible that our experiment was not sufficiently powerful to detect recombination suppression. Given our sample size, we should have been able to detect a recombination rate difference of about 2.2 cM for the pooled data (Table 2). We determined this value using Fisher's exact test and determining for increasingly more extreme tables, when we would achieve a probability less than 0.05 of obtaining these data by chance, given our sample size. If the *S*-locus is truly located within an inversion/rearrangement, it is possible that the inverted/rearranged chromosomal segment is small causing a low degree of recombination suppression that falls below our detection limit.

In *Brassica* spp., recombination suppression may occur in chromosome regions possessing the *S-locus* (Boyes and Nasrallah, 1993; Boyes *et al*., 1997; Casselman *et al*., 2000; Shiba *et al*., 2003). Using population genetic and segregation data, Kamau and Charlesworth (2005), Hagenblad *et al.* (2006) and Kawabe *et al.* (2006) found evidence for recombination suppression in a narrow region around the *S*-locus of *Arabidopsis lyrata* (Brassicaceae). Once the *S*-locus gene(s) in *T. subulata* are discovered, it might then be possible to use these methods to explore whether recombination suppression indeed occurs.

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