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A COMPARISON OF ABOVE-GROUND AND BELOW-GROUND POPULATIONS OF *CULEX PIPIENS PIPIENS* IN CHICAGO, ILLINOIS, AND NEW YORK CITY, NEW YORK, USING 2 MICROSATELLITE ASSAYS

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Abstract

Aboveground and belowground populations of the mosquito *Culex pipiens pipiens* are traditionally classified as form (f.) pipiens and f. molestus, respectively, and gene flow between forms is thought to be limited. Relatively few f. molestus populations have been found in the United States, which has hindered their study in North America. In this investigation, we used microsatellites to characterize a recently discovered population of f. molestus in Chicago, IL, and compared levels of genetic diversity and differentiation in above-ground and below-ground populations from Chicago and New York City, NY. Levels of genetic diversity were markedly lower in both f. molestus populations. Pairwise F_{ST} values between populations indicated that f. molestus populations were highly divergent from each other, as well as from their associated aboveground populations. The most likely number of genetic clusters depended on the number of loci used; we began with a set of 8, and reanalyzed the specimens with 17. Using a panel of 17 loci, there were 4 clusters, 1 for each below-ground population, and 1 for each pair of above-ground populations. Our findings are supportive of the hypothesis that f. molestus populations in Chicago and New York City arose from local aboveground populations.

Keywords

population genetics; *Culex pipiens*; *Culex pipiens* form molestus; microsatellites; genetic structure

INTRODUCTION

Members of the *Culex pipiens* complex are difficult to distinguish morphologically. Found in more tropical areas, *Culex pipiens quinquefasciatus* Say can produce interfertile hybrids with the temperate *Culex pipiens pipiens* (Kothera et al. 2009). *Culex p. pipiens* is composed of 2 forms: form molestus Forskål, and form pipiens L. Although morphologically identical, the 2 forms exhibit important biological differences. Form pipiens individuals are anautogenous and undergo seasonal diapause, while f. molestus are autogenous and active all year (Chevillon et al. 1995, 1998; Vinogradova 2000). In the U.S., f. molestus is found underground, mostly in cities, and relatively few populations have been studied to date (Boston, MA, Spielman 1957; New York City, NY, Kent et al. 2007, Huang et al. 2008, Kothera et al. 2010; Marin County, CA, McAbee et al. 2004; Philadelphia, PA, Kilpatrick et

al. 2007 and Chicago, IL, Wray 1946, Mutebi and Savage 2009, Kothera et al. 2010). All members of the *Culex pipiens* complex are efficient vectors of West Nile and other arboviruses (Tahori 1955, Turell et al. 2006, Kramer et al. 2008).

Form molestus populations persist in isolated, enclosed habitats, where presumably there is little migration and therefore a low degree of gene flow. It is possible that European f. molestus populations are the source of f. molestus populations in the U.S. If this is the case, one would expect to observe a high degree of genetic similarity among U.S. f. molestus populations. On the other hand, U.S. populations of f. molestus could be derived from local aboveground f. pipiens populations. Under this scenario, f. molestus populations would be expected to be genetically divergent from one another, each reflecting its own history of colonization, isolation, selection, and drift. Each f. molestus population would also be expected to share alleles with the local above-ground population.

With regard to whether the 2 forms hybridize, 1 well-characterized autogenous population in the U.S. was studied by Spielman (1957, 1964, 1971, 1973). He found limited hybridization between forms and concluded that while the 2 forms are interfertile, behavioral and ecological factors reduce opportunities for hybridization, and thus serve as effective reproductive isolating mechanisms. Huang et al. (2008) and Kothera et al. (2010) also found evidence for low levels of hybridization.

Gene flow tends to make populations homogenous, and there is an inverse relationship between the amount of gene flow between populations and the degree of genetic differentiation observed. Genetic differences are quantified within and between populations using neutral genetic markers, such as microsatellites, which look at changes that result from genetic drift. The 2 kinds of population data that are standard in population genetic studies are concerned with genetic diversity and genetic differentiation. Genetic diversity refers to the variety of alleles and genotypes present in a population (Frankham et al. 2002). It is measured within populations, and often expressed as Expected Heterozygosity (HE), the proportion of loci expected to be heterozygous in an individual, and Allelic Richness, which describes the number of alleles per locus per population. Differentiation portrays the degree of genetic difference between populations, is denoted by the statistic F_{ST}, and can range from 0–1. To give a qualitative idea of what constitutes significant amounts of genetic differentiation (and hence restricted gene flow), Wright (1978) stated that values over 0.05 suggested “moderate” genetic differentiation.

In this paper, we use the tools of population genetics to describe a f. molestus population collected from Chicago, Illinois (Mutebi and Savage 2009), and compare above and belowground populations of *Cx. p. pipiens* from Chicago and New York City, New York. The methods, data analyses and much of the data have been reported in Kothera et al. (2010), but here we include a comparison of the original analyses with additional ones performed with new microsatellite markers.

MATERIALS AND METHODS

In each city, 2 above-ground (f. pipiens) and 1 below-ground (f. molestus) populations were sampled (Table 1; Fig. 1). Originally, each individual was surveyed with a panel of 8 previously-published microsatellite loci. We have since developed an additional 11 loci, and dropped 2 of the original 8 from our assay. The resulting panel consists of 17 loci that we allocated to 2 multiplexes, the details of which are published elsewhere (Molecular Ecology Resources Primer Development Consortium et al. 2012).

Specimens were collected in the winter where possible, so the presence or absence of diapause could be noted and the collection of the correct form confirmed. The exception was the sampling of the above-ground populations in Chicago, which were collected as part of another study. Autogeny or anautogeny was confirmed by colonizing the Chicago and New York City f. molestus populations, as well as the above-ground f. pipiens populations from New York City. The above-ground specimens from Chicago were presumed to be anautogenous.

Data analysis was the same as Kothera et al. (2010). Briefly, specimens were examined morphologically and screened with the ITS assay to confirm membership in the *Cx. pipiens* complex (Savage et al. 2007). After multiplexed polymerase chain reaction (PCR) with fluorescently labeled forward primers, fragments were visualized on a Beckman Coulter (Fullerton, CA) CEQ8000 sequencer, and a multilocus genotype was generated for each individual. Individuals missing more than 3 of the 17 loci were not included in the analyses. Convert (Glaubitz 2004) was used to format the data for analysis in Arlequin 3.1 (Excoffier et al. 2005) which was used for HE and F_{ST} calculations, FSTAT 2.9.3 (Goudet 1995) which was used for the calculation of Allelic Richness, and Structure (Pritchard 2000, Falush et al. 2003) which was used to determine the most likely number of genetic clusters in the data. Distruct (Rosenberg 2004) was used to visualize the Structure results. The program Bottleneck (Piry et al. 1999) was used to determine whether any of the populations showed evidence of a recent reduction in effective population size (Ne). Microsatellite Analyzer (MSA; Dieringer and Schlotterer 2003) was used to generate a matrix of percent shared alleles between pairs of populations. Phylip was used to generate a neighbor-joining tree from the resulting matrix (Felsenstein 1993).

RESULTS

Table 2 shows a comparison of genetic diversity measures, using 8 and 17 loci, for each of the 6 populations in this study. For both sets of loci, the genetic diversity in the below-ground populations is lower than in the above-ground ones. The allelic richness, which is adjusted to account for the smallest sample size, ranged from 2.616 in the $ChiMolG_0$ population with 17 loci to 7.291 in the NYFT population with 8 loci. The average HE followed a similar trend, ranging from 0.383 for 8 loci in the $NYMolG_0$ population to 0.670 in both the Chi16 and NYGC populations for 8 loci.

Table 3 shows pairwise F_{ST} values between populations using 8 and 17 loci. For this measurement, there was a difference in the significance of the results when more loci were

used. For example, with 8 loci none of the 4 above-ground populations are statistically significantly differentiated from each other. However, with 17 loci, the 2 above-ground populations in each city were differentiated from the above-ground populations in the other city. Regardless of which panel was used, the 2 belowground populations were highly differentiated from each other (F_{ST} = 0.394 and 0.248 for 8 and 17 loci, respectively), as well as from each above-ground population. Similarly, for either number of loci, the above-ground populations from each city were not statistically significantly differentiated from each other (F_{ST} range 0.000–0.006).

Table 4 shows results from the Bottleneck program. With 8 loci, 1 population, ChiMolG$_0$, exhibited a signature of a genetic bottleneck, having a significant Wilcoxon Test P-value, and a mode shift in allele frequency. When 17 loci were used, all populations had a significant Wilcoxon Test P-value, and 4 of 6 displayed a mode shift.

The neighbor-joining tree produced from the matrix of percent shared alleles (using 17 loci) between pairs of populations is shown in Fig. 2. Trees for 8 and 17 loci were virtually identical. The configuration of the tree is consistent with the pairwise F_{ST} results between populations, with the 2 below-ground f. molestus populations highly diverged from the above-ground ones. In addition, the above-ground populations form a larger group, with each city's f. pipiens populations in closest proximity.

The program Structure was used to determine the most likely number of genetic clusters represented by the data. Figure 3 shows results using 8 loci. The top panel shows the most likely number of clusters (K = 3) with the 2 belowground populations each occupying their own cluster, and the 4 above-ground populations in 1 cluster. The middle and bottom panels of Fig. 3 illustrate the stability of assignments of the 2 below-ground clusters. As the number of clusters is arbitrarily increased (K = 4, K = 5), the belowground population clusters remain intact, and the 1 above-ground cluster is further subdivided. Figure 4 shows Structure results using 17 loci. When additional loci are used, there is sufficient discriminating power to divide the single aboveground cluster seen with 8 loci into 2 clusters, 1 for each city.

DISCUSSION

In general, the use of additional loci further elucidated genetic relationships within and among populations in this study. Several lines of evidence suggest that the f. molestus populations experience low amounts of gene flow, which has resulted in them showing low genetic diversity and a high degree of genetic differentiation. First, the values for H_E and Allelic Richness are markedly lower for the f. molestus populations compared to the above-ground f. pipiens populations. The H_E, for example, was approximately a third less in the f. molestus populations. Reduced genetic diversity can result from a genetic bottleneck, and there was evidence of a genetic bottleneck in several populations when 17 loci were used. Genetic bottlenecks reduce genetic diversity by reducing the N_e in the population (Nei et al. 1975).

If there was periodic gene flow from the aboveground populations, it would have a homogenizing effect, and the above- and below-ground populations would not be expected

to exhibit a high degree of differentiation. Instead, significant F_{ST} values were found between all pairs of populations except each city's pair of above-ground f. pipiens. Although the below-ground populations share alleles with the local above-ground populations (Kothera et al. 2010), these results reinforce the idea that the below-ground populations are largely reproductively isolated from the local aboveground populations.

The clustering program Structure was useful in illustrating the degree of divergence among populations. Using 8 or 17 loci, both analyses resulted in the 2 below-ground populations occupying their own cluster, and there was a high degree of stability with regard to assignments of individuals to these clusters. One difference that resulted from using additional loci was an ability to obtain a finer resolution on differences among the f. pipiens populations. With 8 loci, all of the f. pipiens populations occupied 1 cluster, while with 17, there was a higher posterior probability associated with having each city's f. pipiens populations occupy its own cluster. This suggests that additional loci can provide further resolution with populations that share many alleles but are geographically positioned such that there is a low probability of gene flow between them.

In addition to genetically characterizing a recently-discovered f. molestus population ($ChiMolG_0$; Mutebi and Savage 2009), a purpose of this study was to seek evidence in support of a hypothesis regarding the origin of f. molestus in the U.S. The results from this study indicate that the 2 f. molestus populations do not appear to be recently derived from a common ancestor, which should be the case if all of the f. molestus in the U.S. is European in origin. Instead, the 2 f. molestus populations show a very high degree of divergence, although they still share alleles with the associated above-ground populations. For this reason, the results suggest that U.S. f. molestus populations are more likely to be derived from local, above-ground f. pipiens populations that have undergone a reduction in genetic diversity, perhaps as a result of selection for the traits that distinguish f. molestus, namely autogeny and a lack of winter diapause.

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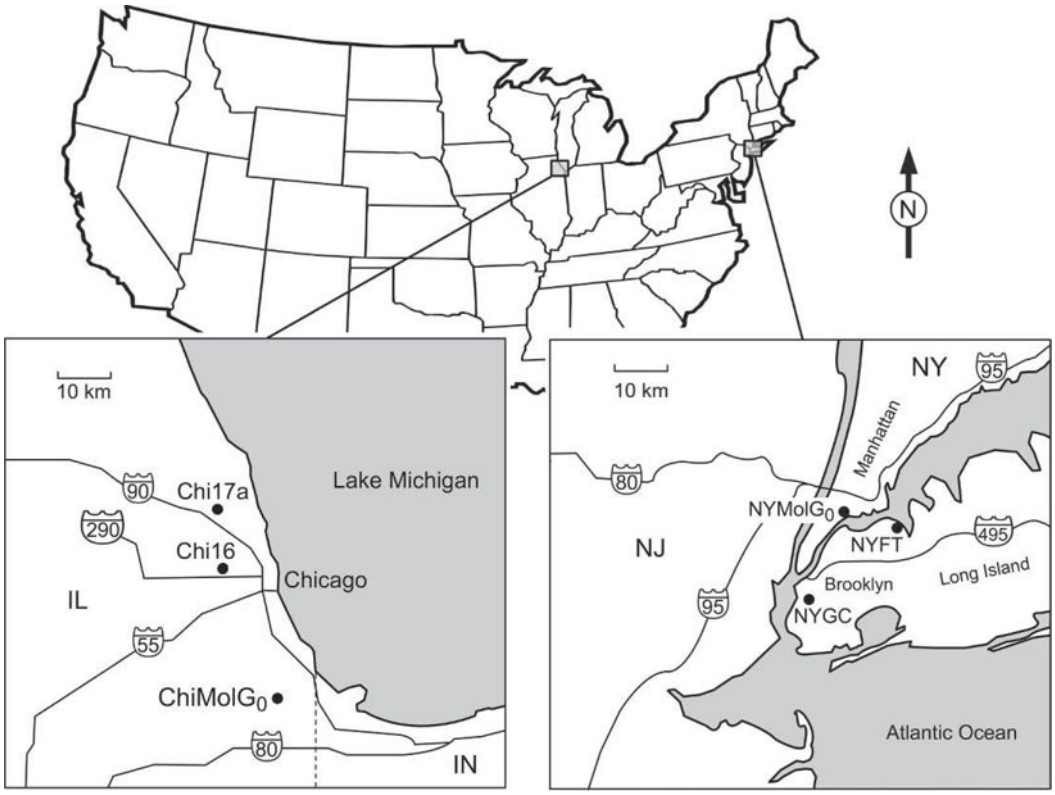

Fig. 1.

Map of sites sampled in this study. Maps of individual cities are shown at the same scale.

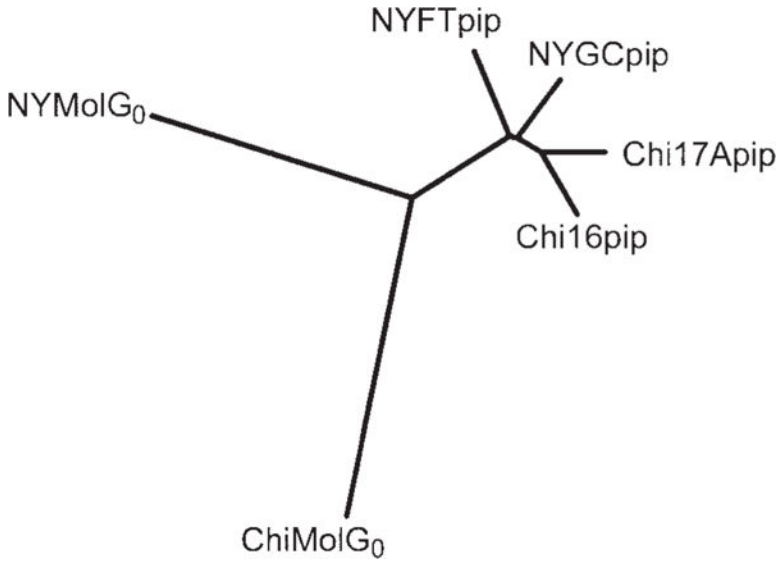

Fig. 2.

Neighbor-joining tree of 6 populations based on proportion of shared alleles. See Table 1 for site descriptions.

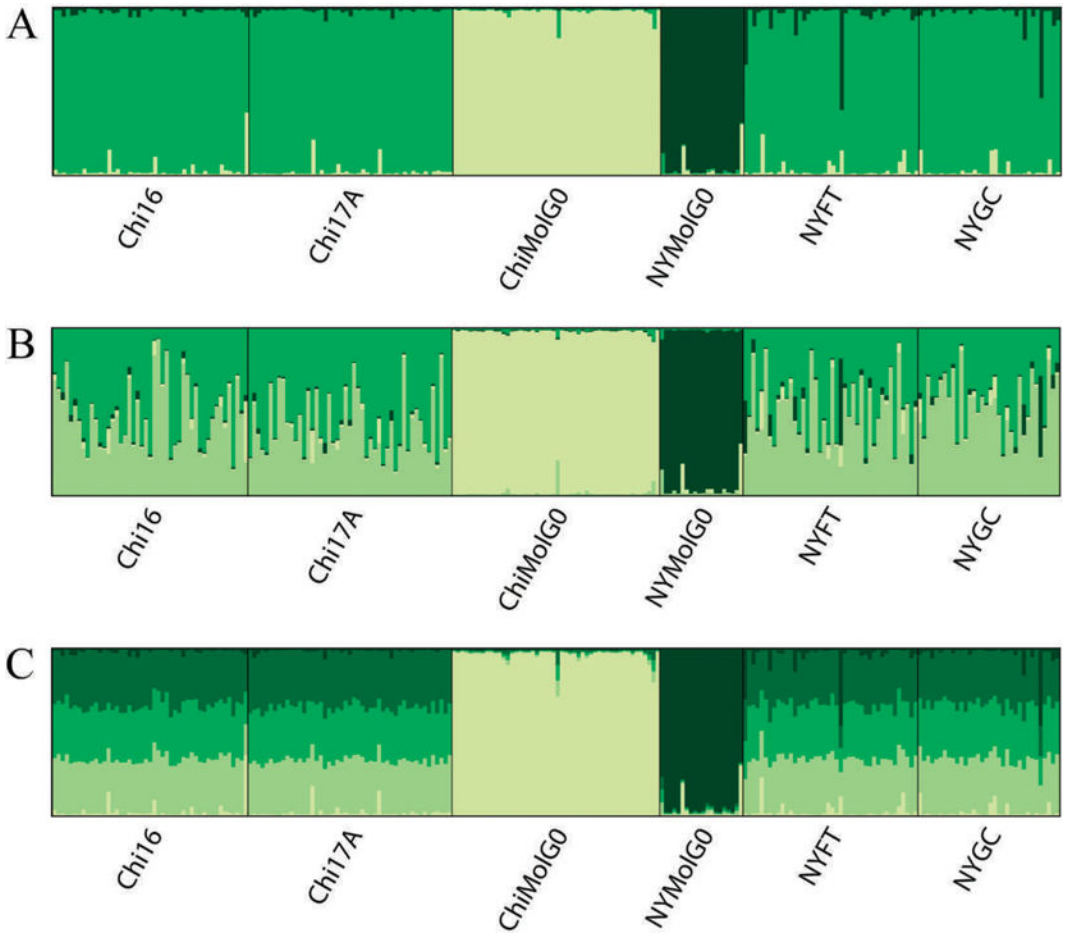

Fig. 3.

Results from Structure analysis, with 8 loci, default settings and no prior population information. Panel A shows individual assignments for the most likely number of clusters, $K = 3$. B and C illustrate the stability of assignments to the 2 f. molestus clusters when K is arbitrarily set at $K = 4$ and $K = 5$, respectively.

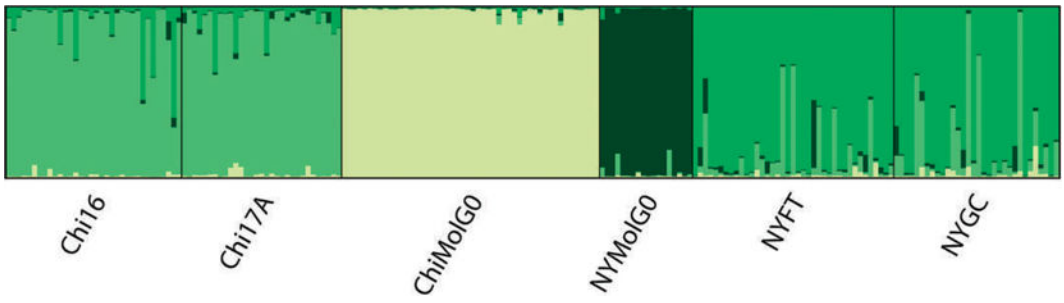

Fig. 4.

Results from Structure analysis with 17 loci, showing $K = 4$. Additional loci allow the program to discriminate between the 2 pairs of above-ground populations.

Table 1

Site and population information.

Site Name	N[1]	City	State	Latitude	Longitude	Location[2]	Trap Method
Chi16	46	Chicago	IL	42.0317	−87.7414	Above	Gravid Trap
Chi17A	50	Chicago	IL	41.9825	−87.7506	Above	Gravid Trap
$ChiMolG_0$	50	Chicago	IL	41.6633	−87.6083	Below	Aspiration, Dippers
$NYMolG_0$	20	New York	NY	40.7833	−73.9500	Below	CDC Light Trap
NYFT	47	New York	NY	40.7900	−73.7808	Above	CDC Light Trap
NYGC	34	New York	NY	40.6574	−73.9862	Above	CDC Light Trap

[1] Number of individuals sampled.

[2] Above-or below-ground.

Table 2

Among-population genetic diversity measures using 8 loci (top panel) and 17 loci (bottom panel) for 3 populations (2 above-ground, 1 below-ground) each from Chicago, IL and New York City, NY.
See Table 1 for site description.

	Chi16	**Chi17A**	**ChiMolG$_0$**	**NYMolG$_0$**	**NYFT**	**NYGC**
Average Allelic Richness	6.958	6.526	2.742	3.533	7.291	7.214
Average Expected Heterozygosity	0.670	0.654	0.427	0.383	0.665	0.670

	Chi16	Chi17A	ChiMolG$_0$	NYMolG$_0$	NYFT	NYGC
Average Allelic Richness	5.075	5.097	2.616	3.156	5.531	5.283
Average Expected Heterozygosity	0.642	0.624	0.445	0.448	0.655	0.653

Table 3

Pairwise F_{ST} values for pairs of populations in this study using 8 (top panel) and 17 (bottom panel) loci.

	Chi16[1]	**Chi17A**	**ChiMolG$_0$**	**NYMolG$_0$**	**NYFT**
Chi17A	0.001				
ChiMolG$_0$	**0.215**[2]	**0.240**			
NYMolG$_0$	**0.169**	**0.174**	**0.394**		
NYFT	0.003	0.003	**0.232**	**0.152**	
NYGC	0.007	0.004	**0.253**	**0.158**	0.003

	Chi16	Chi17A	ChiMolG$_0$	NYMolG$_0$	NYFT
Chi17A	0.006				
ChiMolG$_0$	**0.207**	**0.195**			
NYMolG$_0$	**0.179**	**0.174**	**0.248**		
NYFT	**0.044**	**0.049**	**0.217**	**0.137**	
NYGC	**0.031**	**0.029**	**0.215**	**0.129**	0.000

[1] See Table 1 for site descriptions.

[2] Bold values are significant, **P** < 0.05.

Table 4

Results from the program Bottleneck for 8 (top panel) and 17 (bottom panel) loci.

Population	**Expected Het. Excess**[1]	**Observed Het. Excess**[2]	**Wilcoxon P-value**[3]	**Mode shift**[4]
Chi16	4.61	3	0.84400	NO
Chi17A	4.62	3	0.84400	NO
ChiMolG$_0$	4.27	6	0.02000*	YES
NYMolG$_0$	4.36	6	0.25000	YES
NYFT	4.60	5	0.31300	NO
NYGC	4.65	4	0.84400	NO
Population	Expected Het. Excess	Observed Het. Excess	Wilcoxon P-value	Mode shift
Chi16	8.71	15	0.00004*	YES
Chi17A	9.10	14	0.00042*	NO
ChiMolG$_0$	7.59	14	0.00314*	YES
NYMolG$_0$	8.68	13	0.01161*	YES
NYFT	9.06	16	0.00003*	YES
NYGC	8.96	15	0.00034*	NO

[1] Expected number of loci showing heterozygote excess.

[2] Observed number of loci showing heterozygote excess.

[3] One-tailed Wilcoxon's test * $P < 0.05$.

[4] Whether a shift in allele frequency distribution occurred.