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Wild sorghum from different eco-geographic regions of Kenya display a mixed mating system

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Abstract Knowledge of mating systems is required in order to understand the genetic composition and evolutionary potential of plant populations. Outcrossing in a population may co-vary with the ecological and historical factors influencing it. However, literature on the outcrossing rate is limited in terms of wild sorghum species coverage and eco-geographic reference. This study investigated the outcrossing rates in wild sorghum populations from different ecological conditions of Kenya. Twelve wild sorghum populations were collected in four sorghum growing regions. Twenty-four individuals per population were genotyped using six polymorphic simple sequence repeat (SSR) markers to compute their indirect equilibrium estimates of outcrossing rate as well as population structure. In addition, the 12 populations were planted in a field in a randomised block design with five replications. Their progeny (250 individuals per population) were genotyped with the six SSR markers to estimate multi-locus outcrossing rates. Equilibrium estimates of outcrossing rates ranged from 7.0 to 75.0%, while multi-locus outcrossing rates (t_m) ranged from 8.9 to 70.0% with a mean of 49.7%, indicating that wild sorghum exhibits a mixed mating system. The wide range of estimated outcrossing rates in wild sorghum populations indicate that environmental conditions may exist under which fitness is favoured by outcrossing and others under which selfing is more advantageous. The genetic structure of the populations studied is concordant with that expected for a species displaying mixed mating system.

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Introduction

Flowering plants exhibit a diversity of mating systems, ranging from complete selfing through cleistogamy to obligatory outcrossing through dioecy (Leblanc et al. 1995; Ross-Ibarra et al. 2007). However, the extent to which the observed variation is induced genetically or environmentally is in most of the cases unknown. This is a serious lack of knowledge, given the contrasting effects the mating system may have on the genetic structure, ecological adaptation and the fitness of plant populations (Booy et al. 2000). Outcrossing leads to a high level of heterozygosity

and, coupled with recombination, constantly generates new potentially adaptive genotypes. Self-pollination, on the other hand, increases the level of homozygosity and may preserve adaptive combinations of both linked and unlinked genes (Allard 1975). The rate of selfing can vary widely among closely related species and even among populations within species (Jain 1976). Therefore, variable outcrossing rates exhibited by a species may reflect a characteristic that has evolved in response to several ecological variables.

As populations become smaller and habitat disturbance increases, there is a trend towards increased inbreeding and greater variation in outcrossing (Coates et al. 2007). Therefore, changes in the mating system can be useful indicators of population genetic processes and provide valuable insight into the consequences of conservation strategies following anthropogenic disturbances (Neel et al. 2001). Indicators include the outcrossing rate, biparental inbreeding, and the correlation of outcrossed paternity. Selfing or biparental inbreeding leads to an increase in the frequency of homozygotes within a population. Subsequent effects may include inbreeding depression and reduced fitness. Moreover, a loss of genetic diversity is expected from genetic drift.

The majority of wild sorghums found in Africa are classified, along with cultivated sorghum, into the species *Sorghum bicolor* (L.) Moench (De Wet 1978; Doggett 1988). Cultivated sorghum is further classified as *S. bicolor* spp *bicolor* (L.) Moench and wild sorghum as *S. bicolor* spp *verticilliflorum* (Steud.) De Wet. *S. bicolor* is considered a predominantly autogamous and hermaphroditic species. Flowering starts with the terminal flowers of a panicle and continues downwards in a fairly regular manner over a period of 6–15 days (Doggett 1988), providing great opportunity for different mating systems under varying environmental conditions. Literature on outcrossing rates of wild sorghum is limited both in terms of species coverage and eco-geographic reference. Outcrossing rates in wild sorghum species ranging from 0 to almost 100% have been reported. Hogg and Ahlgren (1943) calculated a mean of 7% natural outcrossing in johnsongrass (*S. halepense*) in Wisconsin, USA, Garber and Antwood (1945) found 18–77% outcrossing in sudangrass (*Sorghum bicolor* subsp. *drummondii*) in Pennsylvania, USA, and Pedersen et al. (1998) reported 0–100% outcrossing on individual sudangrass plants in Nebraska, USA.

Wild sorghum is an important gene reservoir for sorghum crop improvement programmes. For example, resistance mechanisms against the parasitic weed *Striga*, such as low germination stimulant production, germination inhibition, and low haustorial initiation activity have been found in wild sorghum (Rich et al. 2004). Wild sorghum has novel grain starch properties that could be used to improve digestibility of crop-sorghum for intensive livestock production (Dillon et al. 2007). However, due to the ever-increasing anthropogenic changes to agricultural and natural ecosystems as well as loss of natural habitat due to increasing human and livestock population pressure, wild sorghum may be at risk of genetic erosion. There is therefore a need to study the mating system of wild sorghum in order to develop in situ conservation strategies that will maintain its rich genetic diversity.

Estimating the level of outcrossing in wild sorghum has attracted attention in the context of biosafety of genetically engineered sorghum cultivars. Pollen-mediated gene flow can potentially occur between sorghum cultivars and nearby wild sorghum populations. This is especially important if the genetically engineered traits that, if transferred to wild sorghum populations, may lead to invasiveness, evolution of more aggressive weeds, or the most extreme cases the extinction of wild sorghum populations. Since cultivated and wild sorghum populations occurs sympatrically in Africa (Mutegi et al. 2010; Tesso et al. 2008), pollen-mediated gene flow belongs to the most important concerns pertaining to the introduction of genetically engineered sorghum cultivars.

The mating system of a plant species can be determined either directly or indirectly. The direct method is based on a multi-locus mating system model, which allows for the progeny of a single maternal individual to be genotyped at each of several loci (Ritland 2002). The indirect method is based on the frequency of heterozygotes in a population assumed to be in inbreeding equilibrium (Brown and Allard 1970). Under this assumption, equilibrium outcrossing rates can be calculated from Wright's inbreeding coefficient. The direct measure refers to a particular year and environment, while the indirect measure reflects the mating behaviour over many proceeding generations. In the present study we applied both the direct and indirect approaches. We analysed 12 wild sorghum populations from four eco-geographically different regions in Kenya using six simple sequence repeat (SSR) markers.

Materials and methods

Sample collection

Twelve wild sorghum populations (three populations per region) were collected in four distinct sorghum growing regions in Kenya (Fig. 1; Table 1). At each sampling site, 24 individuals were collected.

Population estimates of outcrossing rates

Outcrossing rates of wild sorghum populations were assessed at the Kiambere research station of the Kenya

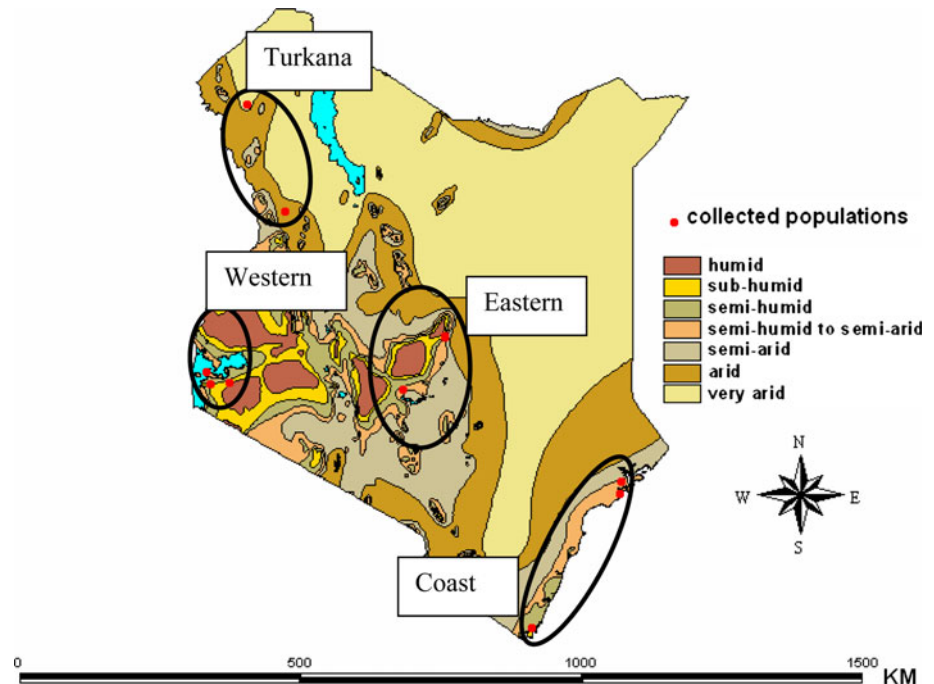**Fig. 1** Four sorghum growing regions in Kenya (*encircled*) where wild sorghum populations were sampled

Table 1 Agro-climatic characteristics of the 12 wild sorghum populations sampling sites and of the KARI station chosen for evaluating them

Population	Region	Alt (m)	AEZ	Classification	Moisture (%)	Rain (mm)	Temp (°C)
T3	Turkana	570	VII	Very arid	<15	150–350	22–40
T4	Turkana	568	VII	Very arid	<15	150–350	22–40
T10	Turkana	706	VI	Arid	15–25	300–550	22–40
W17	Western	1,479	II	Sub-humid	65–80	1,200–1,600	15–30
W26	Western	1,143	III	Semi-humid	50–65	800–1,200	20–35
W107	Western	964	III	Semi-humid	50–65	800–1,200	20–35
C13	Coast	3	IV	Semi-humid to semi-arid	40–50	>500	22–40
C32	Coast	11	IV	Semi-humid to semi-arid	40–50	>500	22–40
C49	Coast	19	II	Sub-humid	65–80	>500	15–30
E40	Eastern	892	IV	Semi-humid to semi-arid	40–50	751–1,000	22–40
E6	Eastern	869	IV	Semi-humid to semi-arid	40–50	751–1,000	22–40
E78	Eastern	1,169	IV	Semi-humid to semi-arid	40–50	1,001–1,250	22–40
All	Kiambere	848	IV	Semi-humid to semi-arid	40–50	751–1,000	22–40

Source: agro-ecological classification and data were obtained from the Ministry of Agriculture (MOA), Kenya

Alt, altitude; AEZ, agro-ecological zone; Rain, annual rainfall range; Temp, growing season temperature range; All, all 12 populations evaluated at the same location (KARI field station at Kiambere, located in Eastern Region)

Agricultural Research Institute (KARI), during the short-rain season of 2007/2008. The 12 wild sorghum populations collected were planted in the field. A randomised complete block (RCBD) design with five replicates and one-row plots was used. Each plot consisted of 30 plants with a spacing of 75 cm between rows and 50 cm between plants within rows. At maturity, five plants per plot were randomly selected and harvested. Thus each population consisted of 25 individual plants (panicle). Seeds harvested from the same panicle constituted a family.

DNA extraction and genotyping

A single seed from each of 24 individuals per collected population was planted in a greenhouse at the University of Hohenheim. In addition, a random sample of ten seeds per harvested panicle (progeny of the original wild sorghum populations) that were grown at Kiambere was also planted. Thus each population consisted of 250 individual plants (25 families, each with 10 progenies). A special protocol was followed for germination in order to overcome physiological

dormancy in wild sorghum (Muraya and Parzies 2009, unpublished data). Seeds were deglumed and placed in Eppendorf tubes arranged in a rack and warm water (37°C) was added to each tube. The rack was subsequently incubated overnight (12 h) in the dark in a water bath at 37°C. The seeds were placed individually in pots in compost in a greenhouse. Total genomic DNA was extracted from tissues of 5 cm young lyophylized leaves using a modified CTAB protocol (Mace et al. 2003). Concentration and quality of the DNA was assessed using electrophoresis of 1 μl of extracted DNA on a 0.7% (w/v) agarose gel followed by normalisation of the concentration at 50 ng/μl.

SSR selection

The SSR markers used in this study were part of 18 generation challenge project markers (Brown et al. 1996; Taramino et al. 1997; Kong et al. 2000; Schloss et al. 2002; Agropolis-CIRAD-Genoplante unpublished) previously used to genotype 62 wild sorghum populations (Muraya et al., submitted). The six SSR markers worked efficiently and had the highest polymorphism. Furthermore, we compared the equilibrium outcrossing rate of the 62 populations based on the 6 SSR markers to that of the entire set of 18 SSR markers and found no significant differences (Table S2; PROC TTEST; $P = 0.3471$). We therefore considered the six SSR markers sufficient in estimating the outcrosssing rates of the wild sorghum populations. This is especially because the approach used to estimate outcrossing in this study has been shown to be particularly useful in cases where effort in scoring more loci is less than the effort in scoring more progeny (Ritland 2002; Ritland and Jain 1981). Ritland (2002) showed that six loci could provide accurate estimates of multi-locus mating system as demonstrated by low levels of variance. He demonstrated higher level of accuracy for loci with four alleles compared to those with only two. We expect the SSR markers used in this study to be more informative given that between 19 and 28 alleles per loci were observed (Table S1).

SSR analysis

A set of six highly polymorphic sorghum SSR markers (Table S1) was used to genotype the extracted genomic DNA. PCR reactions were performed in 10 μl reaction volume, containing 1× PCR buffer [20 mM Tris–HCl (pH 8.4), 50 mM KCl], 1.5 mM MgCl$_2$, 0.25 μM of each fluorescent labelled forward and unlabelled reverse primers, 0.2 mM dNTPs, 0.5 unit per reaction of Taq polymerase and 100 ng template DNA (2 μl). The amplification reaction consisted of a denaturing step of 3 min at 94°C, followed by 40 cycles beginning with 94°C for 1 min, annealing reaction of 1 min at 55°C, extension at 72°C for 1 min followed by one terminal step at 72°C for 10 min

and consecutive storage of amplification products at 4°C. All PCR reactions were performed on a MJ Research icl PTC-100 thermocycler followed by fragment analysis on automated laser fluorescence sequencer (MegaBACE). The genotype "BTX 623" was included as a control on each sample plate to correct for any shift in allele calling.

Estimation of equilibrium outcrossing rates

Twenty-four individuals per population were used to compute estimates of equilibrium outcrossing rate (t_e) as follows:

$$t_e = \frac{1 - F}{1 + F}$$

where F is the inbreeding coefficient which was computed as in Weir (1996). Correlation between t_e and panicle compactness and shape was also computed.

Estimation of multi-locus and average single-locus outcrossing rates

The multi-locus outcrossing rate (t_m) and the average single-locus outcrossing rate (t_s) were estimated using the procedure of Ritland (1986) implemented in the MLTR software 3.0 (Ritland 2002). Calculations were based on a mixed mating model assuming a selfing rate of s and an outcrossing rate of $1 - s$. Standard errors (SE) of the estimated outcrossing rates were obtained using 1,000 bootstraps by resampling families within populations. The biparental inbreeding ($t_m - t_s$), a measure of mating between relatives, was computed as well as the correlation of paternity (r_p), i.e., the proportion of full sibs in the outcrossed seeds (Ritland 1989). A multiple test of differences between populations t_m and t_s was performed using SAS version 9.1 (SAS Institute 2004). A significance level of 5% was used.

Population structure

To explore differentiation among populations and regions, F-statistics parameters (F_{IS}, F_{IT} and F_{ST}) were estimated in Genetix 4.05 (Belkhir et al. 2004). Cluster analysis of the population was based on Euclidean distances implemented in MEGA versus 4.0 (Tamura et al. 2006). A consensus neighbour joining (NJ) tree was constructed by performing 100 bootstrap replicates.

Results

Polymorphism of SSR markers

All SSR markers scored were highly polymorphic (Table S1). The number of alleles per marker obtained across all

12 populations ranged from 19 to 27 and from 18 to 25 for their progenies planted at Kiambere. All the populations displayed high PIC values, ranging from 0.80 to 0.92 in the collected populations and 0.69 to 0.90 in their progenies.

Estimation of equilibrium outcrossing rates

The average number of alleles per SSR marker ranged among populations from 2 to 9 (Table 2). Observed heterozygosity values (0.03–0.49) were lower than the expected heterozygosity values (0.24–0.78), and inbreeding coefficients ranged from 0.14 to 0.86. The equilibrium estimates of the outcrossing rate varied from 0.07 to 0.75. The correlation coefficient between t_e and panicle compactness and shape was not significant ($r = -0.05$; $P = 0.88$).

Estimation of multi-locus and average single-locus outcrossing rates

The t_s and t_m estimates differed significantly between populations ($P = 0.05$; Table 2). The t_s values ranged from 0.08 to 0.64 and t_m from 0.09 to 0.70. On average t_s and t_m estimates were highest in Coast populations. Eastern region had the lowest t_s and t_m values but showed the highest variation between populations. The overall mean t_s and t_m values across all populations were 0.45 and 0.49, respectively. The overall biparental inbreeding ($t_m - t_s$) mean was low for all populations studied. The standard errors of the estimated

outcrossing rates were not significantly different from zero. Very low values of the correlation of paternity r_p were found in all the populations, ranging from 0.05 to 0.20 (Table 2), i.e., only 5–20% of the outcrossed sibs were full sibs.

Comparison of outcrossing rates of the collected wild sorghum populations and their progeny grown in the field at Kiambere (Fig. 2) showed no consistent outcrossing trend among populations originating from same regions. However, on average Coast and Eastern populations showed increased outcrossing rates in their progeny as compared to progeny of Turkana and Western populations, which showed decreased values. Population E40 had lower than 10% outcrossing rates under both estimates.

Population structure

The estimates of F_{IS}, F_{IT} and F_{ST} were significant at ($P < 0.001$) at both population (0.42, 0.70 and 0.48, respectively) and region (0.63, 0.70 and 0.20, respectively). Cluster analysis based on Euclidean distances estimated from the SSR data revealed that sorghum populations were not strictly clustering to their regions of origin (Fig. 3).

Discussion

Our study revealed great differences in the mating systems of wild sorghum populations. We found multi-locus

Table 2 Diversity statistics, estimated multi-locus and average single-locus outcrossing rates (t_m and t_s), and multi-locus correlation of paternity (r_p) of the 12 Kenyan wild sorghum populations from four eco-geographic regions based on six SSR loci

Population	Region	Collected population						Progeny planted in field			
		A_o	H_e	H_o	F	t_e	PCS	t_s (SE)	t_m (SE)	$t_m - t_s$ (SE)	r_p (SE)
C13	Coast	2	0.29	0.19	0.36	0.47	1	0.520b (0.018)	0.574b (0.02)	0.054 (0.003)	0.200 (0.000)
C32	Coast	6	0.42	0.27	0.37	0.46	5	0.500b (0.0000)	0.554b (0.007)	0.054 (0.007)	0.053 (0.077)
C49	Coast	–	–	–	–	–	1	0.600ab (0.029)	0.656ab (0.03)	0.056 (0.002)	0.136 (0.200)
E6	Eastern	9	0.78	0.27	0.66	0.20	2	0.540b (0.0220)	0.592b (0.023)	0.052 (0.001)	0.200 (0.000)
E40	Eastern	3	0.24	0.03	0.86	0.07	2	0.082d (0.0420)	0.089d (0.045)	0.007 (0.004)	0.057 (0.067)
E78	Eastern	8	0.64	0.30	0.55	0.29	6	0.540b (0.0240)	0.594b (0.022)	0.054 (0.002)	0.200 (0.000)
T4	Turkana	4	0.38	0.15	0.63	0.23	2	0.355c (0.0250)	0.402c (0.030)	0.047 (0.005)	0.200 (0.011)
T3	Turkana	4	0.53	0.46	0.15	0.73	2	0.560b (0.0380)	0.618b (0.041)	0.058 (0.013)	0.200 (0.000)
T10	Turkana	2	0.26	0.21	0.21	0.65	2	0.510b (0.0270)	0.570b (0.032)	0.060 (0.006)	0.064 (0.102)
W26	Western	7	0.61	0.49	0.22	0.65	6	0.388c (0.0430)	0.437c (0.046)	0.049 (0.016)	0.200 (0.000)
W107	Western	4	0.46	0.34	0.28	0.56	2	0.600ab (0.024)	0.592b (0.024)	−0.008 (0.013)	0.200 (0.003)
W17	Western	4	0.45	0.40	0.14	0.75	1	0.640a (0.0380)	0.700a (0.043)	0.060 (0.006)	0.200 (0.000)
Mean		4.82	0.46	0.28	0.40	0.46	4.82	0.454 (0.02200)	0.497 (0.0220)	0.043 (0.009)	0.114 (0.040)

Means followed by the same letter are not significantly different from each other at $P = 0.05$

Region, region in Kenya which the populations were sampled; A_o, average number of alleles per population; H_e, expected heterozygosity; H_o, observed heterozygosity; F, inbreeding coefficient; t_e, equilibrium outcrossing rate; PCS, panicle compactness and shape (where 1 = very lax panicle; 2 = panicle with very loose erect primary branches; 5 = panicle with loose drooping primary branches; and 6 = panicle with semi-loose erect primary branches); t_s, single-locus outcrossing rate; t_m, multi-locus outcrossing rate; r_p, multi-locus correlation of paternity and variance estimate were based upon 1,000 bootstraps and units of resampling was family within population
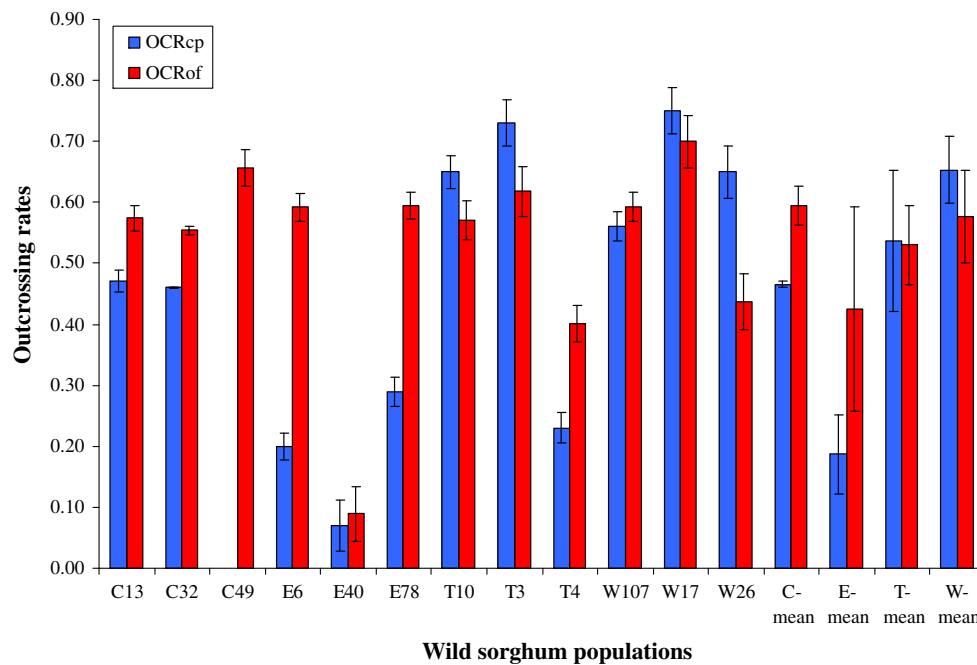
Fig. 2 Estimates of the outcrossing rates of the 12 wild sorghum populations (*OCRcp*) collected in four regions of Kenya and their progenies (*OCRof*) planted in the field at KARI-Kiambere during the 2007/2008 growing season (*C*, *E*, *T* and *W* series are populations collected at Coast, Eastern, Turkana and Western region, respectively, while mean is the average across the populations). *Bars* represent the standard errors
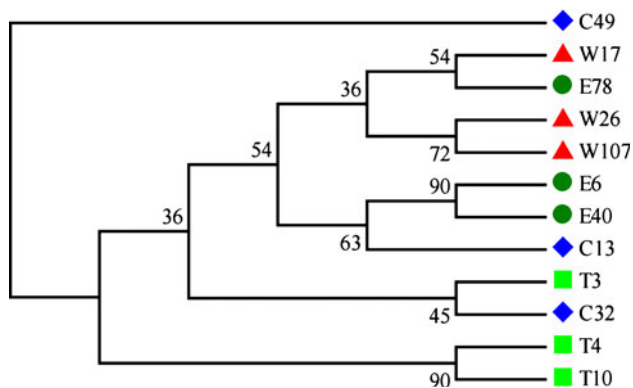

Fig. 3 Neighbour-joining (NJ) consensus tree based on Euclidean distances on allelic data from 6 SSR loci among 12 wild sorghum populations collected from four regions of Kenya. Populations are identified by number and each region is identified by a *different symbol* and *colour*. The *numbers* at the nodes represent the number of times each node was represented in a bootstrap analysis

outcrossing rates ranging from 8.9 to 70%. Equilibrium estimates of outcrossing rates confirmed the results obtained by Ritland's multi-locus approach. The outcrossing rates were considerably higher than those reported for cultivated sorghum. For example, Dje' et al. (2004) reported outcrossing rates in cultivated sorghum in Morocco of 7–16%, whereas Barnaud et al. (2008) reported 5–40% for sorghum landraces in Cameroon. Ellstrand and Foster (1983) and Ollitrault et al. (1997) reported an

average outcrossing rate of cultivated sorghum of 34 and 19% in USA and Burkina-Faso, respectively. In Kenya, Rabbi et al. (2010) reported outcrossing rates of 5–7% have been reported in cultivated Ochuti sorghum. Thus our study is in agreement with phylogenetic analyses suggesting that outcrossing systems are ancestral (Schoen et al. 1997; Goodwillie 1999; Vallejo-Marin and Uyenoyama 2004; Porcher and Lande 2005).

Outcrossing rates are influenced not only by the maternal genotype but also by environmental conditions. In our study, variation among wild sorghum populations occurred within a single season (progenies planted at KARI-Kiambere), in which all plants experienced similar environmental conditions, demonstrating the great outcrossing potential existing in wild sorghum. In the current study, no consistent pattern of outcrossing rate was observed among the agro-ecological zones. Lack of a clearly distinct classification of the populations may bias inferences regarding associations between mating system and ecological trends. In our study we did not identify the species or races of the population studied. However, cluster analysis (Fig. 3) indicate that at least two different populations were found in the same region. Muraya et al. (2010) also showed that at least three species of wild sorghum exist in Kenya and three races of the species *S. bicolor* spp *verticilliflorum*. Morever, they revealed that that two or more species of wild sorghum can be found in the same region. These issues notwithstanding, our results indicate that mixed

mating systems occur frequently in a wide range of wild sorghum populations, motivating continued exploration of the factors that promote their evolutionary stability.

The estimated wide range of outcrossing rates in wild sorghum populations studied indicated that environmental conditions may have existed under which fitness was favoured by outcrossing and others under which selfing was more advantageous. However, the available climatic data for different agro-ecological zones was too variable to deduce any correlation between the outcrossing rates and specific environmental factors. Populations from Eastern region showed the highest variation between populations within the same region. Sites in the Eastern region may considerably differ in altitude (steep fall in altitudinal gradient over short distances) leading to a wide spectrum of climatic conditions which may explain the high variation observed in the outcrossing rates. Abdel-Ghani et al. (2004) showed that outcrossing rates of cultivated and wild barley varied greatly among genotypes and seasons. Comparison of the estimates of equilibrium outcrossing rates between the collected wild sorghum populations and their progenies grown at one location (Fig. 2) indicated that environmental conditions could cause considerable temporal variation in mating behaviour. Low temperatures and light intensity have been found to modify outcrossing in selfing species (Demotes-Mainard et al. 1995; Li et al. 1996). Therefore, studies over successive years are required to measure temporal variation in outcrossing rates of the studied populations.

Beside variation in outcrossing, another important aspect of the mating system is the extent to which mating between relatives contributes to the genetic structure. Estimates of the degree of relatedness between paternal and maternal parents are often missing in studies of mating systems in plants. We observed low biparental inbreeding (overall mean of 4.3%), which fits with values obtained for other species (Jarne and Auld 2006). Estimates of biparental inbreeding allow us to differentiate between self-fertilisation and mating between relatives. If mating had occurred between relatives such outcrossing events could be blurred.

Mixed mating can result from three types of reproductive systems (Cruden and Lyon 1989). First, a genetically based selfing rate polymorphism can exist, for example in populations that contain both self-compatible and self-incompatible individuals (Stone 2002). This was not supported in this study since no significant differences were observed in outcrossing rates between families (progenies of individual plants) within a population. Second, species can exhibit heteromorphic flowering systems (Schoen and Lloyd 1984; Masuda et al. 2004), such as cleistogamous (purely selfing) and chasmogamous flowers (both outcrossing and selfing possible). Thirdly, all flowers are chasmogamous, and progenies may arise from selfing, outcrossing or a mixture of both (Schoen and Brown 1991). Wild sorghum populations in Kenya likely display the second or third type of reproductive system. Cultivated sorghum has considerably lower outcrossing rates (Dje′ et al. 2004; Barnaud et al. 2008; Rabbi et al. 2010) and might have more cleistogamous flowers than wild sorghum. Plants that have both chasmogamous and cleistogamous flowers display high levels of reproductive assurance, because under selfing natural selection reduces inbreeding depression by purge deleterious recessive alleles (Keller and Waller 2002; Weekley and Brothers 2006). Under a mixed mating system displayed by Kenyan wild sorghum, it would be expected that selfing will produce seeds under resource-limited conditions thus ensuring reproduction while outcrossing reduce inbreeding depression causing additional genetic variation when conditions are favourable.

Panicle shape has been considered to play a major role in determining outcrossing rate in cultivated sorghum Dje' et al. (2004) suggested that genotypes with loose panicles display higher outcrossing than those with compact panicles. Our study did not agree with this, as there was no correlation between outcrossing and panicle compactness and shape. Results of this study rather suggest that outcrossing is largely influenced by ecological conditions rather than panicle morphology. Populations with same panicle compactness and shape but originating from ecologically different sites displayed different outcrossing rates.

Cultivation of sorghum in Kenya carries a risk of introgressing genes, including transgene into wild sorghum populations, as wild sorghum occurs abundantly in or near farmers' fields and most genotypes are cross-compatible with cultivated sorghum. Till-Bottraud et al. (1992) showed that even foxtail millet, a largely self-pollinated species, may exchange genetic information with wild relatives at a rate that may cause problems if transgenic cultivars are grown in proximity. Although flowering phenology in sorghum reduces pollen-mediated gene flow, it does not fully prevent pollen exchange unless no overlap in flowering time exists between wild and cultivated sorghum.

with regulations according to the Convention on Biological Diversity (CBD)], planting and data collection in the field (KARI-Kiambere).

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