Dynamics of Gene Introgression in the African Malaria Vector
Anopheles gambiae

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ABSTRACT

Anopheles gambiae is a major malaria vector in Africa and a popular model species for a variety of ecological, evolutionary, and genetic studies on vector control. Genetic manipulation of mosquito vectorial capacity is a promising new weapon for the control of malaria. However, the release of exotic transgenic mosquitoes will bring in novel alleles in addition to the parasite-inhibiting genes, which may have unknown effects on the local population. Therefore, it is necessary to develop methodologies that can be used to evaluate the spread rate of introduced genes in *A. gambiae*. In this study, the effects and dynamics of genetic introgression between two geographically distinct *A. gambiae* populations from western Kenya (Mbita) and eastern Tanzania (Ifakara) were investigated with amplified fragment length polymorphisms (AFLPs) and microsatellite markers. Microsatellites and polymorphic cDNA markers revealed a large genetic differentiation between the two populations (average $F_{ST} = 0.093$, $P < 0.001$). When the two strains were crossed in random mating between the two populations, significant differences in the rate of genetic introgression were found in the mixed populations. Allele frequencies of 18 AFLP markers (64.3%) for Mbita and of 26 markers (92.9%) for Ifakara varied significantly from F_5 to F_{20}. This study provides basic information on how a mosquito release program would alter the genetic makeup of natural populations, which is critical for pilot field testing and ecological risk evaluation of transgenic mosquitoes.

MALARIA, the most important parasitic disease in the world, results in 1–2 million deaths annually worldwide and in >90% of the deaths that occur in sub-Saharan Africa (WORLD HEALTH ORGANIZATION 1998; BREMAN *et al.* 2004). The economic burden caused by malaria is enormous (GALLUP and SACHS 2001). As the Plasmodium parasites become increasingly resistant to antimalarial drugs, and as insecticide-resistant anopheline mosquitoes become increasingly prevalent (WHITE 2004), a strong need to develop new control measures exists. One promising and novel transmission-blocking approach is to use genetically modified mosquitoes that can effectively inhibit malaria parasite development (COLLINS *et al.* 2000; ALPHEY *et al.* 2002). Over the past several years, researchers have made remarkable progress in the development of mosquito germline transformation techniques (CATTERUCCIA *et al.* 2000) and in the identification of parasite-inhibiting molecules (BEERNSTEN *et al.* 2000; BLAIR *et al.* 2000; JACOBS-LORENA 2003; MOREIRA *et al.* 2004). However, little is known

about the spread of transgenes in targeted natural mosquito populations (BRAIG and YAN 2001).

Ideally, the released transgenic mosquitoes should have a genetic background similar to that of the targeted populations in nature. Releasing individuals with a different genetic background would bring novel alleles, in addition to the parasite-inhibiting genes, to the target population. This may change the genetic makeup and fitness of the target population and thus may have unintended consequences on the transmission of malaria or other pathogens vectored by the anopheline mosquitoes. However, no information yet exists on how introducing mosquitoes with a different genetic background would change the genetic makeup of a local population. The aim of this study was to determine the dynamics of allele frequency and gene introgression when two genetically distinct mosquito populations were mixed.

Population hybridization and subsequent gene introgression have been investigated in plants (ARIAS and RIESEBERG 1994; MAO *et al.* 1995; RIESEBERG *et al.* 1995, 1999; BRAR and KHUSH 1997). For example, high rates of population hybridization and gene introgression have been reported between the cultivated sunflower and its wild progenitor, *Helianthus annuus* (ARIAS and

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RIESEBERG 1994; RIESEBERG *et al.* 1995), but only 2.4% of the genome from the 10 rearranged linkages was transferred. Thus, chromosomal rearrangements appear to provide an effective mechanism for reducing or eliminating introgression in rearranged chromosomal segments. The rates of gene introgression vary among species of sunflowers. RIESEBERG *et al.* (1999) reported a low rate of introgression in four sympatric *H. petiolaris* populations, with average marker frequencies per population ranging from 0.006 to 0.026.

In African malaria mosquitoes, gene introgression may be occurring between sympatric *Anopheles gambiae* and *A. arabiensis* species, but introgression is not uniform across the genome (GARCIA *et al.* 1996). For example, X chromosome inversions do not introgress well due to selective elimination in hybrids and backcrosses. However, the consequence of hybridization on gene introgression within strains of the same species has not yet been studied.

This study investigated genetic introgression between two geographically distinct populations of *A. gambiae* mosquitoes. We used microsatellites and polymorphic cDNA markers to determine the degree of genetic differentiation and to test whether random or assortative mating occurred when populations are mixed. We then used amplified fragment length polymorphisms (AFLPs) to test dynamics of genetic introgression in different genome regions. This information aids understanding of the genetic structure of natural anopheline populations and helps develop strategies for field testing of transgenic mosquitoes in the future.

MATERIALS AND METHODS

Mosquito rearing and maintenance: Two *A. gambiae* strains (Mbita and Ifakara) were used in this study. The Mbita strain was from wild mosquitoes collected at Mbita Point (000 25′S, 340 13′E), western Kenya, and it has been maintained in the laboratory since 1999. The Ifakara strain was originally collected from Njage village, 70 km from Ifakara, southeast Tanzania, and it has been maintained under laboratory conditions since 1996. The two strains were maintained at Mbita Point Field Station, International Centre of Insect Physiology and Ecology, Kenya. The mosquito colonies were maintained at ambient tropical temperature (maximum average monthly temperature of 28.4° and minimum temperature of 15.0° during 1970–2000) and under artificial light conditions provided by fluorescent tubes. The detailed protocol for mosquito larvae and adult rearing is described in MENGE *et al.* (2005).

Experimental population: We mixed equal proportions of 1-day-old Ifakara and Mbita strains (100 Ifakara females, 100 Ifakara males, 100 Mbita females, and 100 Mbita males) in a 30 × 30 × 30-cm cage. The F_1 larvae were reared to adulthood under similar conditions. Subsequent generations from F_2 to F_{20} were obtained by mass mating, with a population size of ~1000 individuals.

For genetic introgression analysis, we set up two populations that mixed the Mbita and Ifakara strains. Briefly, the F_1 generation hybrids from the two strains were obtained by mixing a population (F_0) of 100 1-day-old Ifakara females, 100 Ifakara males, 100 Mbita females, and 100 Mbita males to-

TABLE 1

AFLP primer sequences and combinations used for *Anopheles gambiae* genome mapping and genetic introgression

*Eco*RI primers (5′–3′)
    L1: GACTGCGTACCAATTCACA
    L2: GACTGCGTACCAATTCAAC
    L3: GACTGCGTACCAATTCAAG
    L4: GACTGCGTACCAATTCACC
    L5: GACTGCGTACCAATTCACG
    L7: GACTGCGTACCAATTCAGC
    L8: GACTGCGTACCAATTCAGG

*Mse*I primers(5′–3′)
    B1: GATGAGTCCTGAGTAA CAC
    B2: GATGAGTCCTGAGTAA CAT
    A3: GATGAGTCCTGAGTAA CTC
    A4: GATGAGTCCTGAGTAA CTA
    A8: GATGAGTCCTGAGTAA CGA
    A9: GATGAGTCCTGAGTAA CGT
    A10: GATGAGTCCTGAGTAACGG
    A18: GATGAGTCCTGAGTAA TAG
    A19: GATGAGTCCTGAGTAATTC
    A21: GATGAGTCCTGAGTAATTT
    A27: GATGAGTCCTGAGTAATCC

Primer combinations used for genome mapping
    L1/A3, L1A8, L1/A9, L1/A10, L1/A18, L1/A21
    L2/A18
    L3/A8, L3/A19
    L4/B1
    L5/B2
    L7/A3, L7/A4, L7/A18, L7/A27
    L8/A4, L8/A18, L8/A27

gether in a single cage. The F_1 larvae were reared to adulthood under similar conditions. Subsequent generations, F_2–F_{20}, were obtained by allowing an equal number of males and females of the previous generation (100 each) to mate and start a subsequent generation. Two replicate experiments were set up following the same procedure.

Establishment of population for genetic mapping: To determine the genome position of the molecular markers, we set up an F_2 population using pairwise mating between an Mbita male and an Ifakara female and an F_1 intercross. A total of 123 F_2 individuals were used for linkage map construction.

Screening and genotyping of polymorphic AFLP markers: We extracted genomic DNA from individuals in the mapping population (two parents and 123 individuals of the F_2 generation) following the phenol/chloroform method (SEVERSON 1997). All individuals were subjected to genotyping with AFLP markers using the methods described by ZHONG *et al.* (2003). A total of 100 AFLP primer combinations were screened for polymorphism. The majority of the primer combinations (85 pairs) produced polymorphic fragments between the Mbita and Ifakara strains. We selected 18 pairs of AFLP primer combinations for segregation analysis of the F_2 intercross populations on the basis of reproducibility and the extent of polymorphism (Table 1). Only polymorphic fragments that could be scored unambiguously were used for linkage map construction. With the 18 pairs of AFLP primer combinations, we genotyped the Ifakara and Mbita parental populations and the F_1, F_5, F_{10}, F_{15}, and F_{20} populations for a total of 482 individuals. Only clearly amplified fragments were scored (168 AFLP markers).

Screening and genotyping of polymorphic microsatellite markers: To determine the degree of genetic differentiation between the Mbita and Ifakara populations, and to determine whether the two mosquito strains mate randomly when they are mixed in cages, we screened the two strains using 36 microsatellite markers (ZHENG *et al.* 1996). Microsatellite analysis was conducted using the methods described in NYANJOM *et al.* (2003). Ten markers (AGXH503, AGXH131, and AGXH678 on chromosome X; AG2R7, AG2H187, AG2H786, AG2H603, and AG2H117 on chromosome II; and AG3H158 and AG3H29C on chromosome III) were polymorphic in the mating population and thus suitable for mapping. We also screened 13 cDNA markers (DELLA TORRE *et al.* 1996) for polymorphism between the two strains, but only 1 marker (U50474) was found to be polymorphic. Using these 10 microsatellite loci and one cDNA locus as anchor markers, the AFLP linkage map developed in this study was integrated into the previously published genetic maps of *A. gambiae* (DELLA TORRE *et al.* 1996; ZHENG *et al.* 1996). To examine whether the mixed populations were mating randomly, we genotyped the F_1 and F_5 populations using five microsatellite markers for which the two strains exhibited the highest degree of genetic differentiation (AGXH131, AG2R7, AG2H117, AG2H187, and AG3H158).

Data analysis: Chi-square analysis was conducted on all markers to test whether they were segregating according to the expected Mendelian ratio of 3:1 for dominant AFLP markers and 1:2:1 for codominant microsatellite and cDNA markers. Using MapMaker version 3.0 (LINCOLN *et al.* 1992), a genetic linkage map based on AFLP, microsatellite, and cDNA markers was generated from the F_2 mapping population derived from the cross between Mbita and Ifakara strains. A LOD score of 5.0 was used as a threshold value for defining a linkage group. The map distance was calculated using Kosambi's mapping function (KOSAMBI 1944).

To determine whether random mating occurred in the mixed cage populations, the Hardy–Weinberg equilibrium (HWE) was tested for each microsatellite locus using the probability test implemented in the GENEPOP software (RAYMOND and ROUSSET 1995). Significance of deviation from HWE was adjusted for multiple comparisons, using the sequential Bonferroni procedure (RICE 1989). We used Wright's F_{ST} (WEIR and COCKERHAM 1984) to quantify genetic differentiation between the Mbita and Ifakara populations. This analysis of microsatellite genotype data was performed with FSTAT computer software (GOUDET 1995).

Because AFLP loci segregate as dominant markers, they were scored as presence (1) or absence (0) in each population. The frequencies of the null recessive allele and of the dominant allele were estimated using the methods of LYNCH and MILLIGAN (1994). Analysis of molecular variance (AMOVA), which partitions the genotypic variance into components attributable to different hierarchical levels, was implemented in the software program ARLEQUIN (version 2.000; SCHNEIDER *et al.* 2000). Two population structure models were examined. In the first model, the two parental populations were defined as one group and AMOVA partitioned the total variance into components due to differences between parental populations and differences between individuals within parental populations. In the second model, the five progeny populations (F_1, F_5, F_{10}, F_{15}, and F_{20}) were defined as a group to be compared with their parents, and thus AMOVA partitioned the total variance into components due to differences between parents and their progeny and differences between groups within populations. AMOVA was based on the pairwise squared Euclidian distances among the AFLP genotypes. The significance of the variance components at the different hierarchical levels was assessed with a permutation procedure (1000 permutations).

RESULTS

AFLP marker polymorphism in *A. gambiae* populations and linkage map: Eighteen AFLP primer combinations yielded 168 informative markers. Each primer combination generated an average of 9.3 informative markers. The L1A8 and L1A21 primer combinations produced the highest number of qualified polymorphic fragments (13 each), whereas the L8/A18 primer combination had the fewest polymorphic fragments (2). Among the 168 informative AFLP markers, 124 markers were assigned to three linkage groups (20 on chromosome X, 73 on chromosome II, and 31 on chromosome III). In addition, 10 microsatellite markers (3 on chromosome X, 5 on chromosome II, and 2 on chromosome III), and 1 cDNA marker (U50474) were mapped in relation to the AFLP markers (Figure 1). Linkage groups ranged in length from 53.5 to 76.0 cM. The total map consists of 194.7 cM, with a marker density of 1.44 markers/cM (194.7 cM for a total of 135 markers). However, only a partial linkage map consisting of 28 traceable AFLP markers, with notable change in allele frequency observed up to the F_{20} generation, is presented (Figure 1).

Genetic and HWE analyses: Mbita and Ifakara populations were significantly different, as revealed by the 14 codominant microsatellite markers ($F_{ST} = 0.093$, $P < 0.001$). Ten markers (AGX1D1, AGX H131, AG2R7, AG2H117, AG2H187, AG2H79, AG2H46, AG3H158, AG3H29C, and AG3H33C) exhibited a large genetic differentiation, with the F_{ST}-value ranging from 0.061 to 0.266 (Table 2). Five of these were thus used to test whether the mixed populations exhibited any assortative mating. A random-mating test was based on genotype data of 50 individuals from each population (a total of 300 individuals). Among the microsatellite markers used, the AGXH131 locus was in good agreement with HWE in all the tested populations (parents, F_1, and F_5), whereas the AG2H187 locus exhibited significant departures from the HWE in all populations (Table 3). Because not all markers consistently showed significant deviation from HWE in F_1 and F_5 populations (Table 3), there was no evidence to suggest that assortative mating had occurred in the mixed populations.

Genetic differentiation between the parental population and the F_1–F_{20} generations: AMOVA results indicated a strong differentiation between the Ifakara and Mbita populations. Partitioning the variation within and between populations using AMOVA showed that 10.0% of the genetic variability existed between the two populations ($F_{ST} = 0.0994$, $P < 0.001$; Table 4). A comparison of parents and progeny populations showed that 4.3% of the variation existed between the two groups ($P = 0.47$). Approximately 7.8% of the variation was partitioned among the progenies ($F_{ST} = 0.0125$; $P < 0.001$). These results suggest that parental strains affect introgression rates of AFLP alleles.
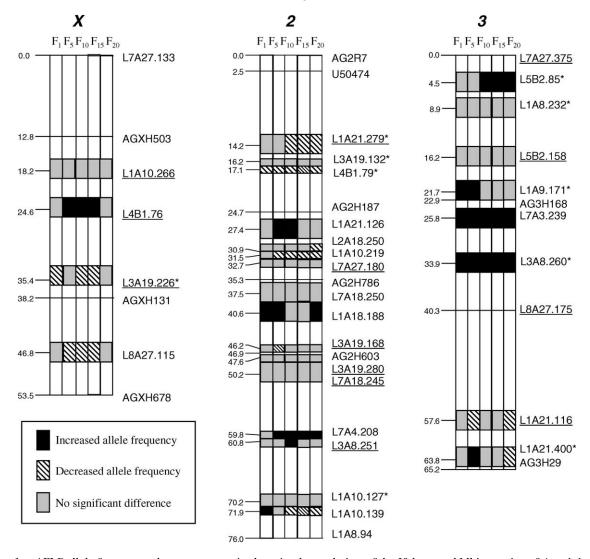
FIGURE 1.—AFLP allele frequency change patterns in the mixed population of the Ifakara and Mbita strains of *Anopheles gambiae.* Solid markers represent significantly higher allele frequency than that of the Mbita parent strain, and hatched markers are those with significantly lower frequencies at $P < 0.001$ after a sequential Bonferroni correction. Shaded markers show no significant change in allele frequencies from that of the Mbita parent strain. An asterisk (*) indicates markers with alleles specific to one parent strain. Microsatellite markers (names beginning with AG) are used as anchors for AFLP marker orientation. AFLP markers are designated by the *Eco*RI primer name, the *Mse*I primer name, and the molecular size of the fragment. For example, marker L7A27.133 represents the 133-bp fragment amplified by *Eco*RI primer L7 and *Mse*I primer A27, whereas L1A10.266 represents the 266-bp fragment amplified by *Eco*RI primer L1 and *Mse*I primer A10.

Pairwise genetic differentiation (F_{ST}) between the parents and progeny generations was highly significant (Table 5), with a maximum F_{ST} of 0.1889 (between Ifakara and the F_{15} generation) and a minimum F_{ST} of 0.0574 (between the F_{15} and F_{20} generations). It should be noted that the pairwise genetic difference between Ifakara and the progeny generations (mean $F_{ST} = 0.1584$) is bigger than the pairwise genetic difference between Mbita and the progeny generations (mean $F_{ST} = 0.0892$). The F_{15} generation showed the highest F_{ST} values (0.1889 for Ifakara and 0.1229 for Mbita). These results indicate that the Mbita strain contributed more alleles to the progeny of the mixing populations.

Allele frequency changes in the progeny of the mixing populations: Significant differences in allele frequency were found between the two parental populations and five progeny populations ($F_{ST} = 0.0924, P < 0.001$). Starting from the F_1 up to the F_{20} generations, 18 of the 28 markers were found to have significantly changed allele frequency (Figure 1). Frequencies of alleles specific to the Mbita strain were significantly changed from F_5 to F_{20} in 18 markers (64.3%), including 9 with increased allele frequency and 7 with decreased allele frequency. The allele frequency of 1 marker (L1A21.400) first increased from F_1 to F_5 and then decreased from F_{10} to F_{20}. Another marker (L1A10.139) had increased allele frequency at F_1 and then decreased from F_5 to F_{20}

TABLE 2

Number of microsatellite alleles (*n*) and F_{ST} values indicate genetic differentiation between the Ifakara and Mbita strains of *A. gambiae*

Marker	Chromosome location	*n* Ifakara	Mbita	F_{ST}	*P*
AGX1D1	X: 1D	4	5	0.081	<0.001
AGXH503	X: (4)	3	3	0.026	0.094
AGXH131	X: 3	3	3	0.266	<0.001
AG2R7	2L	2	3	0.146	<0.001
AG2H117	2L	3	3	0.061	<0.001
AG2H187	2R	2	2	0.129	<0.001
AG2H79	2R	7	5	0.122	<0.001
AG2H46	2L: 7A	7	10	0.106	<0.001
AG2H603	2L: 25	3	3	0.000	0.896
AG2H786	2R: 12	2	3	0.018	0.075
AG3H158	3R	4	4	0.148	<0.001
AG3H29C	3R: 29C	4	3	0.228	<0.001
AG3H33C	3R: 33C	2	9	0.045	<0.001

(Figure 1). However, frequencies of alleles specific to the Ifakara strain changed significantly from F_5 to F_{20} in 26 markers (92.9%); most of them showed decreased frequency. These results reveal that Mbita alleles showed higher genetic introgression and spread rate in the progeny of this mixing population in *A. gambiae*.

DISCUSSION

This study describes methods that can be used to evaluate the spread rate of introduced genes in a target population. We used 28 AFLP markers that exhibited a large difference in allele frequencies between two geographically distinct strains of *A. gambiae* to investigate genetic introgression dynamics when the mosquito populations were mixed. We found that alleles specific to the Mbita strain had a higher genetic introgression and spread rate than those specific to the Ifakara strain. This study provides basic information on how release of mosquitoes with a genetic background different from that of a targeted population may alter the genetic makeup of the targeted population. This is critical for ecological risk evaluation of future release of transgenic mosquitoes.

Microsatellite markers were used as anchor markers to produce an AFLP linkage map and to determine whether any assortative mating occurred in our experimental populations. Due to high frequencies of allele sharing between the Kenyan and Tanzanian *A. gambiae* populations, we could not use the microsatellite markers to track allele frequency changes in the mixed populations. Therefore, we screened AFLP markers to identify strain-specific molecular markers or traceable markers that exhibited highly contrasted frequencies between the two strains. AFLP analysis is a DNA fingerprinting technique that gives several hundred fragments in a single PCR reaction, and it is possible that some strain-specific fragments may be identified (Rieseberg *et al.* 1999). AFLP markers have been widely used in genetic diversity and genetic mapping studies and are useful for studying genetic introgression in wild populations (Maughan *et al.*1996; Zhu *et al.* 1998; Yan *et al.* 1999; Zhong *et al.* 2004). AFLP's dominant nature is a disadvantage because each marker is represented by only two alleles, one of them being unamplifiable (null), thereby decreasing their accuracy in allele frequency estimation in comparison with codominant markers (Lynch and Milligan 1994). However, considerable progress has been made in the development of algorithms that provide less biased estimators, especially at low null-allele frequencies (Lynch and Milligan 1994; Zhivotovsky 1999). In this study, the AFLP procedures proved to be a useful tool for assessing genetic variability and genetic introgression in *A. gambiae* because band profiles with the primer combinations were reproducible and followed Mendelian inheritance patterns. We identified a substantial number of polymorphic, strain-specific, traceable markers that are useful for studying gene introgression in mixed mosquito populations. Our results indicated that allele frequency change varied among genome regions, as shown by significant increases in allele frequencies from generation F_5 to F_{20} in some of the AFLP loci and by demonstrable decreases in other loci.

TABLE 3

Inbreeding coefficients (F_{IS}) in parental populations and in F_1 and F_5 mixed populations

Microsatellite marker	Mbita	Ifakara	F_1 Population 1	Population 2	F_5 Population 1	Population 2
AGXH131	0.22	0.08	0.22	0.04	0.17	0.22
AG2R7	0.07	—	0.14**	−0.03	−0.01	0.12*
AG2H117	0.05	−0.14	0.00	−0.04	−0.32***	0.23*
AG2H187	0.78***	0.80***	0.85***	0.93***	0.95***	0.74***
AG3H158	0.00	−0.07	0.32***	0.21	0.53***	0.09***

*P < 0.05, **P < 0.01, and ***P < 0.001 for the Hardy–Weinberg equilibrium test.

TABLE 4

Partitioning of molecular variance within and among parent populations (Ifakara and Mbita) and mixed populations (F$_1$–F$_{20}$ generations) using the analysis of molecular variance (AMOVA) method

Source of variation	d.f.	SS	MS	VC	%	*P*-value
Between Ifakara and Mbita	1	117.29	117.29	2.39	10.00	<0.001
Among progeny populations	4	713.28	178.32	1.95	7.80	<0.001
Among parents and progeny populations	1	265.15	265.15	1.11	4.29	0.047
Within populations	475	10,831.04	22.80	22.80	88.01	<0.001

SS, sums of squares; MS, mean squares; VC, variance component; %, apportionment of genetic variability; *P*-value, level of significance based on 1000 permutations.

Unlike the western Africa *A. gambiae* populations that often exhibit a higher level of chromosomal inversion polymorphisms, the eastern Africa population has lower chromosomal inversion variability (COLUZZI *et al.* 2002). In our study, the two strains (Ifakara and Mbita) were derived from different divisions of the gene pool from two areas in eastern Africa; they were separated by a high genetic differentiation ($F_{ST} > 0.1$). Knowledge of the karyotype of these two populations would have been helpful, especially for interpreting the genetic diversity results from microsatellite or AFLP markers. However, this information is not available for the particular strains that we used.

Our results are consistent with other findings (DELLA TORRE *et al.* 1997) that gene introgression due to exotic mosquito introduction occurs at different rates across the mosquito genome. The alleles of the Mbita strain showed a higher introgression rate than those of the Ifakara strain. This may be because the study was conducted at Mbita Point in Kenya where the Mbita mosquito strain originated. It may be better adapted to the local climatic or rearing conditions and thus exhibited higher fitness than the Ifakara strain from Tanzania. The rates of gene spread in the subsequent mixed populations depended on genome location, fitness of the hybrid generations and the parental populations, the selection pressure on different loci, and other factors (BESANSKY *et al.* 1994; DELLA TORRE *et al.* 1997; BLACK and LANZARO 2001; MEZZERA and LARGIADÈR 2001). Using the same two strains of *A. gambiae,* we found that

population hybridization led to increased fecundity, increased adult longevity, larger body size, and increased blood-feeding ability up to the F$_{10}$ generation (MENGE *et al.* 2005). Increased hybrid fitness would favor intermediate allele frequencies in mixed populations.

In conclusion, we have used AFLP methods to evaluate the spread rate of introduced genes in *A. gambiae* populations. We found that 28 mapped AFLP markers showed a significant difference in allele frequencies between the Mbita and Ifakara populations and that allele frequencies of 18 markers changed significantly from generation F$_5$ to F$_{20}$ in the mixed populations. The methods developed in this study may be adapted for pilot semifield studies on the gene introgression effects of transgenic mosquito release programs. An urgent need exists for methods and schemes that will be useful for evaluating the ecological and public health impacts of transgenic mosquito release programs. Field trials should be conducted only if the likelihood of achieving public health benefits is high while undesirable ecological or environmental impacts are very low (ENSERINK 2002). Studies in fully contained laboratory conditions and semifield systems are important first steps (SCOTT *et al.* 2002). Further studies are needed to understand the factors influencing the spread rate of introduced genes in seminatural and natural conditions.

TABLE 5

Pairwise genetic differentiation index (F_{ST}) among parental and progeny populations based on AFLP data

	Ifakara	Mbita	F$_1$	F$_5$	F$_{10}$	F$_{15}$	F$_{20}$
Mbita	0.0994	—					
F$_1$	0.1110	0.0371	—				
F$_5$	0.1671	0.0904	0.0724	—			
F$_{10}$	0.1752	0.0955	0.1010	0.0613	—		
F$_{15}$	0.1889	0.1229	0.1127	0.0793	0.0430	—	
F$_{20}$	0.1496	0.1001	0.0971	0.0788	0.0706	0.0574	—

All F_{ST}-values are statistically significant at $P < 0.001$.

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Communicating editor: J. A. BIRCHLER