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Population diversity of sheep bot fly, *Oestrus ovis* (Linné, 1758) (Diptera: Oestridae: Oestrinae), using SDS polyacrylamide gel electrophoresisMustafa Kamal^{a,*}, Ghazala Yasmeen^b, Noor Us Saher^c, Farah Naz^c, Naseem Rafiq^a, Wali Khan^d, Muhammad Anwar Iqbal^e, Muhammad Kabir^f, Safia Mushtaq^g, Habib Ul Hassan^{b,g}, Tahir Usman^{h,*}^a Department of Zoology, Abdul Wali Khan University, Mardan 23200, Pakistan^b Department of Zoology, University of Karachi, Karachi 75270, Pakistan^c Centre of Excellence in Marine Biology University of Karachi, Karachi-75270, Pakistan^d Department of Zoology, University of Malakand, KPK, Pakistan^e Department of Zoology, Gomal University, Dera Ismail Khan, Tank Campus, Pakistan^f Department of Biological Sciences, University of Sargodha, Sub-Campus, Bhakkar 30000, Punjab, Pakistan^g Fisheries Development Board, Ministry of National Food Security and Research, Pakistan^h College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University, Mardan 23200, Pakistan

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ABSTRACT

The study was planned to evaluate the inter, and intra population genetic variation in general protein banding pattern in *Oestrus ovis* larvae, by using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). The larvae were collected from slaughtered goats head from five different locations (AAS, PN, LA, GM, and BC) of Karachi, Pakistan. The data obtained was subjected to POPGENE (Population Genetic Analysis) software for analysis. The polymorphic loci within populations ranged from 45.45% to 90.91%. Polymorphic loci observed in all populations were 90.91%. The expected heterozygosity observed was 0.182 ± 0.096 in all populations. The chi-square test showed 5 out of 11 loci at H-W equilibrium. The overall fixation index (FST) value was 0.108, showing that the likelihood of subpopulations being differentiated from one another is about 11 percent. The gene flow value ($Nm = 2.065$) was higher, showing that genes flow occurs between populations. The values of genetic identity were greater, and genetic distance were smaller among all the populations, which means that all the populations were more alike and closer to each other. It was concluded that there was no sympatric and parapatric population differentiation observed among all the population of *O. ovis* and the populations of the five different locations were not genetically and reproductively isolated from each other.

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1. Introduction

Oestrus ovis larvae is a widely distributed parasite, mostly live in the horns, frontal sinuses, and nasal cavities of goats and sheep. This parasite consequently leads to myiasis, termed nasal Oestrosis (Kamal et al., 2021a,b). Oestrosis infections spread worldwide, predominantly in the Mediterranean regions of Europe and Africa

(Ahaduzzaman, 2019). Oestrosis infection starts when adult flies lay eggs in the nostrils of the sheep and goats. Tiny transparent white 1st stage larvae hatch and move inside the nasal cavity and feed on the mucous membrane for their development and growth (Tabouret et al., 2003). The larval period is short (1–10 months), when larvae fully developed it shrinks its size and changes to pupae and gets down from nasal cavity to pupariate in the ground litter, and after 23–28 days, adults come out prepared for mating (Cepeda-Palacios and Scholl, 2000). This infection causes acute clinical manifestations, including breathing disorders (snoring respiration, breathlessness), mucopurulent discharge, anorexia, and weakness (Hoyer et al., 2016). Afterward, Myiasis abscess in the lungs leads to starvation that might cause death (Kamal et al., 2021a,b). Occasionally, larvae move from frontal sinuses and nasal cavity to the brain leading to false gid (Patra et al., 2018). Ultimately the pathogenic effects lead to considerably

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less animal production and significant economic losses to the agricultural industry as estimated roughly as 200–500 gm of wool, 1.1–4.6 kg of meat, and up to 10 % milk per animal (El-Tahawy et al., 2010; Ipek, 2018).

The electrophoretic study of proteins is used to clarify species taxonomic position and the evolutionary relationships of populations within and between species. Protein gel electrophoresis is used to determine genetic diversity within the populations of the same species as well as within different species (Odhano et al., 2018). This investigation is further used to differentiate and identify two morphologically similar species on the genetic level (Naz et al., 2017). This study was planned to discover inter and intra population genetic variation in general protein banding patterns in *O. ovis* larvae collected from five different areas of Karachi, Pakistan.

2. Materials and methods

2.1. Sample's collection and identification

O. ovis larvae were collected from infected goats head from five locations [(Al-Asif Square (AAS), Paposh Nagar (PN), Liaquatabad (LA), Gol Market (GM), and Bhains Colony (BC)] of Karachi, Pakistan (Fig. 1). A total of 110 larvae (22 larvae from each location) were collected. The larvae were collected in small bottles, labeled accordingly, immediately stored in the icebox, and transferred to the laboratory. The larvae were studied under a microscope and identified as *O. ovis* larvae based on the keys described by Zumpt (1965). Then the larvae were stored at -20°C for further processing.

2.2. Protein extraction and SDS PAGE

PAGE was used to study the total protein banding pattern of *O. ovis* larvae. Before electrophoresis analysis, the process of protein extraction was carried out. *O. ovis* larvae were crushed and homogenized in Tris-citrate buffer by hand homogenizer. The homogenate was drawn into a 1.5 ml Eppendorf tube and labeled accordingly. Then all the Eppendorf tubes were centrifuged at

15000 rpm for 15 min. The supernatants were transferred to new labeled Eppendorf tubes. The extract was either used instantly for electrophoresis or store at -20°C until further analysis (Naz et al., 2017). For general protein, 10% SDS PAGE was carried out as described by Laemmli (1970) in the discontinuous buffer system under reducing conditions.

2.3. Staining and de-staining of gel

Staining solution: Dissolve 0.5 g Coomassie brilliant blue in 12.5 ml methanol and 18.75 ml acetic acid. Make volume up to 250 ml with distilled water. Thereafter, the solution was stored at room temperature. Destining solution: Mix together 150 ml of acetic acid and 100 ml of methanol. Make up to two liters. Thereafter, the solution was stored at room temperature (Ali et al., 1995).

The obtained gel was transferred to a petri dish and washed with distilled water. Then the staining solution was added, and the gel was left in for 24 h. After removing the stain solution, the gel was rinsed with distilled water. Then the de-staining solution was added and allowed the gel to de-stain for 24 h.

2.4. Banding pattern analyses

After getting visible band patterns on the gel, the protein banding patterns were documented through photography with the help of a camera. The bands were considered as locus following the nomenclature system of Harris and Hopkinson (1976).

2.5. Data analysis

All the data calculations for genetic variation were carried out with PopGene (version 1.32) software (Yeh et al., 1999). The software was used to find out the percentage of polymorphic loci, observed homozygosity and expected homozygosity (Levene, 1949), observed heterozygosity and expected heterozygosity (Levene, 1949) and Nei's Heterozygosity (Nei, 1973). The observed number of allele, effective number of allele (Kimura and Crow, 1964), Shannon's Information index (Lewontin, 1972),

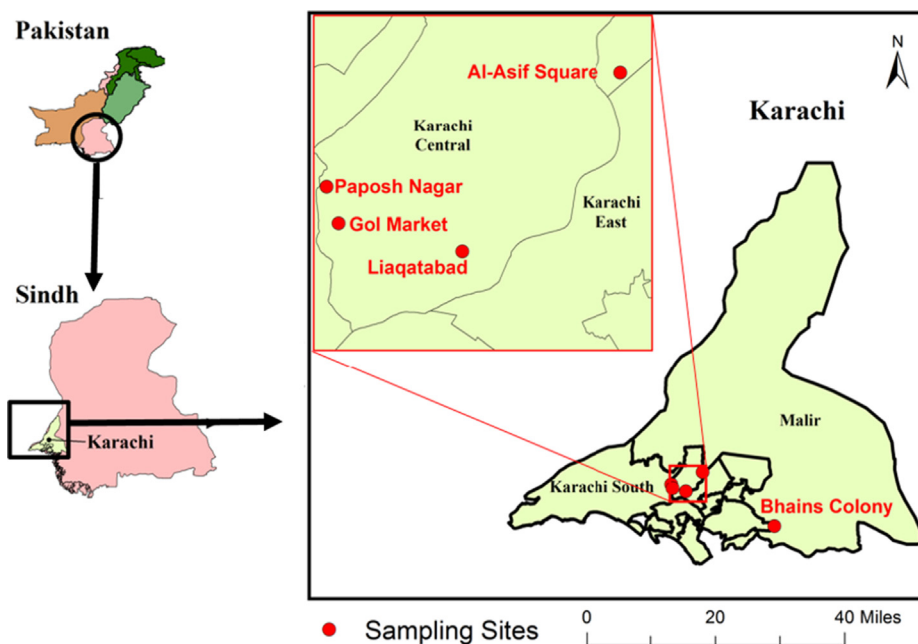


Fig. 1. Map of the five sampling sites of Karachi, Pakistan.

Summary of gene flow and F-statistics (Nei, 1978), Genotype frequency, Chi-square test for Hardy-Weinberg equilibrium were also calculated. The mean unbiased genetic distance (D) and genetic identity (I) were calculated from the allelic frequencies according to Nei (1972).

3. Results

General protein banding patterns were used to examine the genetic variability among the five populations of *O. ovis* (AAS, PN, LA, GM, and BC) using 10% SDS PAGE. A total of eleven loci were determined among these five populations. The highest percent (90.191%) of polymorphic loci was observed in the population of PN. In comparison, the lowest and same percentage (45.45%) of Polymorphic loci was observed in three populations (LA, GM, and BC). The polymorphic loci observed in all populations were 10 (90.91%) (Table 3). Allele A was found in higher frequency than that of allele B among all the populations (Table 1). Total five loci appeared with non-significant value during the Hardy-Weinberg equilibrium (χ^2) test. While the other six loci showed a significant difference, which meant that they deviated from the Hardy-Weinberg equilibrium (Table 2).

The F statistics were also calculated among *O. ovis* populations for genetic structure and gene flow. The average FIS value across the entire loci was -0.293 . The FIS value of all loci was negative, indicating an excess of observed heterozygotes. The overall fixation index value was ($F_{ST} = 0.108$), showing that the likelihood of sub-populations being differentiated from one another is about 11 percent. The gene flow value ($N_m = 2.065$) was higher, showing that genes' flow occurs between populations (Table 2).

Nei's heterozygosity was estimated in 5 populations from the overall mean allele variability and was observed different in all the populations. The maximum Nei's heterozygosity was observed in the AAS population, which was 0.233 ± 0.178 , and the minimum 0.104 ± 0.134 was observed in the LA population. The mean Nei's heterozygosity observed among all the populations was 0.181 ± 0.096 (Table 3).

Among all the five populations, maximum allele variation (I) was found in AAS population, which was 0.360 ± 0.257 . The lowest one 0.174 ± 0.215 was observed in the LA population (Table 4).

The maximum value of Nei's genetic identity ($I = 0.992$) and the minimum value of genetic distance ($D = 0.008$) was observed

between GM and LA populations, which means that these two populations were more similar and less distant from each other. The values of genetic identity were greater and genetic divergence were smaller among all the populations (Table 5). The dendrogram based on Nei's (1972) genetic distance showed that the LA, GM, and BC populations were close to each other as they were in the same cluster (Fig. 2).

4. Discussion

Protein gel electrophoresis is used to determine genetic diversity within the populations of the same species and different species. This investigation is further used to differentiate and identify two morphologically similar species on the genetic level (Naz et al., 2017; Sujatha et al., 2011). The current investigation was designed to discover population differentiation and genetic variation in five populations of *O. ovis*.

In the current study, the percentage of polymorphic loci within populations ranged from 45.45% to 90.91%. The percent polymorphic loci observed in all populations was 90.91%. The observed percentage of polymorphic loci was higher than that previously recorded in the populations of other dipteran insects such as 67.2% and 56.8% in *Aedes aegypti* (Shi et al., 2017; Gokhale et al., 2015), 27.5% in *Culex tritaeniorhynchus* (Kanojia et al., 2010), 54.17% in *Musca domestica* (Taşkin et al., 2011), 63.6% in *Simulium maroniense*, 36.4% in *Simulium rorotaense*, 54.5% in *Simulium trombetense*, 18.2% in *Simulium perflavum* (Scarpassa and Hamada, 2003), and 61.5% in *Lutzomyia shannoni* (Cárdenas et al., 2001).

In the current study, the Nei's heterozygosity calculated was observed higher (0.233 ± 0.178) in the population of AAS and observed lower (0.104 ± 0.134) in the LA population. The expected heterozygosity observed in the five populations ranged from 0.109 ± 0.140 to 0.245 ± 0.187 . The overall expected heterozygosity in all populations was 0.182 ± 0.096 . This expected heterozygosity was higher than the mean value observed in 31 species of *Drosophila* ($H_{exp} = 0.135$), 39 species of other dipterans ($H_{exp} = 0.115$) (Graur, 1985). The heterozygosity observed was also high from that observed in other dipteran such as *Culex tritaeniorhynchus* (Kanojia et al., 2010), *Simulium maroniense*, *S. rorotaense*, *S. trombetense*, *S. perflavum* (Scarpassa and Hamada 2003). The present result was found less than that calculated in populations of *Aedes aegypti* (Shi et al., 2017), *Lutzomyia shannoni* (Cárdenas et al., 2001). The

Table 1
The description of each locus allele frequency observed in the five *O. ovis* populations.

| Locus | Alleles | PN | AAS | LA | GM | BC |
|-------|---------|--------|--------|--------|--------|--------|
| GP-1 | A | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| | B | | | | | |
| GP-2 | A | 0.8636 | 0.7273 | 1.000 | 0.8636 | 1.000 |
| | B | 0.1364 | 0.2727 | | 0.1364 | |
| GP-3 | A | 0.8545 | 1.000 | 0.8636 | 0.7727 | 0.7727 |
| | B | 0.1455 | | 0.1364 | 0.2273 | 0.2273 |
| GP-4 | A | 0.8909 | 0.7727 | 1.000 | 0.8636 | 0.8182 |
| | B | 0.1091 | 0.2273 | | 0.1364 | 0.1818 |
| GP-5 | A | 0.8364 | 0.5455 | 1.000 | 1.000 | 0.8182 |
| | B | 0.1636 | 0.4545 | | | 0.1818 |
| GP-6 | A | 0.8545 | 0.7273 | 0.8636 | 0.8636 | 0.8182 |
| | B | 0.1455 | 0.2727 | 0.1364 | 0.1364 | 0.1818 |
| GP-7 | A | 0.8364 | 0.8182 | 0.7727 | 0.9091 | 1.000 |
| | B | 0.1636 | 0.1818 | 0.2273 | 0.0909 | |
| GP-8 | A | 0.8909 | 1.000 | 0.8636 | 1.000 | 0.7273 |
| | B | 0.1091 | | 0.1364 | | 0.2727 |
| GP-9 | A | 0.9727 | 0.9091 | 1.000 | 1.000 | 1.000 |
| | B | 0.0273 | 0.0909 | | | |
| GP-10 | A | 0.8909 | 0.8182 | 0.9545 | 1.000 | 1.000 |
| | B | 0.1091 | 0.1818 | 0.0455 | | |
| GP-11 | A | 0.9636 | 0.9091 | 1.000 | 1.000 | 1.000 |
| | B | 0.0364 | 0.0909 | | | |

Table 2

The probability of Chi-square statistical analysis calculated F statistics values for the overall total population (FIT), individual in total population (FIS), among subpopulations (FST), and gene flow (Nm) of *O. ovis* Larvae from five different populations.

| Locus | Sample size | H-W Equilibrium | | FIS | FIT | FST | Nm |
|-------------|-------------|-----------------|---------|---------------|---------------|--------------|--------------|
| | | χ^2 | P-value | | | | |
| GP-1 | 110 | 0.000 | 1.000 | – | – | 0.000 | – |
| GP-2 | 110 | 13.89 | 0.007* | –0.325 | –0.158 | 0.126 | 1.729 |
| GP-3 | 110 | 6.143 | 0.188 | –0.239 | –0.170 | 0.056 | 4.226 |
| GP-4 | 110 | 9.914 | 0.041* | –0.234 | –0.122 | 0.090 | 2.524 |
| GP-5 | 110 | 22.31 | 0.000* | –0.500 | –0.196 | 0.203 | 0.982 |
| GP-6 | 110 | 6.875 | 0.142 | –0.248 | –0.170 | 0.063 | 3.750 |
| GP-7 | 110 | 9.698 | 0.045* | –0.311 | –0.196 | 0.088 | 2.586 |
| GP-8 | 110 | 11.78 | 0.019* | –0.257 | –0.122 | 0.107 | 2.083 |
| GP-9 | 110 | 5.482 | 0.241 | –0.082 | –0.028 | 0.050 | 4.766 |
| GP-10 | 110 | 17.39 | 0.001* | –0.333 | –0.122 | 0.158 | 1.331 |
| GP-11 | 110 | 6.226 | 0.182 | –0.100 | –0.038 | 0.057 | 4.167 |
| Mean | 110 | | | –0.293 | –0.153 | 0.108 | 2.065 |

Nm = Gene flow estimated from $FST = 0.25(1 - FST)/FST$.

H-W Equilibrium = Hardy-Weinberg equilibrium

* = Significant P-value

Table 3

The inter-population genetic variations among the five populations of *O. ovis* larvae.

| Population | Obs-Hom±SD | Exp-Hom±SD | Obs-Het±SD | Exp-Het±SD | Nei±SD | PL | % PL |
|---------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|-----------|---------------|
| PN | 0.792 ± 0.115 | 0.818 ± 0.096 | 0.208 ± 0.115 | 0.182 ± 0.096 | 0.181 ± 0.096 | 10 | 90.91% |
| AAS | 0.678 ± 0.286 | 0.756 ± 0.187 | 0.322 ± 0.286 | 0.245 ± 0.187 | 0.233 ± 0.178 | 8 | 72.73% |
| LA | 0.877 ± 0.164 | 0.891 ± 0.140 | 0.124 ± 0.164 | 0.109 ± 0.140 | 0.104 ± 0.134 | 5 | 45.45% |
| GM | 0.868 ± 0.165 | 0.884 ± 0.141 | 0.132 ± 0.165 | 0.117 ± 0.141 | 0.111 ± 0.135 | 5 | 45.45% |
| BC | 0.810 ± 0.224 | 0.844 ± 0.182 | 0.190 ± 0.224 | 0.156 ± 0.182 | 0.149 ± 0.174 | 5 | 45.45% |
| Overall mean value | 0.792 ± 0.115 | 0.818 ± 0.096 | 0.208 ± 0.115 | 0.182 ± 0.096 | 0.181 ± 0.096 | 10 | 90.91% |

PL = Polymorphic Loci, Hom = Homozygosity, Het = Heterozygosity, Obs = Observed, Exp = Expected, SD = Standard deviation

Table 4

Summary of genetic variation statistics of all loci Nei (1978) in five populations of *O. ovis*.

| S. No | Population | na ± SD | ne ± SD | I ± SD |
|---------------------------|------------|----------------------|----------------------|----------------------|
| 1 | PN | 1.909 ± 0.302 | 1.234 ± 0.133 | 0.312 ± 0.150 |
| 2 | AAS | 1.727 ± 0.467 | 1.371 ± 0.324 | 0.360 ± 0.257 |
| 3 | LA | 1.454 ± 0.522 | 1.142 ± 0.191 | 0.174 ± 0.215 |
| 4 | GM | 1.454 ± 0.522 | 1.151 ± 0.191 | 0.185 ± 0.219 |
| 5 | BC | 1.456 ± 0.522 | 1.225 ± 0.266 | 0.231 ± 0.268 |
| Overall Mean value | | 1.909 ± 0.302 | 1.234 ± 0.133 | 0.312 ± 0.150 |

na = Observed number of alleles

ne = Effective number of alleles Kimura and Crow, (1964)

I = Shannon's Information index Lewontin (1972)

SD = Standard deviation

Table 5

Nei's genetic distance (below diagonal) and genetic identity (above diagonal) of *O. ovis* larvae estimated from populations.

| Population | PN | AAS | LA | GM | BC |
|------------|-------|-------|-------|-------|-------|
| PN | **** | 0.968 | 0.978 | 0.971 | 0.959 |
| AAS | 0.033 | **** | 0.956 | 0.963 | 0.960 |
| LA | 0.022 | 0.045 | **** | 0.992 | 0.985 |
| GM | 0.029 | 0.038 | 0.008 | **** | 0.986 |
| BC | 0.042 | 0.041 | 0.015 | 0.014 | **** |

expected heterozygosity observed in *O. ovis* was nearly the same as that observed in *Aedes aegypti* (Gokhale et al., 2015) and *Musca domestica* (Taşkin et al., 2011).

The frequency of allele A was higher than allele B. The observed number of alleles (na), effective number of alleles (ne), and Shannon's Information index (I) within the populations ranged from 1.909 to 1.454, 1.371 to 1.142, and 0.360 to 0.174, and among all the populations were 1.909, 1.234 and 0.312. The effective number of alleles (ne) observed in other dipteran was 1.56 in *Aedes aegypti*

(Gokhale et al., 2015), 1.23 in *Culex tritaeniorhynchus* (Kanojia et al., 2010), and 1.31 in *Musca domestica* (Taşkin et al., 2011).

The genetic identity and genetic diversity calculated in five populations of *O. ovis* ranged from 0.956 to 0.992 and 0.008 to 0.045. Overall, the genetic identity calculated was high and the genetic diversity observed was very low, which indicated that all these populations were genetically close to each other. The exact values of genetic identity and genetic distance were also observed among the populations of other dipterans such as in *Musca domestica*

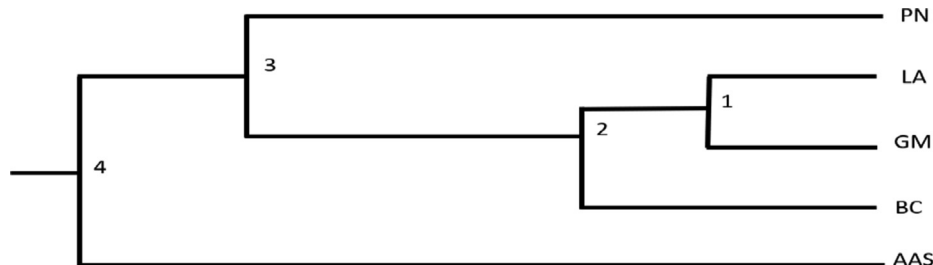


Fig 2. The dendrogram of genetic distance between five populations of *O. ovis* based on Nei's (1972) genetic distance [Method = Unweighted Pair-Group Method with arithmetic mean (UPGMA)].

(Taşkin et al., 2011), and *Lutzomyia shannoni* (Cárdenas et al., 2001).

5. Conclusion

The genetic identity calculated was high, and the genetic diversity observed was very low, which indicates that all these five populations of *O. ovis* were genetically close to each other. Heterozygosity was observed in all five populations of *O. ovis*. Results confirmed that there was no sympatric and parapatric population differentiation observed among all the population of *O. ovis* and the populations of the five different locations were not genetically and reproductively isolated from each other.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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