GENETIC DIVERSITY OF *STRIGA HERMONTHICA* POPULATIONS IN ETHIOPIA: EVALUATING THE ROLE OF GEOGRAPHY AND HOST SPECIFICITY IN SHAPING POPULATION STRUCTURE

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Striga hermonthica, a root hemiparasitic Orobanchaceae, severely constrains grain production in sub-Saharan Africa. Host specificity and geography may play important roles in shaping the population structure of *S. hermonthica*, with the Rift Valley potentially presenting a significant barrier to dispersal. Genetic diversity was assessed in 12 *S. hermonthica* populations from locations in Ethiopia. Of these, seven populations were parasitic on sorghum, two each on tef and maize, and one on finger millet. Genetic variation was detected using four amplified fragment length polymorphism (AFLP) primer combinations. After correcting for repeatability, 385 fragments were detected across all primer combinations. The percentage of polymorphic loci was relatively high, ranging from 53.2% to 76.4%. Expected heterozygosity ranged from 0.168 to 0.279. Genetic differentiation between populations was relatively high, and all populations were significantly different from each other. F_{ST} values ranged from 0.032 to 0.293 and averaged 0.146. Genetic differences between populations could not be attributed to host specificity. Instead, geography was the main determinant of population structure. There was a correlation between geographic and genetic distance. A significant portion of the genetic variance could be apportioned on either side of the Rift Valley (5%; $P = 0.001$). Also, a significant geographic barrier was identified in the southern portion of the sampled region.

Keywords: *Striga hermonthica*, genetic, AFLP, host specificity, geography, Ethiopia.

Introduction

Striga hermonthica (giant witchweed), a parasitic plant native to Ethiopia and Sudan (Musselman 1987), is known to cause substantial losses in cereal crop production across Africa and south Asia. Corn (*Zea mays* L.), sorghum (*Sorghum vulgare* Pers.), finger millet (*Eleusine coracana* [L.] Gaertn.), and tef (*Eragrostis tef* [Zucc.] Trotter) are among the staple foods threatened by giant witchweed. This parasitic plant currently affects up to 40% of Africa's crop production, and the annual crop yield losses in West African savannas alone account for $7 billion, affecting more than 100 million people (Emechebe et al. 2004).

The giant witchweed can adapt very quickly to different hosts and environments. Dawoud and Sauerborn (1994) showed that *S. hermonthica* can attain up to 50% germination under moisture regimes described as the permanent wilting point for its host, illustrating the potentially serious consequences this parasite can have in arid regions. Additionally, witchweed can tolerate wide ranges of day/night temperatures (25°/15°C–40°/30°C; Patterson et al. 1982), making it a successful parasite throughout its range. These characteristics render *S. hermonthica* a serious pest to cereal production, especially in the Sahel region (Senegal to Ethiopia), where it has developed two host-specific strains. The first is specific to millet, occurring in the drier and more northerly region of

the Sahel, and the second attacks sorghum and is found farther south, in wetter regions (Musselman and Hepper 1986). In addition, this species has spread in Africa south to Angola and north to the Delta in Egypt, extending its range outside the continent to Yemen and Saudi Arabia (Mohamed et al. 2001).

The ability of *S. hermonthica* to withstand a wide range of climatic conditions (Patterson et. al. 1982; Dawoud and Sauerborn 1994) and parasitize different hosts (Ali et al. 2009) qualifies it to be considered among the most widely distributed known witchweeds with real invasive potential threatening cereal production worldwide (Mohamed et al. 2006). Therefore, it is difficult to develop universally resistant host crops, and crop breeding efforts toward obtaining resistant cultivars may need to take the view that *Striga* species are diverse at the intraspecific level. Instead, it may be better to focus efforts on controlling witchweed itself, particularly its spread. Host specificity and geography could potentially influence the spread of *S. hermonthica*. If host specificity and geography are significant evolutionary forces for witchweed, the population structure of the species should reflect genetic differences based on host or geographic barriers.

Limited studies on witchweed genetic diversity have been conducted, especially considering its wide range (Mohamed et al. 2007). *Striga hermonthica* is an obligate outbreeder (Safa et al. 1984), and its hybridization with *Striga aspera* has caused some taxonomic confusion (Aigbokhan et al. 2000). Allozyme electrophoresis of nine loci in two populations of *S. hermonthica* (pearl millet–adapted and sorghum-adapted populations) collected from Burkina Faso and one

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sorghum-adapted population from Sudan revealed high heterozygosity within each population ($H = 0.261-0.365$; Bharathalakshmi et al. 1990). That study showed that geographic distance played a greater role in genetic differentiation of *S. hermonthica* populations than host specialization. Similar evidence was presented for *S. hermonthica* parasitizing sorghum, pearl millet, maize, and wild grasses in Burkina Faso (Olivier et al. 1996, 1998), for six West African (Benin, Mali, and Burkina Faso) and nine Kenyan populations of *S. hermonthica* (Kuiper et al. 1996), and for *S. hermonthica* parasitizing maize, millet, and sorghum in Sudan (Ali et al. 2009). These studies indicate that low selectivity for hosts may be the trend in *S. hermonthica*. Genetic differentiation based on host specificity was shown to be greater between the millet and sorghum strains than between any of the maize strains (Ali et al. 2009). In summary, the genetic studies done to date show a general correlation between geographic distance and genetic distance and little evidence for host-specific witchweed populations.

The Rift Valley is a potential geographic barrier to the dispersal of *S. hermonthica* and may result in genetic differences between populations on the east and west sides of the valley. The Rift Valley is thought to have formed more than 2.5 million years ago, bisecting Ethiopia and essentially creating two land masses (Kingdon 1990). Previous studies on various plant and animal species have demonstrated the significance of this barrier in determining population structure in Ethiopia (e.g., Belay and Mori 2006; Kebede et al. 2007; Silvestrini et al. 2007).

Amplified fragment length polymorphisms (AFLPs; Vos et al. 1995) have been widely used in plant species, especially in crop plants (Bensch and Akesson 2005). The advantage of AFLPs is that hundreds of loci can be targeted, and no prior genetic information about the species is required. Additionally, many statistical methods and software packages are now available for AFLP analysis, allowing for the exploration of many population genetic questions (Bonin et al. 2007). Gethi et al. (2005) demonstrated the utility of AFLP analysis in *S. hermonthica* and identified four primer combinations that generated a large number of diverse fragments in the species.

The objective of our study was to identify which evolutionary force—host specificity or geography—is playing the greatest role in shaping genetic diversity within *S. hermonthica* in Ethiopia. By assessing the level of genetic differentiation between different geographic locations and between witchweed using different hosts, the influences of host specificity and geographic isolation on *S. hermonthica* evolution can be evaluated. We hypothesize that geographic barriers to dispersal, specifically the Rift Valley as a significant barrier, played a major evolutionary role in genetic differentiation in *S. hermonthica* populations.

Material and Methods

Sample Collection

Samples were collected in Ethiopia at the end of the growing season in November 2006. Samples were collected randomly from different *Striga* individuals parasitizing different individual host plants (table 1). Each sample came from a single *Striga* growing on a different individual host within the population. Young leaves were collected in the field, immediately placed in paper bags, and kept on dry silica gel. Upon return to the laboratory, the samples were placed on new and dry silica and were then refrigerated at 5°C. We collected samples from 12 populations of *Striga hermonthica* from 10 locations in central, northern, and eastern Ethiopia (table 1; fig. 1). Of these 12 populations, seven were parasitizing sorghum (*Sorghum vulgare* Pers.), two were parasitizing tef (*Eragrostis tef* [Zucc.] Trotter), two were parasitizing maize (*Zea mays* L.), and one was parasitizing finger millet (*Eleusine coracana* [L.] Gaertn.).

Laboratory Procedures

DNA was extracted from 10 randomly selected individuals from each of the sampled populations, using a standard CTAB extraction procedure (Doyle and Doyle 1987; Cullings 1992). Several representative samples were quantified, using a fluorometer to ensure that the extraction procedure worked and to optimize subsequent reactions. AFLPs were then analyzed. Each 10-μL digestion-ligation reaction consisted of 1X buffer, 0.05 M NaCl, 0.045 M BSA, 2 μM MseI adapter, 0.2 μM EcoRI adapter, 5 U MseI restriction enzyme, 5 U EcoRI restriction enzyme, 1 U T4 DNA ligase, and ~100 ng DNA. Samples were incubated at 37°C for 2 h. Each 20-μL preselective PCR reaction consisted of 15 μL of AFLP core mix (Applied Biosystems), 0.5 μM MseI-C primer, 0.5 μM EcoRI-A primer, and 4 μL of the digestion-ligation product. Thermocycler conditions were 72°C for 2 min; 20 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 2 min; 72°C for 2 min; and 60°C for 30 min. Each 10-μL selective PCR reaction consisted of 7.5 μL of AFLP core mix (Applied Biosystems), 0.25 μM MseI selective primer, 0.05 μM dye-labeled EcoRI selective primer, and 1.5 μL of the preselective PCR reaction. Selective primer combinations were EcoACT/MseCTC, EcoAGC/MseCTC, EcoACC/MseCTC, and EcoACC/MseCAT

Table 1

Striga hermonthica Collection Sites in Ethiopia

Sample name	Locality and elevation (m)	Sorghum cultivar sampled
Sorghum1	10°03′N, 38°11′E, 1703	Land race
Sorghum2	12°59′N, 37°46′E, 1860	Land race
Sorghum3	13°17′N, 37°51′E, 1449	Land race
Millet4	13°32′N, 38°07′E, 1489	Land race
Tef5	13°32′N, 38°07′E, 1496	Improved variety
Tef6	14°12′N, 39°15′E, 1884	Land race
Sorghum7	13°05′N, 39°44′E, 1910	Meko, an improved variety
Maize8	12°11′N, 39°44′E, 2194	Farmer's variety
Sorghum9	10°17′N, 40°02′E, 1792	Land race
Sorghum10	08°44′N, 40°33′E, 1551	Land race
Maize11	09°08′N, 42°25′E, 1067	Farmer's variety
Sorghum12	09°08′N, 42°25′E, 1067	Gubiye, an improved variety

Note. Land races were farmer's domesticated indigenous sorghum, pearl millet, and tef varieties. Improved varieties were high-yielding varieties selected through breeding for certain areas. Farmer's varieties were nonindigenous maize cultivars but were left to hybridize naturally.
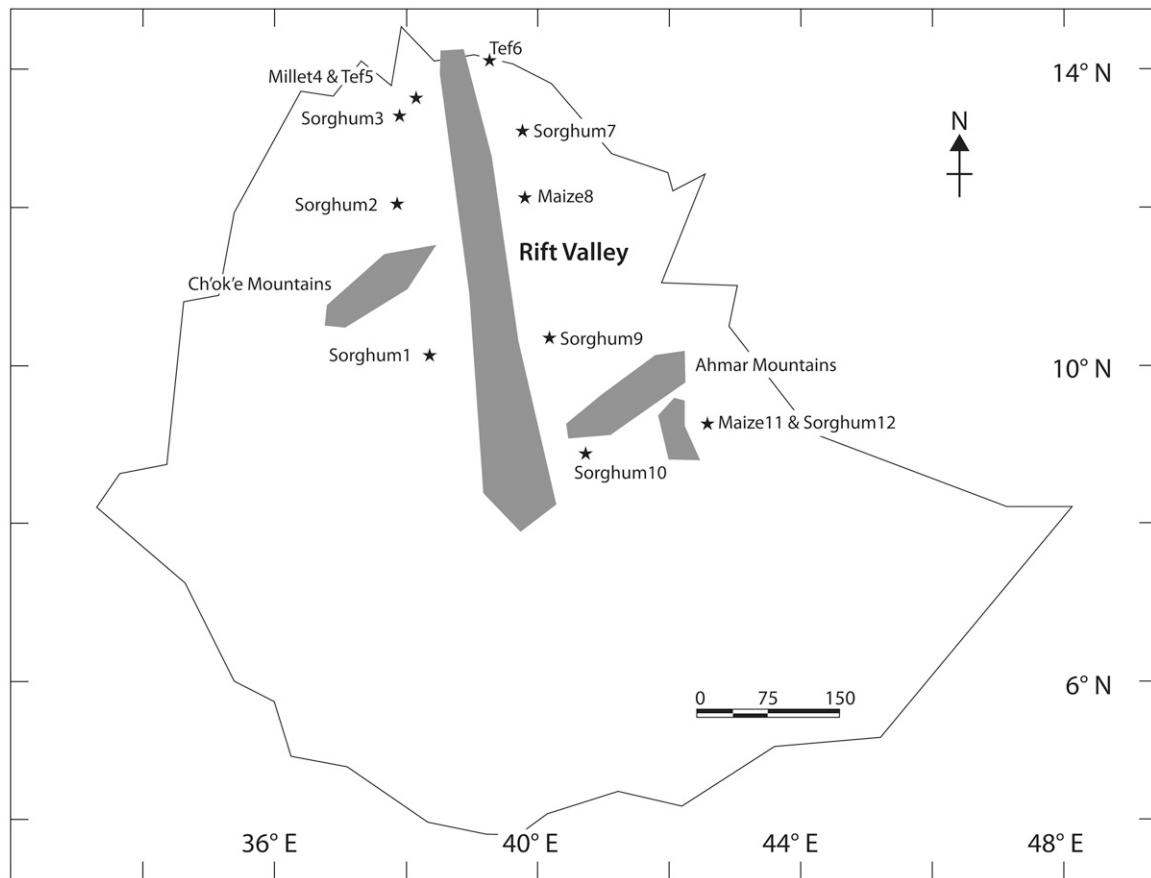
Fig. 1 Map showing locations of sampling sites (represented by stars) and hosts from which *Striga hermonthica* samples were collected in Ethiopia. Samples 4 and 5 were separated by 8 km, and samples 11 and 12 were collected from one site. Shaded areas represent potential geographic barriers.

(Gethi et al. 2005). Thermocycler conditions were 94°C for 2 min; 10 cycles of 94°C for 20 s, 66°C for 30 s with a 1°C decrease each cycle, and 72°C for 2 min; 20 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 2 min; and 60°C for 30 min.

Fragments were visualized on a Beckman Coulter CEQ8000 Genetic Analysis System. Analysis of resulting fragments was conducted with the Beckman Coulter CEQ software, where the presence of a fragment of a particular size was denoted by 1, and the absence of the fragment was denoted by 0. Three samples (one sorghum sample, one tef sample, and one maize sample) were replicated with each primer combination to assess repeatability. Fragments that had a mismatch in scores in any of the replicated samples were dropped from subsequent analyses. All designations by the software were manually checked by a single individual.

Statistical Analyses

Levels of genetic diversity within each population were measured by calculating expected heterozygosity (assuming no inbreeding) and percentage of polymorphic loci, based on a Bayesian method with uniform prior distribution of allele frequencies (Zhivotovsky 1999). Genetic differences between populations were measured by F_{ST} (Wright 1978). These values can range between 0 (no differentiation) and 1 (complete differentiation). Significance of the F_{ST} values was assessed by permutation tests (1000 replicates). Nei's genetic distance (after Lynch and Milligan 1994) was also computed, and these genetic distances were used to construct a neighbor-joining tree to evaluate which populations were most genetically similar. Bootstrapping (based on 1000 replicates) was conducted to determine the statistical support for each group present in the tree. All these genetic measures were calculated using the software AFLP-SURV (Vekemans 2002). The tree was constructed using the software PHYLIP (Felsenstein 1993).

To test for the role of host specificity in determining population structure, an analysis of molecular variance (AMOVA) was conducted to determine the proportion of variance attributable to differences in host species, with significance based on 999 permutations. To test the role of geography in determining structure, a Mantel test was performed to determine if there was a significant correlation between geographic and genetic distances. Significance was determined based on 999 permutations. An AMOVA (with 999 permutations) was conducted to see if a significant proportion of genetic variance could be apportioned between populations on

the east and the west sides of the Rift Valley. We also calculated Φ_{ST} values, a genetic differentiation measure similar to F_{ST} (Peakall et al. 1995), during the AMOVA analyses. The software GenAlEx was used for the Mantel and AMOVA tests (Peakall and Smouse 2006). An additional test on the role of geography was also performed using the software BARRIER (Manni and Guérard 2004). This is a spatial autocorrelation approach that uses Monmonier's maximum difference algorithm (Monmonier 1973) to identify genetic barriers in a landscape, specifically those locations where genetic differences are largest.

Results

A sufficient amount of genetic variation was detected using the described AFLP primer combinations (fig. 2). The total number of bands detected across all primer combinations after correcting for repeatability was 385 fragments. The average percentage of polymorphic loci was 60.9%, ranging from 53.2% to 76.4% (table 2). Average expected heterozygosity was 0.204, ranging from 0.168 to 0.279 (table 2).

Genetic differentiation between populations was relatively high, and all populations were significantly different from each other ($P < 0.05$; table 3). F_{ST} values ranged from 0.032 (Sorghum10 vs. Maize11) to 0.293 (Sorghum9 vs. Maize11). The average F_{ST} was 0.146.

Genetic Diversity due to Geography

Geography appeared to play a significant role in shaping the genetic diversity of *S. hermonthica*. The neighbor-joining tree showed three distinct groupings with high statistical support that corresponds to geographic location (fig. 3). The first group included Sorghum2, Sorghum3, and Sorghum4, lying in the northwestern portion of the study area to the west of the Rift Valley. The second group consisted of Tef5, Tef6, Sorghum7, Maize8, and Sorghum9, all to the east of the Rift Valley, with the exception of Tef5, which was collected 8 km north of Sorghum4. The third group included Sorghum1, Sorghum10, Maize11, and Sorghum12, all in the southern portion of the study area. Samples 10–12 were collected in highlands in the Ahmar Mountain Range to the east of the Rift Valley.

The Rift Valley appeared to be a significant barrier to *S. hermonthica* dispersal. The AMOVA results suggest that a significant proportion of the variance can be attributed to the Rift Valley, with the rest of the genetic variance being partitioned among populations on either side of the barrier and within populations (table 4). Spatial autocorrelation also indicates that there is a significant genetic barrier in the southern portion of the study area that separates populations Sorghum1, Sorghum10, Maize11, and Sorghum12 from the rest of the populations (fig. 4), corroborating one of the three groups in the neighbor-joining tree.

There appears to be a small isolation-by-distance effect. Genetic distance slightly increased with geographic distance.
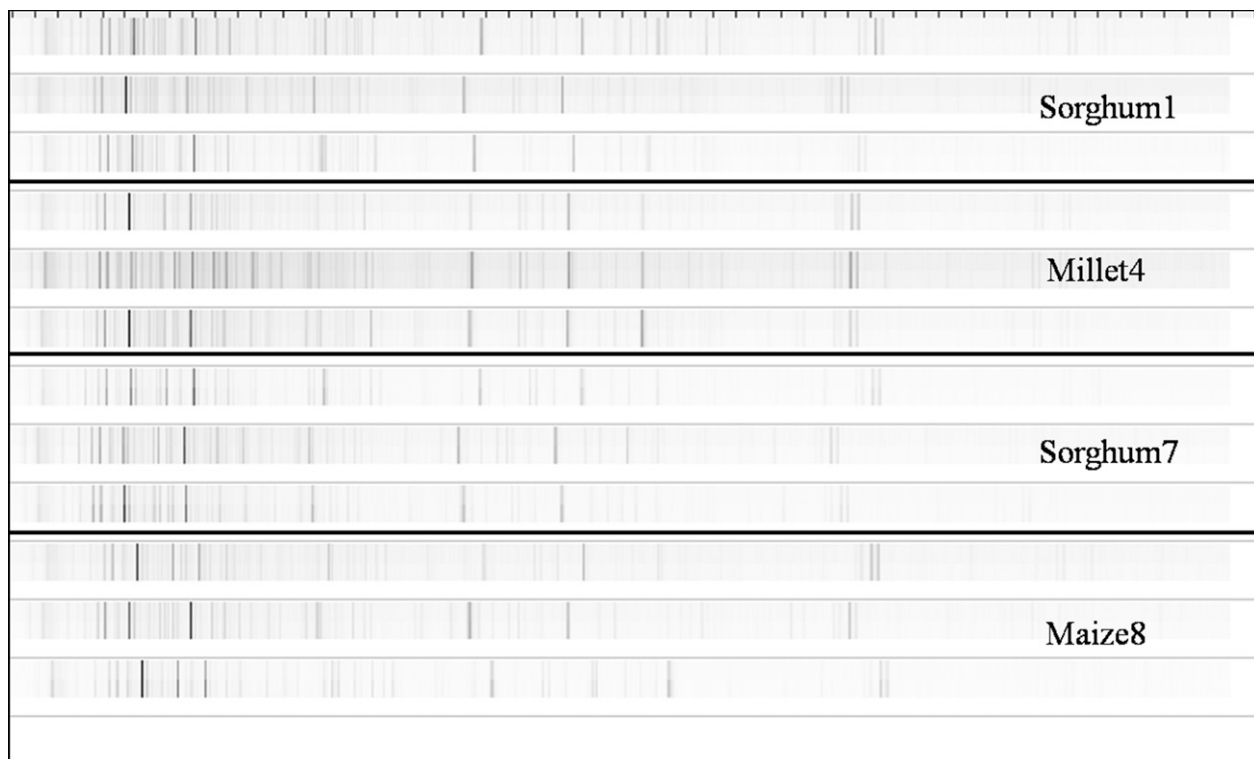

Fig. 2   Sample AFLP gel image. Three individuals from four populations (Sorghum1, Millet4, Sorghum7, Maize8) are represented. Image is a pseudogel created from raw capillary electrophoresis data using the software GelQuest (SequentiX Digital DNA Processing).

Table 2

Genetic Diversity of Each *Striga hermonthica* Population

Population	Sample size (n)	% polymorphic loci	Expected heterozygosity (SE)
Sorghum1	10	53.8	.168 (.009)
Sorghum2	8	76.4	.279 (.009)
Sorghum3	9	67.3	.233 (.009)
Sorghum7	8	55.6	.172 (.009)
Sorghum9	10	64.7	.200 (.009)
Sorghum10	9	53.2	.175 (.009)
Sorghum12	10	59.5	.194 (.009)
Millet4	8	66.5	.254 (.010)
Tef5	9	60.3	.200 (.009)
Tef6	9	59.7	.196 (.009)
Maize8	8	59.7	.204 (.010)
Maize11	9	53.8	.177 (.010)

Note. Percentage of polymorphic loci calculated based on analyzed fragments across all primer combinations (EcoACT/MseCTC: 102 fragments; EcoAGC/MseCTC: 79 fragments; EcoACC/MseCTC: 97 fragments; EcoACC/MseCAT: 107 fragments).

There was a weak but significant correlation between genetic and geographic distance ($R^2 = 0.025$, $P = 0.001$). In general, those populations that were farthest from each other geographically were also the most genetically distinct.

Genetic Diversity due to Host Specificity

A correlation between host specificity and genetic differentiation was not detected. The groupings on the neighbor-joining tree did not correspond to hosts (fig. 3). The results of the AMOVA suggested that host specificity was not a significant factor in explaining genetic differences between populations (table 4). The variance that could be attributed to host differences was 0%, while differences among populations contributed 27% and among individuals contributed 73%.

Discussion

The results from this study show that the sampled *Striga hermonthica* populations were characterized by high levels of genetic diversity, as indicated by the high level of polymorphism and moderate levels of heterozygosity. These values are comparable to the values obtained using allozymes for *S. hermonthica* parasitizing different hosts (Bharathalakshmi et al. 1990; Kuiper 1996; Olivier et al. 1996, 1998). Our results are also consistent with the genetic diversity of plants that share a similar life-history and ecology with *S. hermonthica*. Hamrick et al. (1979) and Loveless and Hamrick (1984) indicate that the heterozygosity for annuals is 0.116; for dicot species, 0.113; for outcrossed species, 0.185; and for weedy species, 0116.

The levels of genetic differentiation observed between the sampled populations span a broad range. According to the standards presented by Wright (1978), populations with F_{ST} values ranging from 0.15 to 0.25 are highly differentiated, and populations with values ranging from 0.05 to 0.15 are moderately differentiated. Genetic differentiation in our samples was relatively high, and all populations were significantly different from each other. Thirty-two of the 66 (48%) population comparisons were highly differentiated, and the average F_{ST} between all populations represents a high level of genetic differentiation. Lower genetic distance values (range = 0.007−0.025, mean = 0.015), using similar AFLP techniques, were obtained for 24 populations of *S. hermonthica* in Kenya (Gethi et al.

Table 3

Genetic Differentiation between the Populations, as Measured by F_{ST}

	Sorghum1	Sorghum2	Sorghum3	Millet4	Tef5	Tef6	Sorghum7	Maize8	Sorghum9	Sorghum10	Maize11
Sorghum1											
Sorghum2	.188										
Sorghum3	.241	.039									
Millet4	.144	.041	.062								
Tef5	.147	.121	.151	.041							
Tef6	.185	.138	.185	.077	.044						
Sorghum7	.270	.192	.244	.146	.123	.092					
Maize8	.202	.104	.146	.076	.062	.046	.063				
Sorghum9	.283	.186	.193	.139	.127	.113	.172	.100			
Sorghum10	.137	.154	.217	.133	.172	.167	.216	.167	.281		
Maize11	.180	.127	.189	.129	.188	.200	.240	.171	.293	.032	
Sorghum12	.161	.120	.188	.085	.107	.087	.118	.084	.205	.088	.091

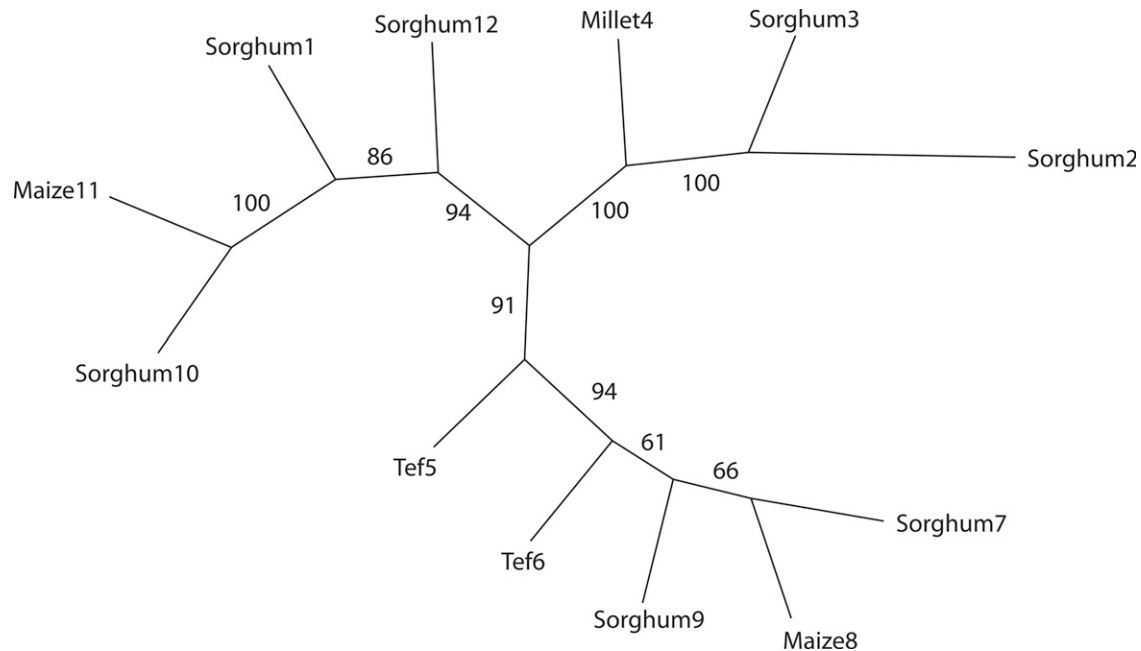Note. All values are significant at $P < 0.05$.

Fig. 3 Neighbor-joining tree based on Nei's genetic distance (after Lynch and Milligan 1994). Numbers on the tree represent bootstrap percentages, based on 1000 replicates. Labels correspond to populations in fig. 1.

2005). Those authors attributed the homogeneity of the Kenyan populations of *S. hermonthica* to recent colonization (a founder event) from the Lake Victoria basin east into Kenya and to its allogamous breeding system. The lack of evidence for genetic differentiation in the Kenyan populations could also be due to the small area sampled, which covered only 0.5° latitude and <1° longitude, in contrast to our study, which extends over a wider and more variable geographical area covering ~5° latitude and longitude.

Genetic Diversity due to Geography

Geography appears to play the greatest role in determining genetic differences between *S. hermonthica* populations, with the most substantial genetic barrier being in the southern portion of the study area. The southern barrier corresponds to the populations collected on the Ahmar Mountains. These

mountains have an elevation of up to 2134 m and are one of the many mountain islands resulting from the topography in Ethiopia. The populations collected from the Ahmar Mountains also have the lowest genetic diversity, indicating that these populations may be isolated on the highlands and may experience a loss of diversity due to genetic drift. Sorghum1 also grouped with samples 10–12, despite the presence of the Rift Valley between the groups and the geographic distance. Sorghum1 also had lower genetic diversity compared to the other groups, perhaps due to its isolation between the Rift Valley and the Ch'ok'e Mountains with a peak of 2470 m. The genetic similarity between Sorghum1 and samples 10–12 may be due to the dispersal route of *S. hermonthica* in the southern region, with Sorghum1 being the potential source for samples 10–12.

The Rift Valley also appears to be a genetic barrier for *S. hermonthica*, resulting in genetic differences between popula-

Table 4

Results of AMOVA Analyses

	Variance	% variation	Φ_{ST}[a]	Probability[b]
Among Rift Valley groups[c]	2.21	5	.05	.001
Among populations within Rift Valley groups	10.08	23	.24	.001
Among host groups[d]	0.00	0	0.00	.993
Among populations within host groups	12.19	27	.27	.001
Within populations	32.46	73	.28	.001

Note.   AMOVA = analysis of molecular variance.

[a] Significant Φ_{ST} values ($P < 0.05$) are underlined.

[b] Probability values are based on 999 permutations.

[c] Rift Valley groups consist of two groups: populations to the east of the valley and populations to the west.

[d] Host groups consist of four groups: sorghum, tef, maize, and millet.

tions on the east and west sides of the valley. Differentiation from this geographic barrier is probably a recent phenomenon and reflects the spread of *S. hermonthica* in recent evolutionary time. Although the proportion of genetic variation that could be attributed to the presence of the Rift Valley was significant, it was relatively low (5%), indicating a recent genetic divergence and probably reflecting the current spread of *S. hermonthica*. In contrast, the giant lobelia (*Lobelia giberroa*) had 58% of the genetic variance attributable to geography, suggesting that the populations diverged long ago and likely survived several glacial episodes (Kebede et al. 2007). One exception to the differentiation between east and west is the grouping of Tef5 with the east side of the Rift Valley, despite its location to the west of the Rift Valley and its close proximity to Millet4. This also may reflect the colonization history and recent divergence of the populations. Colonization may have occurred from the east side of the Rift Valley to the west side, with sufficient time not having passed for complete divergence. The series of highlands in this region may maintain some level of connectivity, allowing for higher levels of dispersal.

There also appears to be a slight isolation-by-distance effect. This would indicate that the most likely mode of dispersal is a stepping-stone model, with those populations that are geographically proximate providing the source for colonizers. However, the isolation-by-distance effect is not as strong as the effect of geographic barriers to dispersal. In contrast, a strong correlation between geographic and genetic distance ($R^2 = 0.61$) was observed by Botanga et al. (2002) for *Striga asiatica*, an autogamous species. In the absence of significant geographic barriers, geographic distance alone may be an even stronger determinant in population structure.

Genetic Diversity due to Host Specificity

In this study, host specificity does not appear to be the primary factor shaping the population structure of *S. hermonthica*. This is consistent with the results obtained for *S. hermonthica* by Bharathalakshmi et al. (1990). Other studies have also demonstrated that geography plays a greater role than host specialization in determining genetic differences between *Striga* populations (Bharathalakshmi et al. 1990; Musselman et al. 1991; Koyama 2000). Previous studies that were taken as strong evidence of host specificity in *S. hermonthica* may in fact be attributed to geographical distance. Among these was the study conducted in Sudan by Musselman and Hepper (1986). They concluded that *S. hermonthica* has two host-specific strains, one for sorghum and another for pearl millet. This is particularly true in areas where only sorghum or pearl millet is used as a food crop, either because of limitations imposed by climatic conditions or because of humans' food preferences. For example, in Sudan, sorghum is most commonly grown in the south, but pearl millet is grown in the north, where it is too dry for sorghum. In these regions, *S. hermonthica* populations developed host specificity to either sorghum or millet. However, in areas where both sorghum and pearl millet were grown, host specificity was not observed (Musselman and Hepper 1986), providing potential evidence for a weak genetic basis for host specificity.

The apparent lack of genetic differentiation based on host specificity may suggest that specialization of *S. hermonthica* to its host may be a recent phenomenon, with insufficient time for genetic differences to arise. This is consistent with the observation that when a sorghum field infested with *S. hermonthica* is replaced by millet, the new crop will be infested by *Striga* after a few years; this is dependent on the intensity with which a particular crop is grown in the absence of others in a given area (Olivier et al. 1998).

Origins of host species may also play a role in genetic differences between populations. Sorghum is native to Ethiopia, and sorghum *S. hermonthica* populations are characterized by fairly high genetic differentiation. *Striga hermonthica* populations associated with historically important and widely grown host crops in Africa, such as sorghum and pearl millet, may have coexisted with their hosts for a longer period than populations associated with unconventional hosts (e.g., finger millet, tef) or with introduced hosts such as maize. To our knowledge, tef and finger millet rarely were grown for food and were not conventional hosts for *S. hermonthica* in Africa; our study is among the first for *S. hermonthica* parasitizing finger millet and tef in Africa. Consistent with this low host specificity in tef and finger millet, the parasite does not seem to seriously damage its hosts in Ethiopia. Furthermore, *S. hermonthica* plants on these two hosts were much smaller and less branched compared to those collected from sorghum fields.

Stronger evidence of host specificity has been observed in autogamous *Striga* species such as *Striga gesnerioides*, where each host-specific strain is adapted to a narrow host range. Mohamed et al. (2001) demonstrated that these host-specific strains have somewhat unique morphology. AFLP markers confirmed genetic differences based on host specificity in populations of *S. gesnerioides* parasitic on *Indigofera hirsuta* in central Florida and populations parasitic on *I. hirsuta* and cowpea from West Africa. The Florida strain and the West African strain parasitic on indigo were more closely related to one another compared to the Florida strain and the West African strain parasitic on cowpea. Race formation in cowpea *S. gesnerioides* has been shown to be largely due to host-driven selection (Botanga and Timko 2005). This high level of genetic differentiation coupled with the species' ability to parasitize different hosts made *S. gesnerioides* the most widely distributed species in the genus, extending its range even to arid habitats in North and East Africa and Arabia, where it is parasitic on *Euphorbia abyssinica*.

Although the results suggest that host specificity is not as significant as geography in shaping *S. hermonthica* population structure, additional studies need to be conducted to confirm the lack of correlation regarding host specificity. Experimental studies in the laboratory involving infection of host species with *S. hermonthica* from different geographic locations are necessary in order to conclusively assess the role of host specialization in shaping genetic diversity. Additionally, in our study, the host species do not necessarily represent the same cultivar, and this could potentially confound the results.

Management Implications

The high genetic diversity of *S. hermonthica* presents a challenge for the development of resistance in its crop
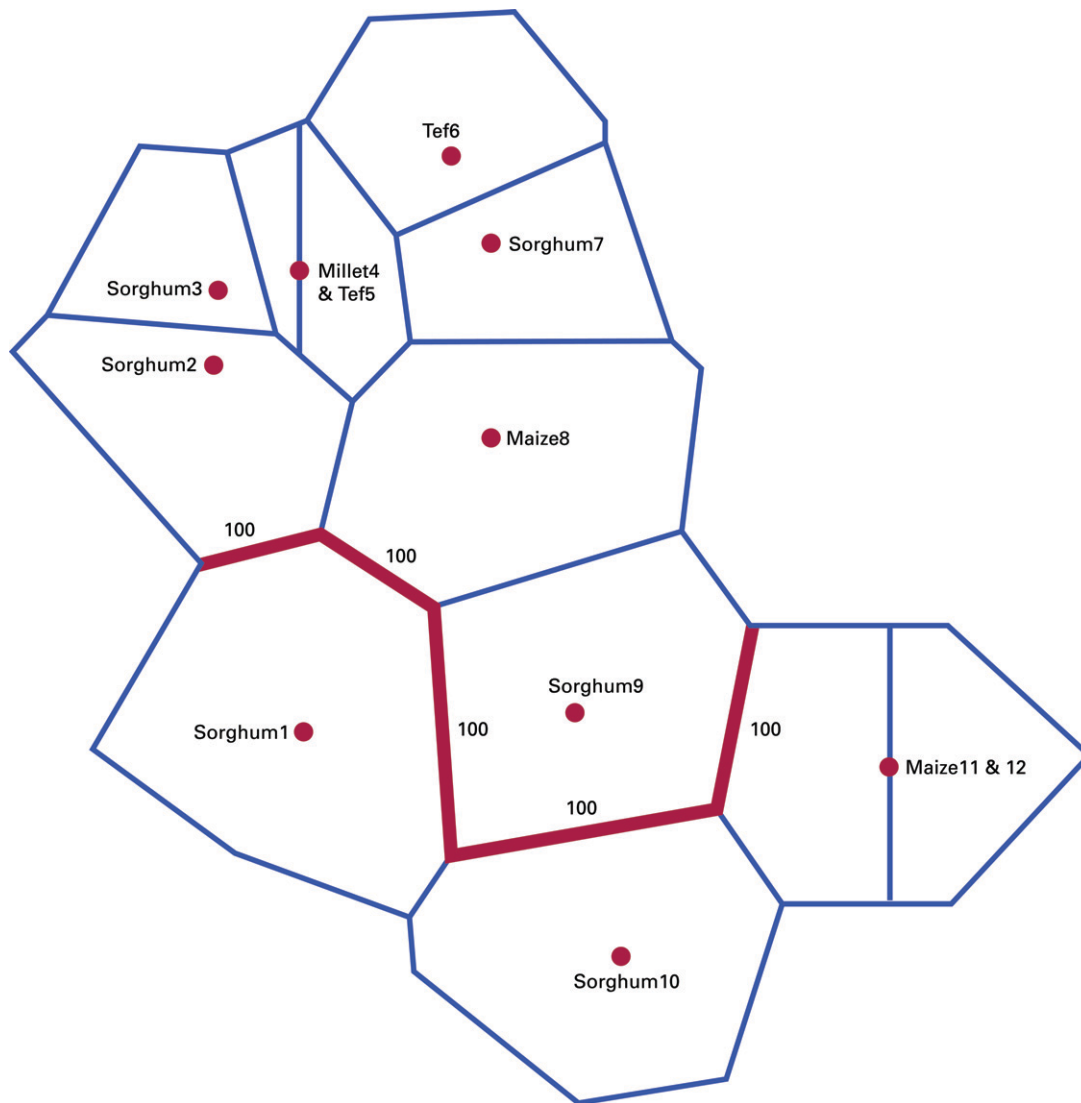
Fig. 4 Results of spatial autocorrelation analysis showing location of sampling sites (labels correspond to fig. 1) and the location of a potential genetic barrier (represented by red line). Numbers along the line represent bootstrap percentages, based on 1000 replicates.

hosts. Our results show that differences among individuals of *S. hermonthica* within the same population contributed to 73% ($P = 0.001$) of the genetic differences. This high level of genetic variability is consistent with obligate outcrosser species (Safa et al. 1984; Bharathalakshmi et al. 1990). The high genetic variability within populations of *S. hermonthica* may make it difficult, if not impossible, to produce reliable resistant varieties. In addition, a single *Striga* plant produces up to half a million seeds that add to the seed bank from previous years. Combined with the fact that seed of *Striga* can live up to 20 years in the soil, plant breeders have a great challenge in developing resistant varieties. These resistant varieties will soon be challenged by the diverse seed bank of *S. hermonthica*. Soils in Ethiopia were highly contaminated with *Striga* seed, and this may preclude cereal cultivation in some areas. The broader genetic background of *S. hermonthica* may enable the species to parasitize a number of cereal crops

under different climatic conditions. *Striga hermonthica* and *Striga aspera* were among the few species proven to hybridize and produce viable seeds (Aigbokhan et al. 2000). This genetic exchange with nonweedy *Striga* species could provide a gene reservoir via hybridization.

Farmers in Ethiopia were instructed to pull and burn *Striga* plants to prevent seed set. However, in practice, the parasites were pulled after seed set and dumped by the roadside. Considering the topography of Ethiopia (i.e., lowlands and highlands), this practice of plant disposal is very harmful and results in spreading *Striga* to new and faraway places through runoff. A second source for the spread of *Striga* seed is the contamination of cereal grains with parasitic plant seed because cereals are threshed on the ground inside contaminated fields using oxen and donkeys. When grains were transported elsewhere, they carried *Striga* seeds with them. The oxen and donkeys provide a secondary source of seed dissemination. These

means of dispersal result in long-distance seed spread of *S. hermonthica* throughout much of Africa and across the Red Sea to neighboring Arabia. In these areas, *S. hermonthica* was able to develop genetically structured populations based on geography.

The most effective method to combat *S. hermonthica* is containment and then eradication of the parasite. There appear to be geographic barriers to *S. hermonthica* migration, providing natural containment areas. Proper disposal of *Striga* pulled by farmers in Ethiopia will also help in containing *Striga* and preventing its spread to new areas. Our study demonstrates the need for a more detailed analysis of genetic diversity in *S. hermonthica* at the level of local populations as well as on a large scale in Africa, in order to understand the parasite well enough for effective management.

Acknowledgments

Literature Cited

Aigbokhan EI, DK Berner, LJ Musselman, HD Mignouna 2000 Evaluation of variability in *Striga aspera*, *Striga hermonthica* and their hybrids using morphological characters and random amplified polymorphic DNA markers. Weed Res 40:375–386.

Ali RA, A El-Hussein, K Mohamed, A Babiker 2009 Specificity and genetic relatedness among *Striga hermonthica* strains in Sudan. Life Sci Int J 3:1159–1166.

Belay G, A Mori 2006 Intraspecific phylogeographic mitochondrial DNA (D-loop) variation of Gelada baboon, *Theropithecus gelada*, in Ethiopia. Biochem Syst Ecol 34:554–561.

Bensch S, M Akesson 2005 Ten years of AFLP in ecology and evolution: why so few animals? Mol Ecol 14:2899–2914.

Bharathalakshmi, CR Werth, LJ Musselman 1990 A study of genetic diversity among host-specific populations of the witchweed *Striga hermonthica* (Scrophulariaceae) in Africa. Plant Syst Evol 172:1–12.

Bonin A, D Ehrich, S Manel 2007 Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. Mol Ecol 16:3737–3758.

Botanga CJ, JG Kling, DK Berner, MP Timko 2002 Genetic variability of *Striga asiatica* (L.) Kuntz based on AFLP analysis and host-parasite interaction. Euphytica 128:375–388.

Botanga CJ, MP Timko 2005 Genetic structure and analysis of host and nonhost interactions of *Striga gesnerioides* (witchweed) from Central Florida. Phytopathology 95:1166–1173.

Cullings KW 1992 Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. Mol Ecol 1:233–240.

Dawoud D, J Sauerborn 1994 Impact of drought stress and temperature on *Striga hermonthica* and *Alectra vogelii* at early growth stages. Exp Agric 30:249–257.

Doyle JJ, JL Doyle 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15.

Emechebe AM, J Ellis-Jones, S Schulz, D Chikove, B Douthwaite, I Kureh, G Tarawali, MA Hussaini, P Kormawa, A Sanni 2004 Farmers' perception of the *Striga* problem and its control in northern Nigeria. Exp Agric 40:215–232.

Felsenstein J 1993 PHYLIP (Phylogeny Inference Package), version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Gethi JG, ME Smith, SE Mitchell, S Kresovich 2005 Genetic diversity of *Striga hermonthica* and *S. asiatica* populations in Kenya. Weed Res 45:64–73.

Hamrick JL, YB Linhart, JB Mitton 1979 Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Annu Rev Ecol Syst 10:173–200.

Kebede M, D Ehrich, P Taberlet, S Nemomissa, C Brochmann 2007 Phylogeography and conservation genetics of a giant lobelia (*Lobelia giberroa*) in Ethiopian and tropical East African mountains. Mol Ecol 16:1233–1243.

Kingdon J 1990 Island Africa: the evolution of Africa's rare animals and plants. Collins, London.

Koyama ML 2000 Genetic variability of *Striga hermonthica* and effect of resistant cultivars on striga population dynamics. Pages 247–260 *in* BIG Haussmann, DE Hess, ML Koyama, L Grivet, HFW Rattunde, HH Geiger, eds. Breeding for *Striga* resistance in cereals. Proceedings of a workshop at IITA, Ibadan, Nigeria, August 18–20, 1999. Margraf, Weikersheim, Germany.

Kuiper E, PLM Koevoets, JAC Verkleij, AH Pieterse 1996 Genetic variability of *Striga aspera* and *Striga hermonthica*: are they really two distinct species? Pages 124–132 *in* MT Moreno, JI Cubero, D Berner, D Joel, LJ Musselman, C Parker, eds. Advances in parasitic plant research. 6th International Congress on Parasitic Weeds, Cordoba, Spain.

Loveless MD, JL Hamrick 1984 Ecological determinants of genetic structure in plant populations. Annu Rev Ecol Syst 15:65–95.

Lynch M, BG Milligan 1994 Analysis of population genetic structure with RAPD markers. Mol Ecol 3:91–99.

Manni F, E Guérard 2004 Barrier, version 2.2. User manual. Distributed by the authors. Population genetics team, Museum of Mankind, Paris.

Mohamed KI, JF Bolin, LJ Musselman, AT Peterson 2007 Genetic diversity of *Striga* and implications for control and modeling future distributions. Pages 71–84 *in* G Ejeta, J Gressel, eds. Integrating new technologies for *Striga* control: towards ending the witch-hunt. World, Singapore.

Mohamed KI, LJ Musselman, C Riches 2001 *Striga* (Scrophulariaceae) in Africa. Ann Mo Bot Gard 88:60–103.

Mohamed KI, M Papeş, R Williams, BW Benz, AT Peterson 2006 Global invasive potential of ten parasitic witchweeds and related Orobanchaceae. Ambio 35:1–10.

Monmonier M 1973 Maximum-difference barriers: an alternative numerical regionalization method. Geogr Anal 3:245–261.

Musselman LJ 1987 Taxonomy of witchweeds. Pages 3–12 *in* LJ Musselman, ed. Parasitic weeds in agriculture: *Striga*. Vol 1. CRC, Boca Raton, FL.

Musselman LJ, Bharathalakshmi, SB Safa, DA Knepper, KI Mohamed, CL White 1991 Recent research on the biology of *Striga asiatica*, *S. gesnerioides* and *S. hermonthica*. Pages 31–41 *in* SK Kim, ed. Combating *Striga* in Africa. Proceedings, International Workshops organized by International Institute of Tropical Agriculture (IITA), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and International Development Research Centre (IDRC), August 22–24, 1988. IITA, Ibadan, Nigeria.

Musselman LJ, FN Hepper 1986 The witchweeds (*Striga*, Scrophulariaceae) of the Sudan Republic. Kew Bull 41:205–221.

Olivier A, J-C Glaszmann, C Lanaud, GD Leroux 1998 Population structure, genetic diversity and host specificity of the parasitic weed *Striga hermonthica* (Scrophulariaceae) in Sahel. Plant Syst Evol 209: 33–45.

Olivier A, J-C Glaszmann, C Lanaud, G Salle, GD Leroux 1996 An insight into the population structure and genetic diversity of *Striga hermonthica* in West Africa. Pages 114–121 *in* MT Moreno, JI Cubero, eds. Advances in parasitic plant research. Cordoba, Spain.

Patterson DT, RL Musser, EP Flint, RE Eplee 1982 Temperature responses and potential for spread of witchweed (*S. lutea/S. asiatica*) in the United States. Weed Sci 30:87–93.

Peakall R, PE Smouse 2006 GENALEX 6: genetic analysis in Excel: population genetic software for teaching and research. Mol Ecol Notes 6:288–295.

Peakall R, PE Smouse, DR Huff 1995 Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss *Buchloe dactyloides*. Mol Ecol 4:135–147.

Safa SB, BM Jones, LJ Musselman 1984 Mechanisms favoring out-breeding in *Striga hermonthica* (Scrophulariaceae). New Phytol 96: 299–305.

Silvestrini M, MG Junqueira, AC Favarin, O Guerreiro-Filho, MP Maluf, MB Silvarolla, CA Colombo 2007 Genetic diversity and structure of Ethiopian, Yemen and Brazilian *Coffea arabica* L. accessions using microsatellites markers. Genet Resour Crop Evol 54:1367–1379.

Vekemans X 2002 AFLP-SURV, version 1.0. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

Vos P, R Hogers, M Bleeker, M Reijans, T van de Lee, M Hornes, A Frijters, et al. 1995 AFLP: new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414.

Wright S 1978 Evolution and the genetics of populations: variability within and among natural populations. University of Chicago Press, Chicago.

Zhivotovsky LA 1999 Estimating population structure in diploids with multilocus dominant DNA markers. Mol Ecol 8:907–913.