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Population genetic structure of *Chrysomya megacephala* (Fabricius) of the Egyptian fauna

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Population genetic patterns and changes in allele frequency within blow flies can provide valuable insight into population structure, diversification, dispersal, gene flow, and population assignments. The population genetic structure of the oriental latrine blow fly, *Chrysomya megacephala* (Fabricius), was investigated using amplified fragment length polymorphisms (AFLP). The goal was to validate the use of this technique to create genetic profile data and determine it is feasibility for inferring postmortem relocation of corpses. The AFLP technique generated 590 polymorphic loci for *C. megacephala*. Analysis of molecular variation (AMOVA) found significantly high genetic variation within individuals of all fly populations, with little variation among populations from different geographic locations. STRUCTURE and principal coordinate analyses (PCoA) revealed no population structure based on geography, with weak correlation between genetic and geographic distances, and moderate temporal differentiation was noted among *C. megacephala* samples. Across the entire data set, the mean relative relatedness coefficients were positive, suggesting that flies arriving at the same bait (carcass) share nonrandom proportions of alleles and comprise of closely related individuals. Genetic assignment of *C. megacephala* flies to a putative source population resulted in a 90.81% success rate, indicating the possibility of using these flies to connect sites between which a corpse had been moved even in the absence of overall geographic population structure.

Keywords AFLP, *Chrysomya megacephala*, Forensic entomology, Population structure, Population assignment

True flies (Order Diptera) have earned a notorious reputation as pests in Egypt. The favorable weather conditions that prevail throughout the year have led to an abundance of various types of flies. Among the many flies of significant medical and veterinary importance that are commonly encountered in Egypt and other regions are blow flies of the Family Calliphoridae.

Although bacteria and other microbes are present from the onset of death and initiate decomposition immediately, blow flies are often considered the first insects to arrive at a crime scene, usually within hours or even minutes of the body's demise[1–4]. It can be asserted that blow flies serve as the initial witnesses to an event. The accurate identification of this species is a crucial component in any investigation to estimate the postmortem interval[5]. To ensure timely and precise species identification, molecular approaches have been utilized in criminal investigations. Various techniques are employed for molecular identification, including direct sequencing of DNA[6], restriction fragment length polymorphism (RFLP) analysis[7], and amplified fragment length polymorphism (AFLP) analysis[8]. Blow flies are known to disperse over several kilometers in a single day to find a mate or a new resource for egg oviposition and development[9–12]. As strong fliers, it would seem that a blow fly population should exhibit little genetic structure and high gene flow[13,14]. However, the incidence of distinct populations of blow flies is a matter of considerable debate as flies collected from different ecosystems develop at different rates under the same conditions. In this context, biological features or life history traits are controlled by loci under selection, particularly among flies from diverse ecosystems or geographic regions[15–18]. Moreover, the flies collected from bait (as a surrogate for carrion) are not random samples of the population, but rather consist of genetically related individuals, suggesting that emerging siblings at the same time and place (carrion) seek out in concert the next carrion source[8,13,19,20]. While one study of widely distributed samples indicated that flies could be assigned to their putative populations using AFLP[19], the question remains whether this can be done on a smaller geographic scale.

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Using methods of source population assignment[19–22], an investigator can associate genetically distinct larvae to their source population (the maggot community on the corpse). There are various approaches that have been used to study genetic diversity within natural populations, AFLP is a PCR-based approach that requires only a small amount of starting genomic material and does not require previous sequencing or probe synthesis. Although more complex sequencing-based methods do exist for studying population genetic diversity, such as RAD sequencing and re-sequencing analyses, these methods remain costly and require specialized training to analyze the data. AFLP remains a consistent method that generates a large number of polymorphic loci. Increasing the number of loci used for analysis is critical in population allocation and genetic assignment tests[23]. In this study, the goal was to characterize the population genetic structure of a common blow fly in Egypt using AFLP to determine if source populations exist and to investigate isolation by distance over both a short time scale and a longer time period.

Materials and methods

Specimen collection

A total of 74 *Chrysomya megacephala* adults were collected between May and June 2013 using fish-baited traps at three geographical locations in Egypt (Fig. 1). Of these, 30 specimens were gathered from Giza, 26 from Dayrout, and 18 from North Sinai. All samples were collected over a short time scale (43 days) during a single event at each location. The distance between the two farthest locations, Dayrout and North Sinai, is approximately 500 km. Additionally, another 24 adults were collected at a single site in Giza in May 2014. All samples from the 2013 and 2014 collections were gathered within a 30-minute period, with the flies immediately preserved in 70% ethanol and stored at -20 °C until genetic analysis could be performed.

Genotyping

The extraction of DNA from the crushed head tissues of the flies was executed to prevent contamination from eggs, ingested proteins, or gut parasites. The DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) was employed in accordance with the manufacturer's protocol, and the eluted DNA was stored at -20 °C. The remaining tissues were retained as voucher specimens at -20 °C in ethanol at Indiana University–Purdue University Indianapolis. AFLP genotyping was conducted in accordance with the protocol outlined in[13]. The amplified DNA fragments were then separated and detected using a 3500 Genetic Analyzer (ThermoFisher, Waltham, MA).

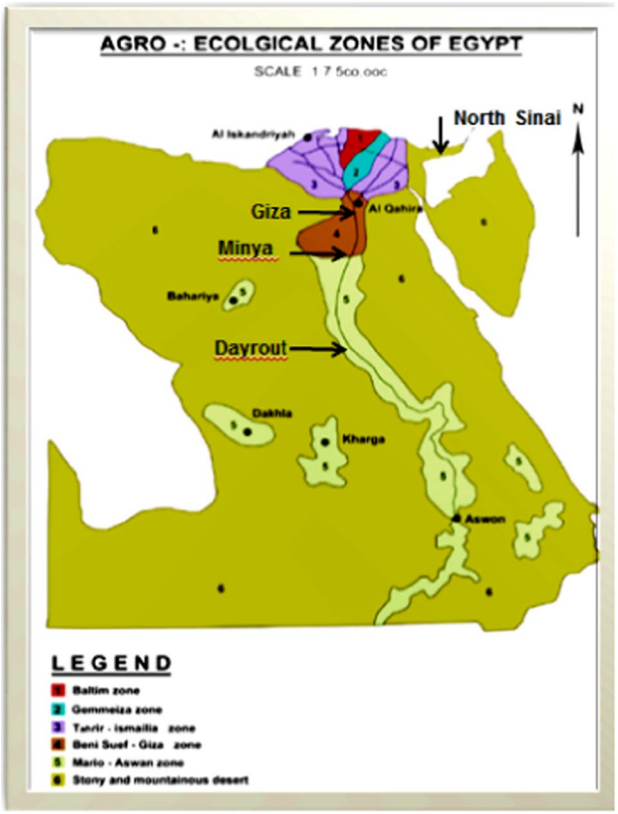

Fig. 1. Places where *Chrysomya megacephala* were trapped in Egypt. (*Source*: http://www.fao.org/ag/AGP/AGPC/doc/Counprof/Egypt/figures/fig10.jpg).

Data analysis

AFLP genotyping

The raw AFLP profiles from the genetic analyzer were imported into GeneMarker v2.4 (SoftGenetics, LLC, State College, PA, USA) for allele scoring using default settings. The analysis range was set from 100 to 500 bp to minimize the effect of size homoplasy[24]. Following the analysis of all samples with GeneMarker, the output binary file for each primer set ($N=4$) was exported to Excel (Microsoft Corp, Redmond, WA, USA). The data were filtered by only considering the loci with allele frequencies between 0.05 and 0.95 to avoid monomorphic loci[25,26]. This resulted in the scoring of 590 polymorphic alleles from the four primer combinations for *C. megacephala*.

Geographical genetic structure

An analysis of molecular variance (AMOVA) was conducted using GenAlEx 6.5 to determine the partitioning of genetic variance within samples = individual flies (ΦPT), among samples = among populations within geographical locations (ΦPR), and among regions = among populations across time (ΦRT). The significance of the resulting variance and intergroup genetic distances was determined through 999 permutations. The Φ statistic is a band-based method that uses the squared Euclidean distance matrix between AFLP profiles and allows for a hierarchical analysis of genetic structures that does not rely critically on specific assumptions that could underestimate genetic variability[27–29]. To explore the possibility of isolation by distance (IBD), GenAlEx 6.5[30] was used to perform a Mantel test for correlation between Euclidean geographic distances among all collection sites according to their GIS coordinates and the calculated matrices of pairwise genetic distance (ΦPT). The statistical significance of the correlation was assessed using SPSS (IPM, Armonk, NY).The geographic genetic structure was further investigated using the Bayesian model-based clustering algorithms implemented in STRUCTURE v2.3.4[31] to detect genetically distinct subpopulations within this dataset. Assuming admixture model and independent allele frequencies and a number of clusters or subpopulations (K) ranging from 1 to 9; 10 runs with a burn-in period of 10,000 replications and a run length of 50,000 Markov chain Monte Carlo (MCMC) iterations were performed to obtain a robust and accurate outcome and to stabilize likelihood parameter estimation. Expecting that a superstructure might include other subpopulations at smaller spatial scales, STRUCTURE was run for the whole data set as well as for the populations within each location individually[32]. Analysis for K was done using the program Structure Harvester v0.6.94[33], according to the method proposed by[32], in which K was plotted against it is rate of change ΔK; and the uppermost peak of ΔK corresponded to the most likely K value.

To validate the sample clustering result of STRUCTURE, principal coordinate analysis (PCoA) using GenAlEx 6.5 was carried out based on the pairwise genetic distances among samples (ΦPR) as input matrix.

Genetic diversity

The genetic diversity of each sample was evaluated by calculating the number of polymorphic loci, and Nei's gene diversity was assessed using AFLP-SURV, following the method described in[34]. The within-sample (H_j) and total population (H_T) genetic diversity were also estimated using this approach.

Relatedness of individuals

Using the program SPAGeDi[35], a spatial pattern analysis of genetic diversity was executed, and relatedness analysis was conducted to calculate the relative relatedness coefficient for all sample pairs within a sample. The relatedness coefficient is the proportion of alleles shared between individuals, and these coefficients are contingent upon reference allele frequencies, which were determined according to[36]. No inbreeding coefficient was specified, but calculations based on inbreeding coefficients over the entire range of 0–1 had no statistically significant impact on the overall mean of R, as determined by[19]. Therefore, we employed the conventional assumed inbreeding coefficient of 0, as prescribed by[36], for this analysis. The software is designed to be robust to moderate violations of this assumption.

Assignment analyses

AFLPOP v. 2.0 software package[37] was used to perform simulation tests in which likelihood statistics were calculated to assign an individual blow fly to a putative source population based on the expected frequencies of it is genotype in this population. And because of it is embodied simulator, AFLPOP can also provide data on the rates and types of incorrect allocations. One thousand random genotypes were generated, and the number of iterations was set to 10. Zero frequency was replaced by 1/ (sample size + 1) in the calculation to account for un-sampled alleles (a correction factor).

The minimum likelihood difference (MLD) serves as a measure of the stringency of assignment, with a higher MLD resulting in a lower rate of incorrect allocations and a greater number of unassigned individuals. To optimize the MLD factor, multiple iterations of the Simulation procedure were conducted with varying MLD values ranging from 0 to 15 until the smallest value was found, which satisfied the desired rate of correct allocations[37]. Subsequently, the Re-allocation run was employed to estimate the expected allocation success rate for each of the source populations. In this process, each individual was removed from it is population's dataset and the allelic frequencies for the most likely population were recalculated across all loci.

Results

The utilization of the four selective PCR primer combinations (PstI + NNN) resulted in a total of 984 loci (261 PstI + AAC, 227 PstI + ACG, 268 PstI + AGT, and 228 PstI + ATC). Following the filtration of loci that were insufficiently polymorphic (less than 5% and greater than 95%), a total of 590 loci were retained for genetic analysis (177 from PstI + AAC, 129 from PstI + ACG, 168 from PstI + AGT, and 116 from PstI + ATC). Every

Collection Site	Coordinate	Date of collection	No. Collected and sampled	%Polymorphism*	Hj ± SE
Giza 2014	30°00 N/31°10 E	September 2014	24 (3♂, 21 ♀)	91	0.12 ± 0.006
Giza 2013	30°00 N/31°10 E	May 2013	30 (4♂, 26♀)	92.6	0.238 ± 0.006
Dayrout 2013	27°34 N/30°49 E	June 2013	26 (2♂, 24♀)	96.3	0.161 ± 0.006
North Sinai 2013	31°02 N/33°00 E	May 2013	18 (2♂, 16♀)	96.2	0.177 ± 0.006
Total		98			

Table 1. *C.megacephala* collection sites, sex ratios, % polymorphic loci, and Nei's gene diversity (Hj). *%polymorphism: the percentage of loci with polymorphism not lower than5% over the total number of loci.

Source	df	SS	MS	Est. Var.	%	Statistics	value	*p*
Among regions	1	1334.694	1334.69	26.847	22	ΦRT	0.217	0.001
Among populations/locations	2	717.67	358.835	11.319	9	ΦPR	0.309	0.001
Within pops	94	8021.36	85.334	85.334	69	ΦPT	0.117	0.001
Total	97	10073.72		123.50	100			

Table 2. AMOVA results for *C. megacephala*.

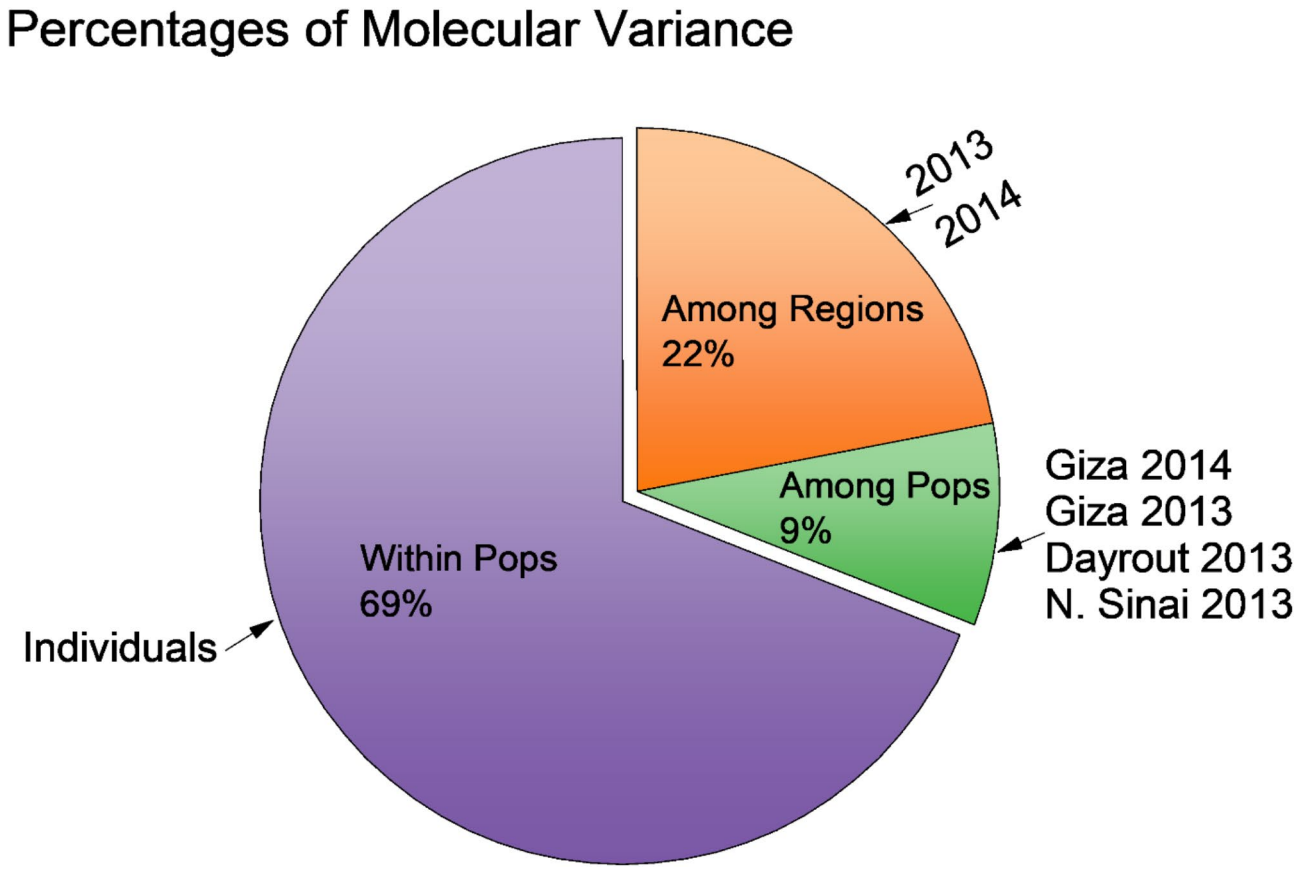

Fig. 2. Percentage of molecular variance for C. *megacephala* population.

individual produced a unique multilocus genotype, and the percentage of polymorphic loci for each sample ranged from 91 to 96.3% (with an average of 93.6%, as presented in Table 1).

Genetic and geographic structure

The results of the AMOVA analysis are presented in Table 2; Fig. 2. The Φ statistic is equivalent to the F statistic in terms of partitioning genetic variation, particularly for haploid data[28]. The within-population genetic variation was found to be significantly high (ΦPT = 69%, $p = 0.001$), as supported by the relatively high Hj values

SSx	SSy	SPxy	Rxy	*P* (rxy-rand >= rxy-data)
101226.600	0.083	− 59.114	− 0.643	0.083

Table 3. Mantel test results for *C. megacephala* population.

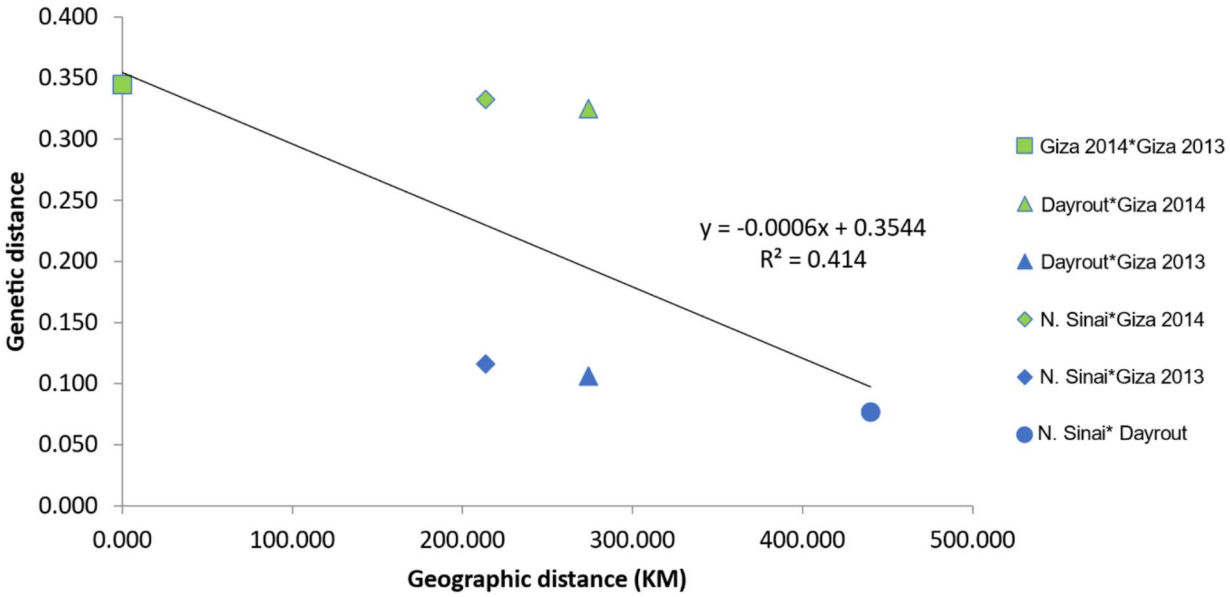

Fig. 3. Correlation between Geographic (km) and genetic distances ΦPT of *C. megacephala* individuals.

presented in Table 1 for each sample. The genetic distinctness of individuals was observed, but when the distance was examined, a small differentiation (ΦPR = 9%, $p = 0.001$) was attributed to among populations/geographic location. When the time factor was included and samples were grouped into two regions (the 2013 and 2014 populations), the percentage of variation increased to ΦRT = 22% ($p = 0.001$), indicating moderate temporal separation.

To evaluate the association between genetic and geographic distance matrices, a Mantel test was carried out, using the Pearson product-moment correlation coefficient R as the test statistic. R ranges from − 1 to + 1, with values close to -1 indicating a strong negative correlation, + 1 indicating a strong positive correlation, and $R = 0$ indicating no correlation. The results showed a non-significant strong negative correlation between geographic and genetic distances, indicating minimal evidence of genetic isolation by geographical distances (as depicted in Table 3; Fig. 3).

The fixation index F_{ST} was calculated to be 0.158 ± 0.422, indicating moderate degree of population differentiation due to genetic structure among *C. megacephala* populations in Egypt. The total gene diversity H_T was found to be 0.207. These results suggest that there are significantly low genetic differences among the various sites of *C. megacephala* in Egypt ($p = 0.001$ and $p = 0.001$ respectively). F_{ST} will approach 0 when allele frequencies are the same between populations, indicating complete sharing of genetic material. Conversely, F_{ST} will rise to 1 when their total separation in allele frequencies between subpopulations, indicating greater genetic diversity and fixation of allele frequencies in subpopulations.

The results of the STRUCTURE simulation indicated that the most probable number of genetic clusters (k) was 2 (as depicted in Fig. 4). Cluster 1 comprised of all individuals collected from various regions in 2013 and represented populations 12, 13, and 15 (Giza, Dayrout, and N. Sinai populations, respectively). Cluster 2 consisted of samples from Giza in 2014 (population 11) (as shown in Fig. 4). The analysis revealed no evidence of isolation by distance and instead, the populations were structured based on temporal aspects. To determine the optimal number of clusters, the STRUCTURE HARVESTER was utilized, which is an extension of the original STRUCTURE software, and was implemented in[33].

The results of the principal coordinate analysis (PCoA) based on genetic distances (ΦPR) among *C. megacephala* populations were found to be consistent and complementary to those of STRUCTURE. The analysis revealed the formation of two distinct clusters. Cluster 1 comprised all individuals that were closely coordinated and collected in 2013 from Giza, Dayrout, and N. Sinai. Cluster 2 represented samples collected in 2014 from Giza. The first two coordinates accounted for 88.32% of the total genetic variation (Fig. 5).

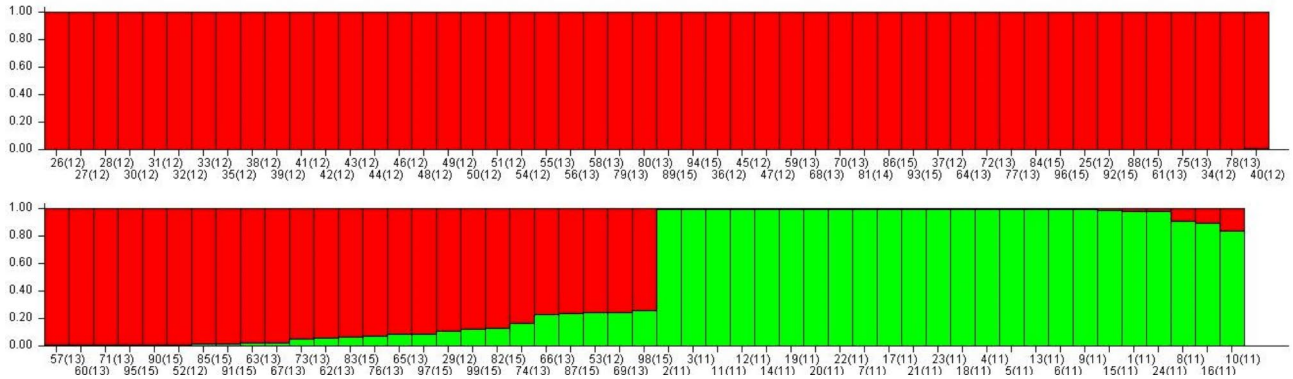

Fig. 4. Bar plot of estimated *C. megacephala* subpopulation when $K=2$. Red color represents cluster 1 including all 2013(12, 13, 15: Giza, Dayrout and N.Sinai populations respectively) individuals and green color represents 2014 (11: Giza population) cluster.

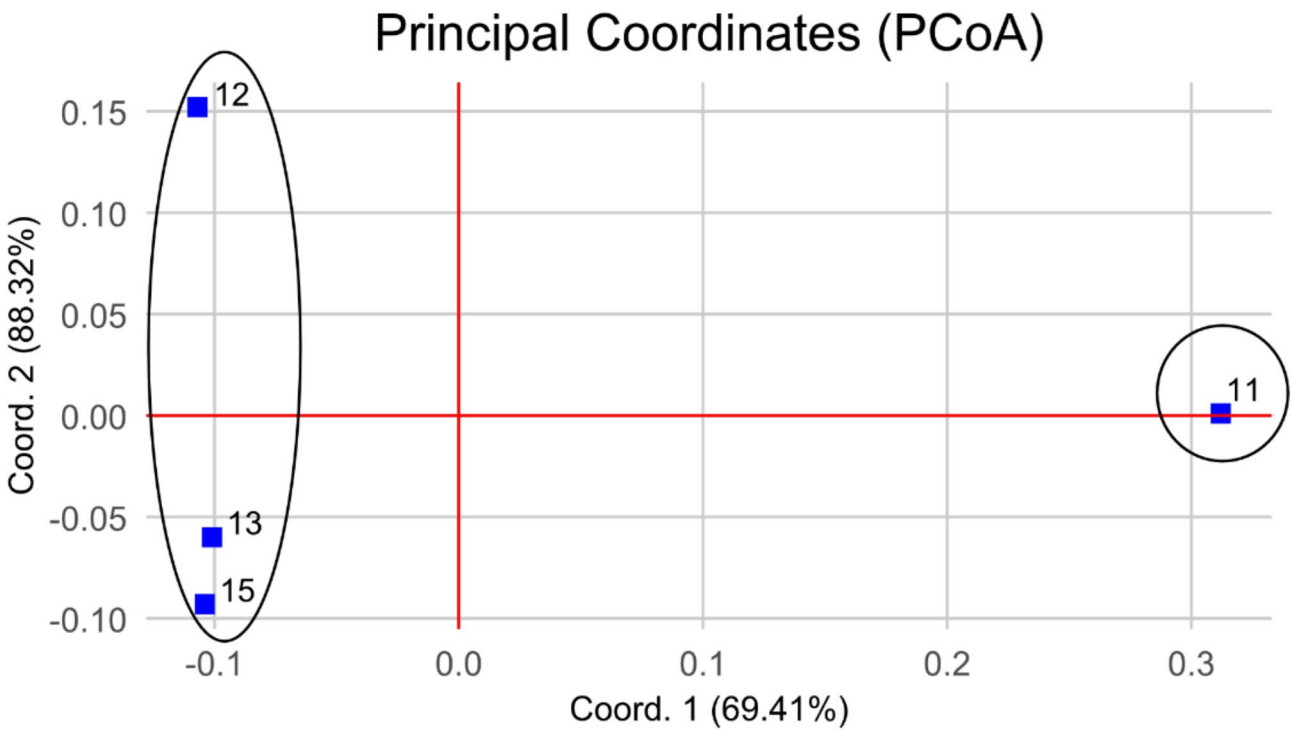

Fig. 5. Results of Principal Coordinate Analysis (PCoA) for *C. megacephala* samples. Coordinates 1 and 2 accounted for 69.41% and 88.32% of the total genetic variation, respectively. Two clusters were identified: Cluster 1 consisted of 2013 samples from all regions (12, 13, 15: Giza, Dayrout and N.Sinai populations respectively), while Cluster 2 contained samples collected in Giza in 2014 (11: Giza 2014 population).

Relatedness of *C. megacephala* samples

The relative relatedness coefficient (r) was determined by SPAGeDi using the entire AFLP dataset as a reference for allele frequency calculation (Fig. 6). These calculations were based on an assumed inbreeding coefficient of zero. Overall, the value of r was larger than zero, indicating a higher degree of relatedness within each *C. megacephala* sample than would be expected by chance. Specifically, the mean value of r was 0.321 ± 0.068 (range: 0.1 to 0.5), as shown in Fig. 6. Moreover, the results revealed that *C. megacephala* individuals share a higher proportion of alleles than would be expected if individuals were selected at random from the collected samples (negative control), indicating excess heterozygosity and outbreeding in the population (Fig. 6).

Assignment analyses

The experimental design utilized the Simulation and Reallocation procedures of AFLPOP v2.0, with the multiplexing level of DNA (MLD) set at 7. This parameter was chosen based on a comprehensive evaluation of

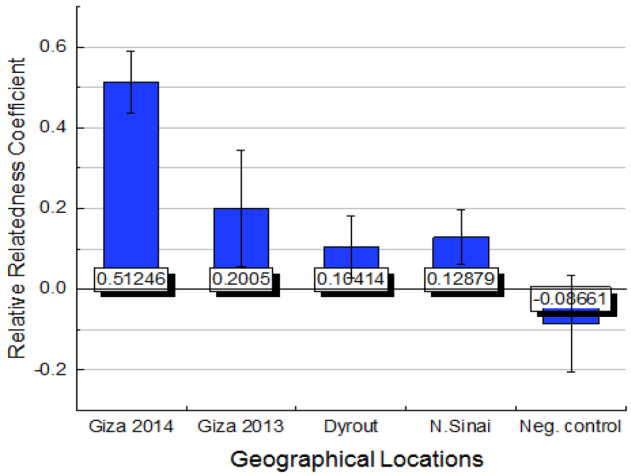

Fig. 6. The relative relatedness coefficient of *C. megacephala* samples is expressed in the graph, with bars indicating the mean and standard deviation of pairwise comparisons within each sample. The negative control bar was generated by selecting one individual from each region at random to minimize relatedness.

Average number of correctly assigned specimens among:				
Allocated to:	**Giza 2013**	**Giza 2014**	**Dayrout**	**N. Sinai**
Giza 2013	1000			
Giza 2014		1000		
Dayrout			999	
N.Sinai				1000
None	0	0	1	0

Table 4. MCMC simulation of 1000 randomly generated genotypes for each *C. megacephala* sample and their allocation success when minimum likelihood difference (MLD) = 7.

Number of specimens among:				
Allocated to:	**Giza 2013**	**Giza 2014**	**Dayrout**	**N. Sinai**
Giza 2013	27	0	1	0
Giza 2014	0	24	0	0
Dayrout	0	0	24	0
N.Sinai	0	0	0	14
None	3	0	1	4

Table 5. *Re-allocation* results of 98 *C. megacephala* individuals when minimum likelihood difference (MLD) = 7.

various MLDs, ranging from 0 to 6, which resulted in 100% correct allocation of all genotypes. The MLD of 7 was found to be the optimal balance between minimizing erroneous non-assignment and incorrect assignment. When the MLD was set at 7, only one genotype was not assigned to any putative population, with the highest number of non-assignments observed for MLD values greater than 7. These findings are presented in Table 4.

When the Re-allocation procedure was executed with MLD = 7, it resulted in 89 individuals (90.81%) being unambiguously assigned to their respective source population, while 1 individual (1.02%) was mis-allocated and 8 individuals (8.16%) could not be assigned to any population of origin, as presented in Table 5.

The calculation of the assignment success rates involved dividing the number of correctly assigned individuals by the total number of assignments. Despite employing a relatively high level of stringency for the MLD, the simulation yielded a high success rate as anticipated, with 98.97% for the Simulation and 90.81% for the Re-allocation. While the success rate of the Simulation run represents an optimistic estimate, the Re-allocation success rate provided a more realistic result for each source sample.

Discussion

The study demonstrated that AFLP can generate high-resolution genetic markers in natural populations of *Chrysomya megacephala* in Egypt. The technique has proven to be a robust and sensitive method for distinguishing genetic profiles from different geographic origins, as reported by[38,39]. AFLP has been successfully applied in various fields requiring accuracy, such as genome mapping[40], fingerprinting[41], genetic diversity studies[42,43], and paternity tests[44,45]. The reliability and reproducibility of AFLP have been established in several studies, including[19,20,27,46,47]. The error rate for *C. megacephala* was low at 3.72%, which agrees with the results obtained by[13] for *Phormia regina* (4.25%) and by[20] for *C. megacephala* (3%).

The results of the molecular variance analysis (AMOVA) indicated a high level of genetic variation within blow fly individuals (ΦPT = 69%). This finding suggests panmixia, as it shows that geographic and temporal groupings do not significantly hinder gene flow among individuals. This result was consistent with previous studies by[13,19,20,48], who found that flies collected from the same region share no more alleles than those collected from different regions. Conversely, a low genetic diversity of 14.38% was observed within *C. megacephala* populations from periurban areas of Malaysia[49]. One possible explanation for this is the decreasing trend of genetic variability in urban habitats[49]. This pattern has also been observed in genetic studies of other insects, such as sand flies, mosquitoes, and Jerusalem crickets[50–52].

The majority of genetic variation was found within the Egyptian *C. megacephala* population, while the overall genetic variation among individuals from different geographic locations was low. This is evidenced by a small ΦPR value of 9% and a low F_{ST} value of 0.158. These findings are expected due to the absence of geographic barriers that would restrict the movement of blow flies, coupled with the extensive daily movement of goods, humans, and other resources that facilitate dispersal and mating, contributing to genetic uniformity within the Egyptian fauna. Similar low genetic variation among sites was reported for invasive *C. megacephala* populations in Florida, where ΦPR was 7% and F_{ST} was 0.02[20]. Furthermore, the endemic *Chrysomya latifrons* in Australian rainforests also showed no significant differences between geographic populations (0.00%, F_{ST} = 0.00)[53]. In contrast, the native American species *P. regina* and *L. sericata* exhibited moderate genetic variation among samples, with Φ_{SC} values of 23% and 22%, and F_{ST} values of 0.112 and 0.1, respectively[13,14]. Conversely, the endemic *C. megacephala* in Malaysia displayed high genetic variation, where 85.6% of the total variation was attributed to differences among populations, supported by a high F_{ST} value of 0.8562, likely due to the Titiwangsa mountain range acting as a natural barrier between the East and West coasts of Peninsular Malaysia[49].

Incorporating time as a factor in the AMOVA analyses in this study revealed a moderate level of genetic variation (ΦRT = 22%, $p = 0.001$), indicating that the genetic profile of the Egyptian *C. megacephala* population changes over time. This observation aligns with findings in *P. regina* from the United States[13] and has also been noted in other organisms, such as gastropods[54] and Atlantic salmon[55].

Our study did not detect isolation by distance, and the low ΦPR and F_{ST} values were corroborated by STRUCTURE and PCoA results, which indicated no geographic structure in the Egyptian *C. megacephala* populations. Notably, samples collected over time showed significant changes, suggesting that individuals collected at one time in a specific location could differ markedly from those collected a year later. Consequently, PCoA and STRUCTURE analyses grouped *C. megacephala* into two clusters based on temporal aspects. Conversely, and despite the low genetic variance among sites and the low F_{ST} values, STRUCTURE and PCoA analyses identified two subpopulations of *C. megacephala* from Florida[20], indicating minimal geographic structure. The average ΦPT values within the two clusters were 0.002 and 0.074, respectively, while the value among clusters was 0.146. The authors[20] noted that cluster 2 predominates in Florida, suggesting that the observed genetic variation between clusters had limited impact on ΦPR and F_{ST}, with samples being homogenous within each cluster. The lack of geographic structure in the Egyptian blow fly populations is consistent with other studies that have similarly identified this pattern in several blow fly species worldwide[53].

The study by[56] found moderate gene flow among individuals of *Lucilia cuprina* populations in New Zealand, suggesting a high degree of migration despite the presence of geographic barriers such as the Ruahine and Tararua ranges. The authors attributed this to the ease of transportation and hitchhiking on fly-stricken sheep. Microsatellite analysis of 60 individuals of *P. regina* collected from 7 different locations in Canada failed to detect any population structure in black blow flies[48]. Random amplified polymorphic DNA (RAPD) was used to investigate population differentiation in *L. sericata* across Europe, but no genetic isolation by distance was detected, and mtDNA confirmed the result[57]. Samples over time may exhibit changes, and therefore samples collected at one time in a certain location may be more different from samples collected later, as observed in the *C. megacephala* data collected in this study. A temporal study of *C. megacephala* collected from Giza in 2013 and 2014 showed moderate temporal separation (ΦRT = 22%, $p = 0.001$). Despite being panmictic, the mean relative relatedness detected for the blow flies studied in this work was $r = 0.321 \pm 0.068$. It was observed that *Chrysomya* individuals in this study coming to a bait over a short period of time were highly related, and the mean relatedness within samples was always positive.

It is postulated that *Chrysomya* siblings hatch from eggs, develop together as larvae, and emerge as adults at the same location and time[58]. It is likely that such adults respond to the same olfactory stimuli of the same decayed animal on which they all commence laying eggs, thereby initiating a new life cycle. This pattern is similar to the findings reported by[13,19,20,48,59]. To ensure that captured specimens contain as much genetic diversity as possible, it is crucial to conduct fly collections over multiple events in both space and time. This is critical for establishing laboratory colonies from which growth data can be used to estimate the postmortem interval. Larvae originating from colonized flies developed from a single collection of founder parents have less variability compared to wild-caught ones[60]. As a result, if the development duration of this colony is longer than that of the crime scene larva's source population, then the PMI could be overestimated. If the development duration of this colony is shorter than the crime scene larva's source population, then the PMI could be underestimated using this data to estimate the larva's age.

In some cases, a larva may be left behind at the original crime scene before the movement of the corpse[19], and if this larva shows the same relatedness pattern to the larval community on the corpse as that observed among adults attracted to a bait, it may be possible to use AFLP and relatedness data to infer postmortem movement of a corpse. Forensic entomologists can associate abandoned larvae with their source population using population assignment methods, particularly when F_{ST} is high, thereby increasing the likelihood of assigning an individual to it is population of origin based on the differences in allele frequency.

This method has an advantage over gut content analysis method[61] for the same purpose because DNA of larvae or pupae is more likely to be found in a suspected crime scene while DNA of a victim is gradually degraded in the gut and can be retrieved only up to two days after movement of a maggot to another food source and up to the fourth day if maggots were kept alive and starved[62]. The weakness point of AFLP assignment procedure is the presence of enormous number of larvae on victim's body which makes it difficult to relate a stray larva to it is potential siblings of the same size and age. However, more work should be done to solve this complication. In this study, the fixation index F_{ST} = 0.158 ± 0.422 and the total gene diversity H_T= 0.207 for *C. megacephala* samples were moderately high and that decreased the discrepancy (8.16%) between the upper bound "*Simulation*" and the realistic "*Reallocation*" success rates leading to a high overall assignment success rate for samples. Also, the high stringency level (MLD = 7) further increased the reliability of the assignment test hence, the possibility to reliably assign a stray larva to it is population of origin increases. So, the low genetic variation observed among the Egyptian populations (ΦPR = 9%) implies a potential conservativeness in the genetic structure of Northern Egyptian populations and the genetic profile of *C. megacephala* could be used to infer corpse postmortem relocation. Our results agree with that obtained by[19] for *L. sericata* population in North America in the possibility of using them to detect movement of a corpse. Unfortunately, this wasn't the case for *C. megacephala* from Florida[20] which showed little genetic variation among samples and low value of F_{ST} (0.021 ± 0.242) resulted in poor performance of assignment analysis and lack of reliability when used to infer corpse movement.

Data availability

All data generated or analyzed during this study are included in this published article.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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