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Genetic Diversity of a Parasitic Weed, *Striga hermonthica*, on Sorghum and Pearl Millet in Mali

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Abstract Eleven populations of witchweed, *Striga hermonthica*, were collected in four regions of Mali and investigated with 12 microsatellite markers. Extensive genetic diversity was observed, with most plants heterozygous for most markers. Allelic diversity was broadly distributed across populations with little genetic differentiation and large amounts of gene flow. Nearby fields of pearl millet and sorghum were found to have indistinguishable witchweed populations. Some population structure was apparent, but did not correlate with the local environment or host genotype, suggesting that seed transportation or other human-driven variables act to differentiate central Malian *S. hermonthica* populations from southern Malian populations.

Keywords Microsatellite markers · *Striga hermonthica* · Sub-Saharan Africa · Subsistence agriculture · Witchweed

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Introduction

Striga hermonthica is a weedy plant that parasitizes grain crops, such as sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) throughout Sub-Saharan Africa. There are four other *Striga* species that can have dramatic effects on staple crop production. Taken together, these witchweeds are the most important biological limitation to food production in Africa (Ejeta 2007b).

The initial stages of the *Striga*::host interaction can be broken down into four key steps: seed germination, host attachment, haustorium formation, and penetration of the root vascular system (Yoshida and Shirasu 2009). *Striga* seeds have evolved to recognize chemical signals, strigolactones, that are involved in attracting a beneficial interaction between an arbusculuar mycorrhizal fungus and the plant host (Akiyama and Matsuzaki 2005; Matusova et al. 2005). Several different strigolactones can induce germination and chemotropic growth of *Striga* seedlings towards the roots of a possible host. *Striga* is an obligate parasite and must attach to a host plant before nutrient stores in the tiny seed are exhausted. Firm attachment of the host root is accomplished via a structure known as a haustorium (Keyes et al. 2001). Once attachment is complete, *Striga* forms a connection with the host vascular system and begins parasitizing the host (Bar-Nun et al. 2008).

Efforts to identify crops with resistance to *Striga* have yielded very mixed results, with essentially no success to date with the hosts maize and pearl millet, ephemeral success with cowpea for resistance to the legume parasite *S. gesnerioides* (Timko et al. 2007) and only partial success with sorghum (Ejeta 2007a), the host species that is most likely to have co-evolved with *S. hermonthica* (Musselman 1987). In order to better understand the diversity of *Striga* species, and whether it impinges on host resistance, several studies have been conducted to examine population-level genetic diversity and to identify the existence of races or structure among *Striga* populations (reviewed in (Mohamed et al. 2007)). For the autogamous (self-fertilizing) species *S. asiatica* and *S. gesnerioides*, genetic diversity analyses have shown distinct races of both species across their ranges (Shawe and Ingrouille 1993; Botanga et al. 2002; Botanga and Timko 2006).

S. hermonthica is an obligate out-crossing species (Safa et al. 1984), so it is expected to show less differentiation between populations and greater diversity within populations than seen in related autogamous species (Hamrick 1982). In agreement with this prediction, multiple genetic diversity studies using allozymes, Randomly Amplified Polymorphic DNA (RAPD), or Amplified Fragment Length Polymorphism (AFLP) markers have observed extensive genetic diversity but no convincing evidence for races in *S. hermonthica* (Bharathalakshmi et al. 1990; Kuiper et al. 1996; Olivier et al. 1998; Koyama 2000; Gethi et al. 2005). In those studies where *S. hermonthica* was collected from different countries (on different sides of the continent), a geographic distance effect was noted. None of these studies demonstrated a genetic component to host specificity. However, the number of populations investigated, the number of loci analyzed, and/or the type of marker employed limited all of these studies.

The most powerful approach to characterizing genetic diversity in *S. hermonthica* would employ a robust set of reproducible, neutrally evolving, and co-dominant markers. Simple Sequence Repeat (SSR) markers with these properties have recently been developed for *Striga* (Estep et al. 2010). In addition to robust markers, an optimal study of genetic diversity in *S. hermonthica* would employ populations collected in multiple years, parasitizing multiple staple crops (e.g., sorghum, pearl millet, maize, rice), in multiple agro-ecosystems (e.g., Sahel grasslands, Sudan savanna), and from across the species range at both macro and micro scales. In this manuscript, results from a first collection year are presented to describe the diversity of *S. hermonthica* across a broad swath of environments and agricultural zones in Mali, a nation that is dramatically impacted by *Striga* parasitism.

Results

Striga samples were collected in October and November of 2008 across four broad regions in Mali (Fig. 1, Table 1). The regions, labeled 3000, 4000, 5000 and 6000, were each represented by samplings from 2 to 3 subsistence farmer fields, with at least 20 individual plants sampled per field. The agricultural environments were very different for these four collection regions. The more southern 4000 and 5000 fields are in the Sudan savanna environment, while the central Malian 3000 and 6000 regions represent a drier Sahelian environment and a wetter forested region, respectively.

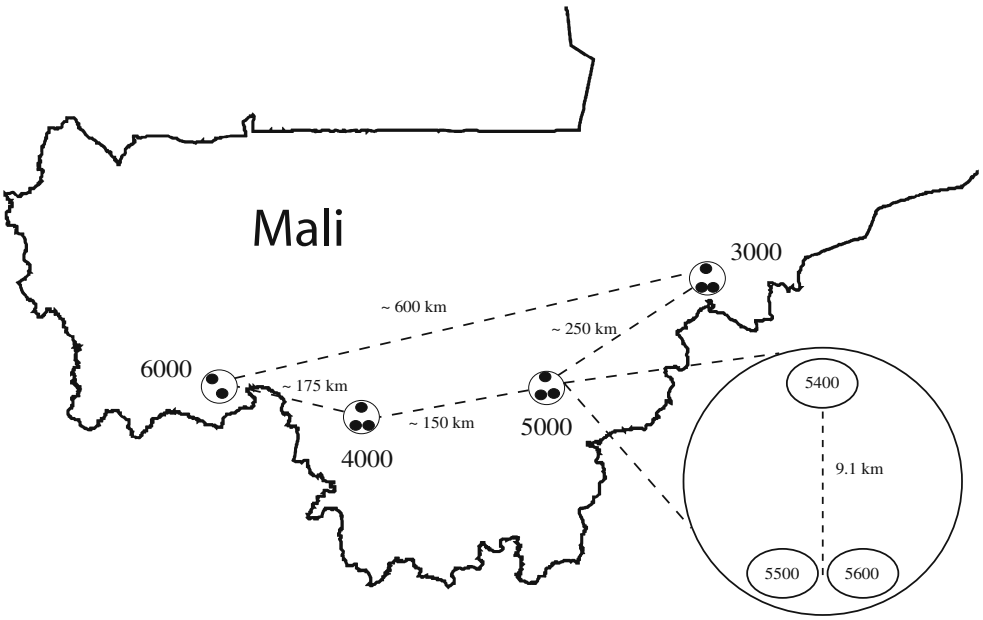

Fig. 1 Map of collection sites, with regional locations and approximate geographic distances indicated

An average of 250 individuals were scored for each of the 12 microsatellite markers and a total of 181 alleles were identified within the sample data (Table 2). Values for the mean allelic diversity (richness) ranged from 7.0 in population 6800 to 10 in population 3900 (Table 2). Mean effective allelic diversity (evenness) values ranged from 4.0 in population 3700 to 5.9 in population 3900. There was no significant difference in the values for richness or evenness between populations. Values for the mean expected heterozygosity (gene diversity) ranged from 0.687 in population 4300 to 0.748 in population 3900 with no significant differences observed between populations (Table 3). Similarly, values for the mean observed heterozygosity ranged from 0.689 in population 3800 to 0.783 in population 3700 with no significant differences between populations. The mean fixation index was calculated to show in which direction populations were trending out of Hardy-Weinberg proportions, with seven populations appearing to have excess heterozygosity (negative values) and the remaining four populations indicating inbreeding or excessive homozygosity (positive values) in comparison to gene diversity (Table 3). The number of region-specific alleles was also counted: region 3000 exhibited 22, region 5000 yielded 20, region 4000 had 6, and region 6000 yielded 2.

An AMOVA was conducted to estimate the Rst (analogue of Fst) using a stepwise mutation model specific for microsatellite data (Slatkin 1995). The resulting Rst value was 0.048 ($P = 0.01$) and Ris and Rit were both 0.991 ($P = 0.01$). A majority of the variance (95%) can be explained by within population variation. The remaining variance can be explained by among region variance (2%) and among population variance (3%). The number of migrants was also calculated from the Rst value using the equation $Nm = (1\text{-Rst})/4*\text{Rst}$, resulting in Nm=4.921.

A principal coordinates analysis was conducted using pairwise comparisons of Nei's standard genetic distance to identify major patterns within the data set (Fig. 2). Coordinate one (x-axis) explains 33.29% of the variance in the data and splits the four collection regions into two distinct groups. Group one (on the left) contains individuals from region 4000 and region 5000. Group two (on the right) contains individuals from region 3000 and region 6000. The second coordinate (y-axis) explains 15.66% of the variance and does not appear to further divide the two groups obtained from coordinate one. While the two major groupings are distinct, one to a few individuals from region 3000 and 6000 appear to be placed within or near group one (Fig. 2).

Table 1 Collection sites with location and host crop

Region	Population	Location		Host
3000	3700	13.2078	−4.6022	sorghum
	3800	13.2099	−4.599	millet
	3900	13.7818	−4.3949	millet
4000	4100	12.4605	−8.1255	sorghum
	4200	12.3845	−8.2999	sorghum
	4300	12.3838	−8.2996	sorghum
5000	5400	12.5925	−6.7081	sorghum
	5500	12.6526	−6.7653	sorghum
	5600	12.6535	−6.7654	sorghum
6000	6700	12.5449	−9.8288	sorghum
	6800	12.5449	−9.8284	sorghum

Table 2 Total number of individuals (N) scored for each microsatellite marker and the total number of alleles (A) identified

Locus	N	A
SH1005	263	6
SH1008	249	9
SH1009	257	24
SH1012	263	6
SH1014	270	6
SH1016	250	28
SH1029	233	25
SH1030	246	22
SH1032	252	9
SH1038	232	26
SH1041	269	13
SH1042	218	7
Alleles total		181

The program STRUCTURE was used to identify population structure within the data set (Fig. 3). The predefined $K = 2$ simulations had the highest value for Delta K among all predefined K values ranging from 1 to 11 (data not shown). This analysis grouped regions 4000 and 5000 as one cluster and grouped regions 3000 and 6000 as a second group. It is important to note that several individuals within these two groups exhibit admixture between the two groups.

A Mantel's test was performed with two data matrices, the first was a pairwise geographic distance matrix (Log transformed) and the second was a pairwise Nei's standard genetic distance matrix (Fig. 4). The resulting R^2 value was 0.2196 and was found to be significant ($P = 0.01$) based on 999 random permutations of the two original data matrices. A second Mantel's test was performed with the same geographic distance matrix and a pairwise matrix of the number of migrants (Fst/1-Fst). The resulting R^2 value was 0.2388 and was found to be significant ($P = 0.01$), based on 999 random permutations of the two data matrices.

Discussion

In this study, populations selected from Mali were characterized with 12 microsatellite markers to describe

Table 3 Mean descriptive population genetic statistics for each population followed by standard deviation values. Allelic diversity (Aa), Effective allelic diversity (Ae), Observed heterozygosity (Ho), Expected heterozygosity (He), and fixation index (F)

Mean diversity statistics by population					
Pop	Aa	Ae	Ho	He	F
3700	7.2(1.0)	4.0(0.7)	0.783(0.052)	0.707(0.042)	−0.110(0.053)
3800	10(1.8)	4.9(1.0)	0.689(0.082)	0.707(0.082)	0.020(0.055)
3900	10(1.9)	5.9(1.3)	0.762(0.067)	0.748(0.064)	−0.022(0.032)
4100	8.1(1.4)	4.3(0.9)	0.735(0.081)	0.697(0.065)	−0.047(0.059)
4200	8.0(1.4)	4.6(0.8)	0.750(0.065)	0.715(0.070)	−0.068(0.0450)
4300	7.6(1.2)	4.2(0.8)	0.735(0.054)	0.687(0.077)	−0.081(0.039)
5400	8.7(1.3)	4.9(0.9)	0.700(0.054)	0.732(0.059)	0.033(0.051)
5500	9.4(1.8)	4.8(1.0)	0.677(0.077)	0.727(0.058)	0.079(0.051)
5600	8.6(1.2)	4.7(0.7)	0.707(0.059)	0.742(0.049)	0.050(0.044)
6700	8.6(1.9)	4.5(1.0)	0.718(0.051)	0.711(0.053)	−0.018(0.044)
6800	7.0(1.0)	4.1(0.7)	0.769(0.054)	0.695(0.057)	−0.155(0.029)

the genetic diversity and structure of *S. hermonthica* in this region. While this is not the first study of genetic diversity within *S. hermonthica* (Bharathalakshmi et al. 1990; Kuiper et al. 1996; Olivier et al. 1998; Koyama 2000; Gethi et al. 2005; Ali et al. 2009; Yoshida et al. 2010), it is the first using reproducible, neutrally evolving, and co-dominant markers.

The analyses show that *S. hermonthica* is rich in allelic variation that is fairly evenly distributed among populations over a large geographic range (>600 km) within multiple ecosystems. It also demonstrates a high level of genetic diversity among populations, with an average gene diversity of 0.715 (range of 0.687–0.748) and a relatively low level of genetic differentiation (Rst=0.048; $P=0.01$). The observed values of heterozygosity and the fixation indices demonstrate that 63% (seven) of the populations appear to have an excess of heterozygous individuals. This can be explained by the negative assortative mating that would be expected for an allogamous (obligate out-crossing) species such as *S. hermonthica* (Safa et al. 1984). All three populations from region 5000 have an excess of homozygous individuals, suggesting an undetected null allele specific to this region. These results are in agreement with previous studies of *S. hermonthica* genetic diversity that indicated a great deal of intrapopulation variability and no evidence of races (Bharathalakshmi et al. 1990; Kuiper et al. 1996; Olivier et al. 1998; Koyama 2000; Gethi et al. 2005). However, we also investigated (and found no differences between) *S. hermonthica* populations on two different host species in nearby plots. Moreover, we also report higher levels of genetic diversity than previous studies, presumably because of the greater sensitivity of the molecular markers employed in this study.

The numbers of specific alleles found in each region are not evenly distributed, with populations in the eastern portion of the study area (region 3000 and 5000) having 3–10 times the number of specific alleles than those in the western portion (region 4000 and 6000). These values can be interpreted as a proxy for the age of a population, due to the high rate of mutation at many microsatellite loci. This

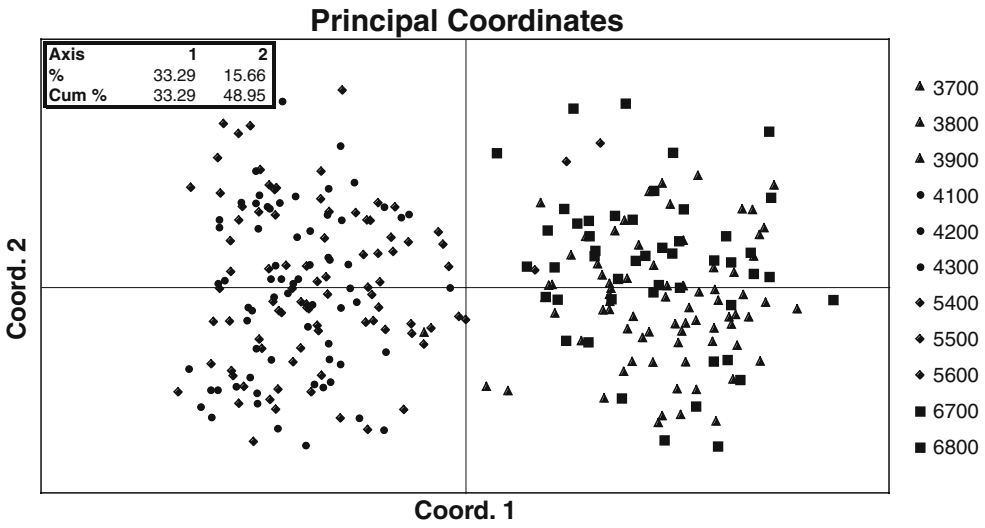

Fig. 2 A principal coordinates analysis of pairwise genetic distance between populations

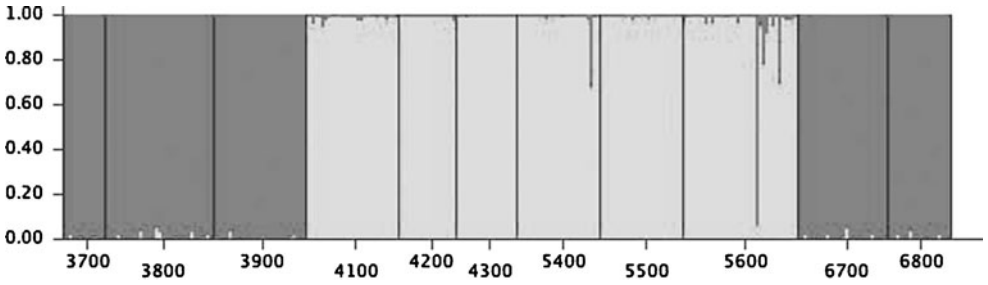

Fig. 3 Results of population structure analysis with $K=2$

would suggest that populations in the Eastern portion of the country have occupied this area for a longer period of time, while those in the Western portion are more recent arrivals. This result is interesting because we did not observe the lower level of genetic diversity that would be expected for a recent founder effect.

While there is a great deal of evenly distributed genetic diversity within the populations studied, the AMOVA analysis indicates "little" genetic differentiation (Rst= 0.048, $P=0.01$) has occurred. Most (95%) of the diversity can be explained by allelic variation within populations. This result also suggests a large amount of gene flow among populations, based on the large number of migrants per generation (Nm=4.921). A small number of migrants per generation is enough gene flow to obscure or overcome the process of drift that causes populations to differentiate over time. Anthropogenic activity, in the form of trading contaminated crop seeds, has been shown to be frequent in subsistence agricultural systems (Berner et al. 1994). Other forms of dispersal like wind, water, and forage animals have also been shown to play a dispersal role in *S. hermonthica*, but the geographic distance examined in this study likely reduces their role.

A PCA was conducted to identify possible differences between populations based on genetic distance. An unexpected result was observed, where the two regions that were most geographically separated were found to group together (regions 3000 and 6000). This observation was further analyzed using an admixture test with the program STRUCTURE where individuals without population designation are grouped based on shared genotypes. Similar results were obtained from the STRUCTURE analysis, suggesting that two distinct "strains" or "races" of *S. hermonthica* exist within the study area.

Two of the three populations from region 3000 were collected in pearl millet fields, while the remaining population was collected from a sorghum field. We saw no differences between these three populations or between the two populations from region 6000 (both from sorghum fields) that group together as one race in the PCA and STRUCTURE analyses. This observation suggests that while distinct races may exist, we cannot infer an association with a host species. It is important to point out that region 6000 is distinctly different from any of the other collection localities. It is difficult to imagine a locally adapted variety of a subsistence crop (sorghum or pearl millet) that would thrive in both the 3000 region with less than 600 mm of rain per year and the 6000 region with more than 1000 mm of rain per year. It has been reported that pearl millet-adapted strains of *S. hermonthica* exist and has been experimentally shown that those strains designated as specific to pearl millet have no problems parasitizing sorghum cultivars while the reverse (sorghum adapted strains parasitizing pearl millet) was not observed (Parker and Reid 1979).

The results reported herein suggest that *S. hermonthica* "race" distribution is not closely related to local ecosystems. If local physical environment were the major determinant of witchweed genotype, then the dry 3000 and wet 6000 region samples would be the most different.

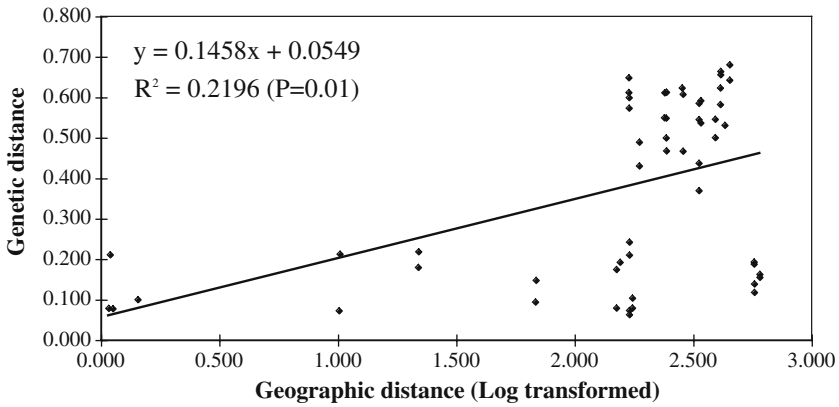

Fig. 4 Graph of Mantel's test results for the relationship between genetic distance and geographic distance

Alternatively, we propose that human-driven variables like road distribution or seed trading might drive gene flow in this parasitic weed. In each scenario, *S. hermonthica* seed may hitchhike along with human-vector transport. If trade and other communication are more active between people in the 3000 and 6000 regions than between these regions and either of the southern collection regions, then this might help explain why, in the northern portions of the study area (even at great geographic distance and on different hosts), one "race" of *S. hermonthica* is found, while a second "race" is found in the southern regions, on the same host species. Sorghum and pearl millet are grown across all four sample regions, but the majority of pearl millet is grown in the 3000 region and further north, while the majority of sorghum is grown in the southern regions. Further population collections and more detailed descriptions of the local cultural, agronomic and infrastructural systems are planned to address these questions.

To better understand what processes are differentiating the "races" identified, the hypothesis of isolation by distance was examined with a Mantel's test. A significant relationship ($p = 0.01$) between genetic distance and geographic distance were observed, arguing that geographic distance is acting to differentiate the two identified "races". The possible relationship between the amount of gene flow (Nm) and geographic distance was also investigated. A significant negative relationship was found in this analysis, suggesting that gene flow is occurring at short geographic distances but is greatly reduced at larger geographic distances. This result seems obvious, but affects the interpretation of long distance dispersal from the older 3000 region populations to the younger 6000 region populations and leads us to further argue that the identified "races" are not primarily determined by the host plant species or the local environment. The results herein on host specificity and the absence of evidence for races are in agreement with several previous studies (Bharathalakshmi et al. 1990; Kuiper et al. 1996; Olivier et al. 1998; Koyama 2000; Gethi et al. 2005), but disagree with an AFLP study conducted in Sudan that identified host-specific races (Ali et al. 2009). It is not clear whether the unique results of Ali and coworkers are caused by their investigating a different source of germplasm or by some other biological or technical factor(s).

Plant breeders are working to produce genotypes of sorghum and pearl millet that are resistant to *Striga* parasitism (Ejeta 2007a). In many plant::pathogen interactions, resistance tends to be specific to a particular pathogen race, thus having a major impact on the durability of the resistance as individual races wax and wane in the agricultural environment. Hence, it would be very useful to identify any possible *S. hermonthica* genotypes (races) that exhibit different parasitic qualities. These genotypes, once discovered and characterized, can then be used to identify individual resistance genes in crop (host) germplasm and can be used to pyramid multiple resistant genes into a targeted crop plant. In order to fully characterize the existence of "races" and the factors driving their formation, further collections of *S. hermonthica* populations and their hosts are needed. Our working hypothesis argues for a Northern "race" in areas where pearl millet is the dominant crop and a Southern "race" in areas where sorghum is the dominant crop. These "races" may not be host species-specific, but instead may be under selective pressures from other environmental factors, yet to be identified, and/or could be host genotype-specific. The amount of precipitation, mean temperature, soil quality, available pollinators, or lengths of growing season are major environmental differences in the ecosystems that were sampled. Many of the same environmental factors are certainly driving the farmers' choice of host genotype in any given region.

This analysis of 11 population of *S. hermonthica* across four regions of Mali demonstrates extensive genetic diversity and gene flow working to homogenize populations, likely caused by the small seed size, impressive parasite fecundity and probable exchange of *Striga*-contaminated host seeds among neighbors. This analysis also demonstrated that populations of *S. hermonthica* growing on sorghum and pearl millet were indistinguishable, suggesting that host crops are not (at least at the location investigated) driving differentiation within *S. hermonthica*. These results also suggest that field screening for resistant varieties in Mali would be most appropriate if conducted in both Northern/central and Southern Malian environments.

Methods

Sampling of *S. Hermonthica* Populations

Individual plants were collected from four regions (designated 3000, 4000, 5000, & 6000) of southern Mali (Fig. 1). A total of 11 populations (agricultural fields) were chosen late in the growing season when *S. hermonthica* was beginning to shed seed, so that both seed and leaf material could be collected from the same plants. Each field chosen was under cultivation by farmers growing sorghum (*S. bicolor*) or pearl millet (*P. glaucum*) as a subsistence crop (Table 1). Each population consisted of a minimum of 20 individuals collected along two linear transects, at 90° angles to each other, in a single agricultural field (~100 m × ~100 m). Leaves from individual plants were air-dried or stored in silica gel and most were shipped to the University of Hohenheim, Germany for DNA extraction. Some DNA extractions were carried out at the University of Bamako in Mali. Each region consisted of two populations within close

proximity and one population ~5–20 km away, except region 6000 where only two populations were collected.

Region 3000 (Segou) supplied 3 populations (3700, 3800, 3900) to this study and is the farthest north and east within the study area. This region is near the southern boarder of the Sahel grassland ecosystem and receives ~600 mm of rain per year. Population 3700 was collected in a sorghum field near the village of Souara in the Tominian district (13.2078 N, −4.60215 W). Population 3800 was collected in a pearl millet field ~100 m from population 3700 (13.2099 N, −4.59895 W). Population 3900 was collected in a pearl millet field near the village of Madiama in the Djenne district (13.7818 N, −4.39491 W).

Region 4000 (Kati) provided 3 populations (4100, 4200, 4300) and is the farthest south within the study area. This region is part of the Sudan savanna and receives 800–1000 mm of rain per year. Population 4100 was collected in a sorghum field near the village of Farabana in the Kangaba district (12.4605 N, −8.12552 W). Population 4200 was collected in a sorghum field near the village of Sindala in the Kati district (12.3845 N, −8.29991 W). Population 4300 was also collected in a sorghum field, ~100 m from population 4200 (12.3838 N, −8.29962 W).

Region 5000 (Dioila) contributed 3 populations (5400, 5500, 5600). This region is also part of the Sudan savanna and receives 800–1,000 mm of rain per year. Population 5400 was collected in a sorghum field near the village of Wakoro in the Dioila district (12.5925 N, −6.70812 W). Populations 5500 and 5600 were collected in sorghum fields separated by ~100 m near the village of Tonga in the Dioila district (12.6526 N, −6.76533 W and 12.6535 N, −6.76541 W, respectively).

Region 6000 (Kayes) supplied 2 populations (6700, 6800). This region is the farthest west within the study area. This region is not part of the Sahel or Sudan ecosystems and is in a mountainous region that receives >1200 mm of rain per year. Both populations were collected in sorghum fields near the village of Sagabari in the Kita district, within ~200 m of each other (12.5449 N, −9.82882 W and 12.5449 N, −9.82844 W, respectively).

DNA Extraction and Marker Amplification

In Mali, DNA isolation was performed using Plant DNAzol Reagent following the manufacturer's protocol (Invitrogen, Carlsbad, CA). Once samples were precipitated, washed, and air-dried, they were shipped to the University of Georgia, USA and re-hydrated in 100 ul of 1× TE buffer (100 mM Tris-Cl, 10 mM EDTA @ Ph 8.0). Samples shipped to the University of Hohenheim were homogenized using a TissueLyzer (Qiagen, Valencia, CA.) and total genomic DNA was extracted using a modified CTAB protocol (Doyle and Doyle 1987).

A set of 12 neutral, non-coding, and co-dominant microsatellite markers was used to access the genetic diversity and genetic structure of the collected individuals (Estep et al. 2010). PCR reactions were 10 ul, using a three-primer system with an M13 universal fluorescent-labeled primer (VIC, FAM, NED, PET) (Schuelke, 2000). Each reaction consisted of 5–10 ng of template DNA, 0.6 U of *Taq*, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP's (each), 1.25 mM forward primer, 1.25 mM fluorescently labeled M13 primer, and 2.5 mM reverse primer. A touchdown PCR program was used on a MJ Research PTC-200 Peltier Thermocycler, consisting of an initial denaturation cycle of 94°C for 5 min; 10 cycles at 94°C for 45 s, 68°C (−2°C per cycle) for 5 min, elongation at 72°C for 1 min; 5 cycles at 94°C for 45 s, 58°C for 2 min, elongation at 72°C for 1 min; 25 cycles of 94°C for 45 s, 50°C for 2 min, elongation at 72°C for 1 min; and a final 30 min elongation at 72°C. Reactions with different fluorescent labels were then multiplexed with a LIZ 500 standard and separated on an ABI 3730 sequencer (Applied Biosystems, Foster City, CA). Resultant chromatograms were scored using ABI GeneMapper software (version 4.0).

Data Analysis

Descriptive population genetic statistics were calculated using GenAlEx and Genepop (Peakall and Smouse 2006; Rousset 2008). These included allelic diversity (richness), effective allelic diversity (evenness), expected heterozygosity (gene diversity), observed heterozygosity, and the fixation index. An Analysis of Molecular Variance (AMOVA) was conducted to estimate F-statistics and to estimate the number of migrants (Nm). Nei's standard genetic distance was calculated between pairs of populations for use in a principal coordinates analysis (PCA). Geographic distance was estimated from location coordinates (Table 1) and Log transformed for use in conjunction with the Nei's genetic distance matrix to test for isolation by distance (Mantel's test). A second Mantel's test was conducted using the same matrix for geographic distance and a second matrix of pairwise comparisons of the number of migrants (Nm).

An analysis of population structure and individual population assignments was conducted with the raw data in the program STRUCTURE (Pritchard et al. 2000). Predefined numbers of populations (K) ranged from 1 to 11 and an initial burn-in period of 20000 replicates and 50000 Markov Chain Monte Carlo (MCMC) iterations were used. Five independent simulations were run for each K value. To identify the number of populations that best reflect the structure of our sample, the average K value was calculated from the five runs and Delta K was calculated as in Evanno et al. (2005).

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