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Population structure and geographical subdivision of the *Leishmania major* vector *Phlebotomus papatasi* as revealed by microsatellite variation

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Abstract. Multi-locus microsatellite typing (MLMT) has been employed to infer the population structure of *Phlebotomus papatasi* (Scopoli) (Diptera: Psychodidae) sandflies and assign individuals to populations. *Phlebotomus papatasi* sandflies were collected from 35 sites in 15 countries. A total of 188 *P. papatasi* individuals were typed using five microsatellite loci, resulting in 113 different genotypes. Unique microsatellite signatures were observed for some of the populations analysed. Comparable results were obtained when the data were analysed with Bayesian model and distance-based methods. Bayesian statistic-based analyses split the dataset into two distinct genetic clusters, A and B, with further substructuring within each. Population A consisted of five subpopulations representing large numbers of alleles that were correlated with the geographical origins of the sandflies. Cluster B comprised individuals collected in the Middle East and the northern Mediterranean area. The subpopulations B1 and B2 did not, however, show any further correlation to geographical origin. The genetic differentiation between subpopulations was supported by *F* statistics showing statistically significant (Bonferroni-corrected $P < 0.005$) values of 0.221 between B2 and B1 and 0.816 between A5 and A4. Identification of the genetic structure of *P. papatasi* populations is important for understanding the patterns of dispersal of this species and to developing strategies for sandfly control.

Key words. *Leishmania major*, *Phlebotomus papatasi*, microsatellites, population structure, sandflies.

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Introduction

The sandfly *Phlebotomus papatasi* (Scopoli) is the vector of *Leishmania major* Yakimoff & Schokhor (Kinetoplastorida: Trypanosomatidae), which is the causative agent of cutaneous leishmaniasis in different regions of the Old World (Ashford & Bettini, 1987; Killick-Kendrick, 1990, 1999). It also transmits other human diseases, such as sandfly fever and Chandipura virus (Lane, 1993; Geevarghese *et al.*, 2005). The developmental stages can be found in a wide range of ecotopes with mild temperatures and relatively high humidity (Modi & Tesh, 1983; Erisoz Kasap & Alten, 2005). Rodent burrows and animal shelters are suspected breeding sites for these sandflies. Adults can sometimes be found in large cracks within walls and dark corners of houses (Feliciangeli, 2004). This sandfly species has a large geographical distribution extending from the western Mediterranean to the Indian subcontinent (Perfil'ev, 1966; Kaul *et al.*, 1973; Schlein *et al.*, 1982; Killick-Kendrick *et al.*, 1985; Sawalha *et al.*, 2003; Guernaoui *et al.*, 2005; Yaghoobi-Ershadi *et al.*, 2005; Shakila *et al.*, 2006). The geographical distribution of *P. papatasi* includes different climatic and ecological discontinuities, where insecticide usage varies greatly, and may set different selection patterns that are favourable to local adaptation among geographically distant *P. papatasi* populations and are associated with patterns of population divergence (Pener & Wilamovsky, 1987; Dhiman & Mittal, 2000). When different genetic markers were used to study genetic variation in *P. papatasi*, such as c*ytochrome b* (Esseghir *et al.*, 1997; Parvizi *et al.*, 2003; Hamarsheh *et al.*, 2007a), internal transcribed spacer 2 (ITS2) (Depaquit *et al.*, 2000, 2008; Hamarsheh *et al.*, 2007b) and isoenzyme electrophoresis (Kassem *et al.*, 1993; Ghosh *et al.*, 1999), it was concluded that widely separated populations of *P. papatasi* were genetically quite homogenous.

It is difficult to determine whether topographic features, such as rivers and mountains, and differences in morphology actually represent natural boundaries in terms of population genetics (Pritchard *et al.*, 2000). Therefore, to understand population differentiation and the forces influencing it, it is useful to analyse DNA sequence variation at multiple loci. This is motivated by the idea that population structure and demography affect the entire genome in a similar way, whereas other evolutionary forces (including selection) leave footprints at individual genes (Avise, 2000).

Microsatellites are tandem repeats of two to five nucleotides which are regarded as excellent molecular markers for studying population genetic structure because they are very abundant throughout the genome, co-dominantly inherited, usually neutral, and relatively easy to score (Bowcock *et al.*, 1994; Estoup *et al.*, 1995; Goldstein & Schlötterer, 1999). This approach has been used for several species, including *Drosophila* (Schlötterer *et al.*, 1997; Glinka *et al.*, 2003).

Microsatellites are currently considered the most discriminating markers used in *Anopheles gambiae* (Lanzaro *et al.*, 1995), *Lutzomyia* (Lanzaro *et al.*, 1998) and *Phlebotomus* (Aransay *et al.*, 2001; Hamarsheh *et al.*, 2006) sandflies, as well as in different *Leishmania* species (Ochsenreither *et al.*, 2006; Schwenkenbecher *et al.*, 2006).

In this study, we examined the population structure of *P. papatasi* from different endemic foci using a recently developed panel of microsatellite markers (Hamarsheh *et al.*, 2006) and identified genetically isolated populations that largely correlated with geographical origin. An understanding of the patterns of dispersal of vector species is important for developing strategies for sandfly control.

Materials and methods

Source of sandflies

A total of 188 *P. papatasi* sandflies were used in this study; 21 originated from Syria, 19 from Turkey, 26 from Palestine, 10 from Italy, 35 from Iran, 30 from Egypt, 10 from Cyprus, four from Tunisia, five from Morocco, seven from Sudan, nine from Nepal, five from India, two from Pakistan and five from Algeria (Table 1). Sandflies of colony and field origin were provided between 2000 and 2006. The locations of the study sites are shown in Fig. 1. All *P. papatasi* males were identified based on external morphological characters of abdominal terminalia (Perfil'ev, 1966). Only intact and randomly selected males and colony-bred females were used for population analysis.

DNA extraction and microsatellite genotyping

DNA was isolated from individual flies by a phenol-chloroform extraction method with slight modifications; individual flies were incubated in liquid nitrogen for 10 min, then ground dry using pre-autoclaved glass rods. DNA extraction and amplification of the five microsatellite markers were performed as described by Hamarsheh *et al.* (2006). Microsatellite fragments were analysed by electrophoresis on metaphor gels; sizes of amplified products were further confirmed by running the fluorescent-labelled polymerase chain reaction products in an automated capillary sequencer (CEQ™ 8000; Beckman Coulter, Inc., Fullerton, CA, U.S.A.). The fragment sizes were measured using the CEQ™ 8000 automated genetic analysis system.

Distance and population structure analysis

The software STRUCTURE Version 2 (Pritchard *et al.*, 2000) was used to reveal potential population structure in the dataset. The program, which uses the Bayesian clustering algorithm, was run using an admixture model with a burn-in period of 20 000 iterations, followed by 200 000 iterations of Markov chain Monte Carlo (MCMC) repeats for each setting of K from 1 to 10. No prior information about the population structure was provided. The program assumes the populations to be in Hardy–Weinberg equilibrium and the loci to be in linkage equilibrium. Based on allele frequencies, this approach probabilistically identifies genetically distinct populations by placing individuals into K populations regardless of their

Table 1. List of genotypes identified for the whole dataset in relation to geographical origin and population structure. Unique genotypes are underlined. n_{Total}, total number of samples. Colony-sourced flies are marked by an asterisk.

	Origin				
Group	Country (code)	Locality	GPS co-ordinates	n_{Total}	Genotypes collected
B	Syria (SY)	Aleppo	N 36.20, E 37.15	21	GEN1, GEN2, GEN3, GEN4, GEN5,
		Raqah	N 35.02, E 39.01		GEN6, GEN7, GEN8, GEN9, GEN10,
		Der Al-Zour	N 35.30, E 40.15		GEN11, GEN12, GEN13, GEN14, GEN15
B	Turkey (TR)	*Sanliurfa	N 37.20, E 38.8	19	GEN16, GEN17, GEN18, GEN10, GEN19, GEN20,
		Nizip	N 37.01, E 37.8		GEN4, GEN21, GEN22, GEN5, GEN23, GEN24,
		Hamdun	N 37.50, E 39.1		GEN25, GEN26, GEN27, GEN28, GEN29,GEN13
B	Cyprus (CY)	Chisochus	N 33.00, E 35.00	10	GEN15, GEN30
		Lapta	N 35.21, E 35.35		
B	Palestine (PS)	Jordan Valley	N 35.45, E 31.87	21	GEN31, GEN2, GEN32, GEN22, GEN33, GEN38,
		Qabatya	N 35.28, E 32.41		GEN10, GEN39, GEN40, GEN41, GEN42, GEN27,
		Raba	N 35.38, E 32.39		GEN43, GEN44, GEN45, GEN46, GEN47
		Al-Yamou	N 32.45, E 35.29		
B	Jordan (JO)	*Jordan valley	N 35.45, E 31.87	5	GEN34, GEN26, GEN35, GEN36, GEN37
B	Italy (IT)	*Rom	N 41.58, E 12.40	10	GEN31, GEN7, GEN36, GEN4, GEN20,
		*Rocca Priora	N 41.48, E 12.40		GEN30, GEN48
B	Egypt (EG)	Qafr Al-Sheikh	N 30.99, E 30.79	30	GEN36, GEN15, GEN3, GEN49, GEN50, GEN51,
		Sinai	N 31.28, E 34.23		GEN52, GEN53, GEN54, GEN33, GEN5, GEN55,
		Alexandria	N 31.19, E 29.91		GEN26, GEN56, GEN57, GEN27, GEN58, GEN4,
		Al-Qalyopia	N 30.00, E 31.21		GEN11, GEN20, GEN59, GEN48, GEN60, GEN29
A1	Tunisia (TN)	Sidi bu Said	N 36.87, E 10.35	4	GEN61, GEN62, GEN63
A1	Morocco (MO)	Marrakesh	N 31.36, W 8.00	5	GEN64, GEN65, GEN66, GEN67, GEN68
B	Sudan (SD)	Burri	N 15.36, E 32.35	7	GEN69, GEN70, GEN71, GEN72, GEN73
A5	Nepal (NP)	Biratnagar	N 26.48, E 87.28	9	GEN74, GEN76, GEN77
A4	India (IN)	Siroa	N 28.45, E 77.23	5	GEN108, GEN109, GEN110
A2, A3	Iran (IR)	Khorasan	N 35.70, E 47.26	35	GEN78, GEN79, GEN80, GEN81, GEN82,
		Khuzestan	N 32.00, E 53.00		GEN83, GEN84, GEN85, GEN86, GEN87,
		Balouchistan	?		GEN88, GEN89, GEN90, GEN91, GEN92,
		Azerbaijan	N 36.20, E 58.79		GEN93, GEN94, GEN95, GEN96, GEN97,
		Isfahan	N 32.43, E 51.34		GEN98, GEN99, GEN100, GEN101, GEN102,
		Tabas	N 32.48, E 51.22		GEN103, GEN104, GEN105, GEN106, GEN107
		Shiraz	N 37.43, E 45.92		
A4	Pakistan (PK)	?	?	2	GEN111, GEN112
A1	Algeria (AL)	M'Sila	N 35.71,W 4.54	5	G113, G114

geographical origin. Individuals can be assigned to multiple clusters with the membership coefficients of all those clusters summing up to 1. To accurately determine the most appropriate number of K clusters, we used the ad hoc quantity ΔK (the mean of the absolute values of the difference between successive values of the second order rate of change of the likelihood averaged over 10 runs divided by the standard deviation) (Evanno *et al.*, 2005). To verify the results, we performed a second run of STRUCTURE; the number of populations in our dataset was estimated following the outline given by (Pritchard *et al.*, 2000). The MCMC scheme was run for different values of MAXPOPS (K). For each K, STRUCTURE provides ln $P(X \mid K)$, from which we calculated the posterior probabilities of K assuming a uniform prior of K (K Є [1,2,3,4,5], as described in the STRUCTURE manual).

Based on the multi-locus microsatellite (MLMT) profiles, we calculated the genetic distance using two different measures: genetic distance based on the proportion of shared alleles (DPS) (Bowcock *et al.*, 1994), and Nei's genetic distance (Nei & Li, 1979) as implemented in the program MSA (Microsatellite Analyser) 3.0 (Dieringer & Schlötterer, 2003). The distance matrix was then imported into the program Splits Tree 4.0 (Huson & Bryant, 2006) as a Nexus file, where phylogenetic analysis was carried out using neighbour-joining (NJ) and a radial tree was re-constructed. Tree topology and branch support were assessed by bootstrapping (1000 iterations).

The genetic differentiation between populations based on (F_{ST}) was calculated using FSTAT Version 2.9.3 (Goudet, 1995). The significance of pairwise F_{ST} values was tested after Bonferroni correction by 1000 permutations. F_{ST} values > 0.25 indicate strong genetic differentiation. In order to estimate if genetic isolation coincides with increasing geographic distance using F_{ST} values, isolation by distance was examined by testing the association of linearized $F_{ST}/(1–F_{ST})$ with geographic distance in kilometres using the Mantel test implemented in the program zt (Bonnet & Van de Peer, 2002). The significance of the tests was obtained by 1000 permutations.

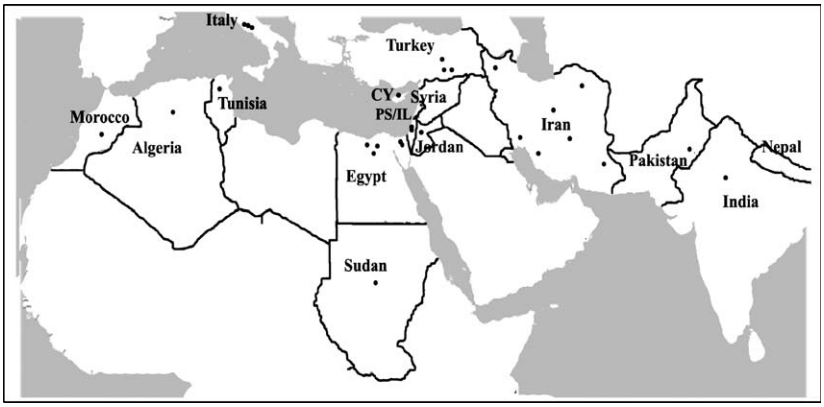


Fig. 1. Geographical origins of the populations studied from 15 different countries in the *Phlebotomus papatasi* range. Dots correspond to collection sites in each country. Detailed information on the origin of sandflies studied is shown in Table 1.

Results

Microsatellite variation

Among the 188 *P. papatasi* individuals tested, 113 different genotypes (individuals with different MLMT profiles) were identified. Of these, 96 were unique and represented by single individuals only, and 17 were shared by more than one individual (Table 1). Primer sequences, number of alleles, observed (H_O) and expected (H_E) heterozygosities are given in Hamarsheh *et al.* (2006). H_O ranged from 0.143 to 1.00 and H_E from 0.702 to 0.876. There was no significant deviation from Hardy-Weinberg equilibrium for all loci and no significant linkage disequilibrium between genotypes at different loci.

Population structure and admixture level

Using the program STRUCTURE on the whole dataset, the most appropriate number of populations was found at K = 2. Successive increases in K did not split the two major groupings into additional clusters (Fig. 2A). The ΔK-value provided support for the division into two clusters with a marked peak at K = 2 and a rapid decline at higher values. The highest posterior probability was found for two clusters (Table 2).

Population A was anchored by 58 sandflies from Tunisia, Morocco, Algeria, Nepal, India, Pakistan and the majority of the Iranian populations (IR2–7). Population B consisted of 128 sandflies from Syria, Turkey, Cyprus, Jordan, Palestine, Italy, Egypt, Sudan and one Iranian population (IR1) originating from Khorasan province. Two individuals from Morocco could not be assigned to either population because they were intermediate between A and B.

Both populations A and B were further tested for hierarchical structure and the existence of subpopulations. Population A was further subdivided into five subpopulations, A1 through A5, as confirmed by the ΔK-value which peaked at K = 5 (Fig. 2B), and the highest posterior probability was found for five subclusters (Table 2). The assignment of individuals to the different subpopulations A corresponded to their geographical origin. Subpopulation A1 included all flies ($n = 12$) from North African countries (Morocco, Algeria and Tunisia). A2 and A3 included flies ($n = 30$) from seven locations in Iran, A4 flies ($n = 7$) from India and Pakistan, and A5 flies ($n = 9$) from Nepal (Fig. 2B; Table 1).

Population B was further subdivided into two subpopulations, confirmed by the ΔK-value which peaked at K = 2 (Fig. 2C). The highest posterior probability was found for two sub-clusters (Table 2). Subpopulations B1 and B2 did not show any clear correlation to geographical origin. A total of 73 flies belonged to subpopulation B1, of which 19 came from Egypt, 16 from each of Syria and Italy, 11 from the neighbouring countries of Palestine/Israel and Jordan, six from Turkey and five from Cyprus. Subpopulation B2 comprised 55 individuals, 15 from the neighbouring countries of Palestine/Israel and Jordan, 11 from Egypt, 12 from Turkey, five from Syria, seven from Sudan and five from Khorasan province in Iran.

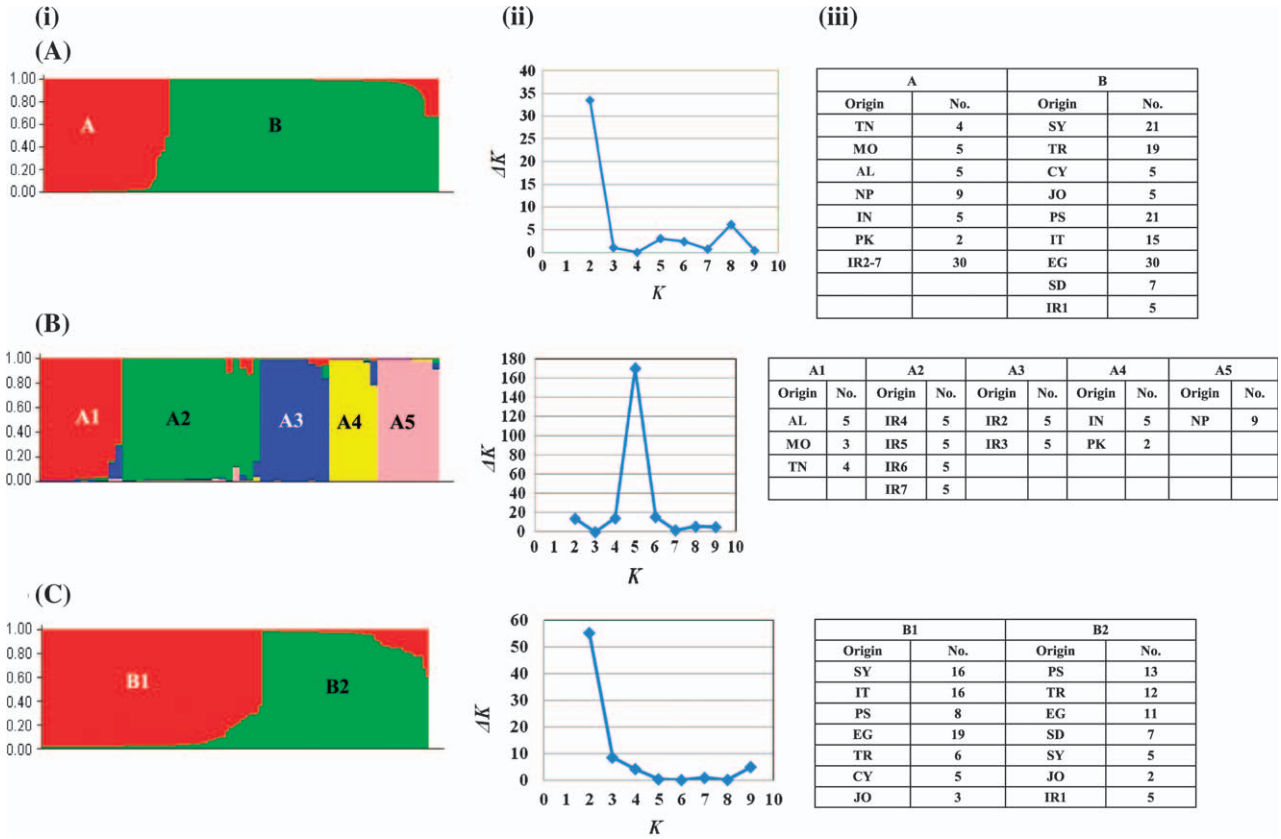
The clustering observed in NJ population tree analysis (Figs 3 and 4) was comparable with the results obtained by STRUCTURE (Fig. 2). Two major clusters, A and B, corresponding to those identified by STRUCTURE, could be recognized in the radial tree. The only difference was that genotypes GEN69–GEN73, originating from Sudan, formed a minor branch close to the 'A' cluster (Fig. 3).

Population differentiation and isolation by distance

Genetic differentiation among subpopulations of *P. papatasi* was defined by *F*-statistics. The F_{ST} estimates were calculated in a pairwise manner for the main populations and revealed high genetic differentiation between A and B ($F_{ST} = 0.292$, $P = 0.001$). When the F_{ST} was calculated at a subpopulation level among the five A subpopulations and the two B subpopulations, very great ($F_{ST} > 0.25$) and significant ($P < 0.005$) population differentiation was observed (Table 3). As illustrated in Fig. 5, the plot of $F_{ST}/(1–F_{ST})$ against geographic distance did not show isolation by distance ($r = 0.1447$, $P = 0.308$) for the seven *P. papatasi* subpopulations studied here.

Discussion

The geographical distribution of *P. papatasi* and its existence in different ecological habitats ranging from arid deserts to agricultural areas and from low- to high-altitude areas strongly



A		B	
Origin	No.	Origin	No.
TN	4	SY	21
MO	5	TR	19
AL	5	CY	5
NP	9	JO	5
IN	5	PS	21
PK	2	IT	15
IR2-7	30	EG	30
		SD	7
		IR1	5

A1		A2		A3		A4		A5	
Origin	No.	Origin	No.	Origin	No.	Origin	No.	Origin	No.
AL	5	IR4	5	IR2	5	IN	5	NP	9
MO	3	IR5	5	IR3	5	PK	2		
TN	4	IR6	5						
		IR7	5						

B1		B2	
Origin	No.	Origin	No.
SY	16	PS	13
IT	16	TR	12
PS	8	EG	11
EG	19	SD	7
TR	6	SY	5
CY	5	JO	2
JO	3	IR1	5

Fig. 2. Population structure of the 188 individuals of *Phlebotomus papatasi*. (i) Plot of estimates of *Q* (estimated membership coefficients for each individual, in each cluster) Each individual is represented by a single vertical line divided into K colours; the length of the coloured segment indicates the individual's estimated degree of kinship to that cluster. (ii) Estimation of the most appropriate population number according to Evanno *et al.* (2005) in which ΔK that evaluates the second order rate of change of the likelihood function was plotted with respect to K. (iii) Frequency of individuals assigned to the populations and subpopulations in (ii). Panel (A) represents the STRUCTURE result for the whole dataset, whereas panels (B) and (C) show substructuring obtained when STRUCTURE was re-run for each main population separately. AL, Algeria; CY, Cyprus; EG, Egypt; IN, India; IR, Iran; IT, Italy; JO, Jordan; MO, Morocco; NP, Nepal; PK, Pakistan; PS, Palestine; SD, Sudan; SY, Syria; TN, Tunisia; TR, Turkey.

suggests the existence of sibling species and/or structured populations. Population structure analysis generated by STRUCTURE (Pritchard *et al.*, 2000; Falush *et al.*, 2003), distance-based methods using the NJ tree, and *F*-statistics analysis generated consistent results, revealing the existence of a hierarchical splitting into two main populations, A and B, which are further subdivided into genetically isolated subpopulations (Fig. 2). The B population, which included the majority of *P. papatasi* sandflies studied in this project, was more homogeneous than the A population. The Bayesian analysis supported the existence of five subpopulations within A, and two within B. This fine-scale substructuring in populations has evolutionary implications. By contrast, poor dispersal capabilities of *P. papatasi* probably lead to high-level substructuring.

The validity of the population structure was further confirmed by the NJ tree that was based on genetic distances calculated from the variation in microsatellite repeat numbers (Figs 3 and 4). In line with the STRUCTURE results, two main populations and distinct subpopulations were found in neighbouring countries, such as in A1, which includes individuals from Morocco, Algeria and Tunisia. Significant genetic differentiation between A1 and A5, and B1 and B2 subpopulations was evident by high F_{ST} values that support the genetic structure in *P. papatasi* as identified by two different approaches to data analysis, namely, individual-level Bayesian cluster and genetic distance analyses. High genetic differentiation could reflect 'isolation by distance', which means that distance-dependent gene flow generally limits the genetic differences among natural populations (Wright, 1943; Slatkin, 1993). Thus, populations which are geographically close will be genetically more similar to one another. In our study, genetic and geographic distances did not correlate significantly, although population structure was detected in *P. papatasi*. The effect of geographic distance on the pattern of differentiation within this species seems to be negligible.

Population B ($n = 128$) comprised most of the sandflies from the eastern Mediterranean area, represented by two subpopulations, B1 and B2, which did not correlate with their geographical distribution (Fig. 2). The absence of geographic barriers such as high mountains, oceans or large deserts between the different *P. papatasi* populations in the eastern Mediterranean, coupled with high levels of human activity, may play a role in the transportation

Table 2. Estimated posterior probabilities of K, the number of *Phlebotomus papatasi* populations and subpopulations.

Populations	K	Ln $P(X\|K)$ 1 million iterations	$P(K\|X)$*
A, B	1	− 2644.8	0.0009
	2	− 2105.2	0.89
	3	− 1974.4	0.0099
	4	− 3252.0	0.0111
	5	− 4517.7	0.0131
A1 to A5	1	− 714.6	< 0.0001
	2	− 627.7	0.0017
	3	− 512.3	< 0.0001
	4	− 411.6	0.0027
	5	− 408.5	0.993
B1, B2	1	− 1155.5	0.0018
	2	− 1008.5	0.90
	3	− 963.0	0.0095
	4	− 1094.6	0.0104
	5	− 1058.5	0.0120

*Assuming a uniform prior of K (K = 1, 2, 3, 4, 5).

of *P. papatasi* sandflies and therefore decrease the genetic differentiation between local sandfly populations.

Recently, three populations of the parasite *L. major* from Africa, the Middle East and central Asia were identified using MLMT; these were all subdivided into two further subpopulations (Al-Jawabreh *et al.*, 2008). Whether the existence in the Middle East of two populations of *L. major* parasites and their vectors *P. papatasi* is purely coincidental or the result of co-adaptations remains to be further explored. Similar observations were made for *L. major* parasites and *P. papatasi* in Iran. Mahnaz *et al.* (2006) identified five genetic groups among strains of *L. major* from different geographic locations in Iran using ITS sequences. These five genetic groups of *L. major* were found to be related to three subpopulations of *P. papatasi* originating from the same geographic areas: parasites from Khuzestan province represented group LmA and *P. papatasi* from the same area represented subpopulation A3; parasites from Isfahan province represented group LmB and sandflies from the same area fell into subpopulation A2, and parasites from Semnan province were assigned to three different genetic groups, LmC, LmD and LmE, whereas flies from the neighbouring province of Khorasan, were assigned to subpopulation B2, which also included sandflies derived from the eastern Mediterranean region and Sudan. The emergence of new sandfly populations in Iran may have resulted from the transfer of sandflies either recently or historically by people who settled in the agricultural area stretching from the Nile River to the southeast coast of the Mediterranean Sea, around the Syrian Desert and north of the Arabian Peninsula to the Gulf. Known as the Fertile Crescent, the area has an impressive record of past human activity and transportation, which includes the migration of some of the earliest known peoples. It was unexpected, however, that individuals from the Sudan would be grouped in cluster B, which mainly consists of genetically unresolved individuals originating from the eastern Mediterranean. Why Sudanese flies could not be resolved at this level and whether more microsatellite markers should be developed that might better discriminate between *P. papatasi* from Sudan and the eastern Mediterranean are questions that remain to be resolved.

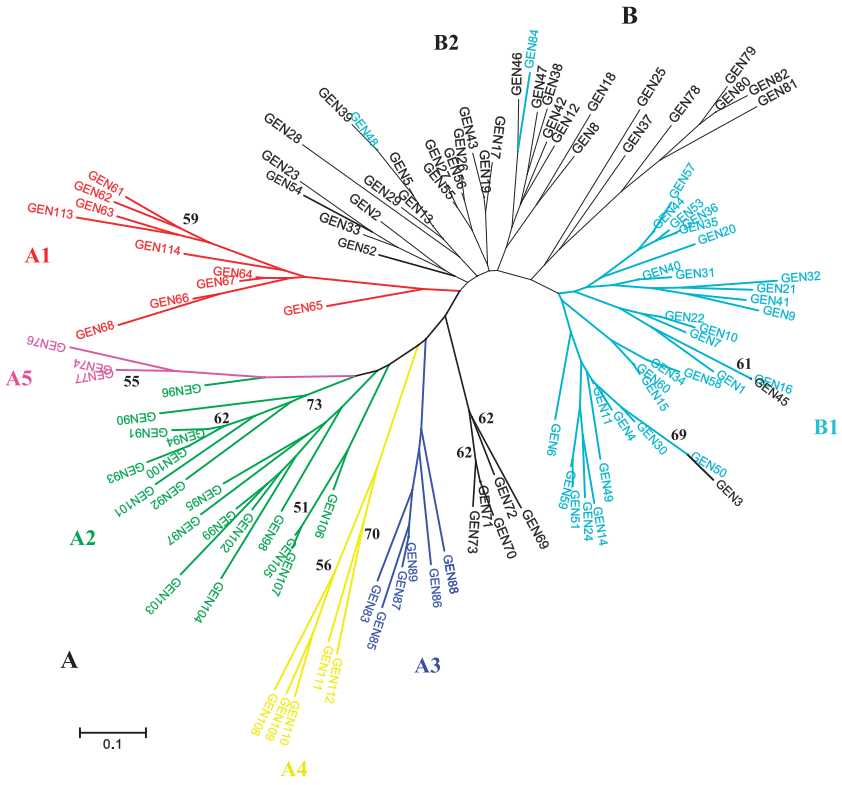


Fig. 3. A neighbour-joining radial tree of the 113 genotypes of *Phlebotomus papatasi*, derived from genetic distances based on the proportion of shared alleles (DPS). The numbers on the branches indicate the bootstrap support. Only bootstrap values > 50% are shown.

Table 3. Pairwise F_{ST} estimates†, a measure of population differentiation among the seven *Phlebotomus papatasi* subpopulations‡, based on five microsatellite loci. Abbreviations are as in Table 1.

	B1	B2	A1	A2	A3	A4	A5
B1		*	*	*	*	*	*
B2	0.221		*	*	*	*	*
A1	0.406	0.527		*	*	*	*
A2	0.374	0.525	0.446		*	*	*
A3	0.434	0.623	0.520	0.409		*	*
A4	0.519	0.652	0.588	0.468	0.641		*
A5	0.573	0.659	0.733	0.555	0.798	0.816	

*$P < 0.005$ (significant after Bonferroni correction).
†F_{ST} estimates calculated as described in (Weir & Cockerham, 1984).
‡Clusters defined according to STRUCTURE estimates for K = 5.

Surprisingly, subpopulation A4, representing individuals derived from India, was not closely related to A5, which derived from Nepal. Although the geographic distance between groups A4 and A5 is rather small, differences in topography and ecological conditions in both countries may exert effects on populations and act as barriers.

The population differentiation of *P. papatasi* could be explained by the fact that sandflies are weak fliers and cannot disperse more than 1 km, but some ecological factors, such as wind speed, humidity and high temperatures, play an important role in sandfly dispersal (Killick-Kendrick, 1999). However, estimates of genetic differentiation can be low even between populations separated by many thousands of kilometres unless there are major hydrographic or geographic barriers to migration. In this study, the F_{ST} values were high enough to suggest population isolation and geographic barriers. Alternatively, the great population differentiation manifested by high F_{ST} values could result from a relatively recent colonization (or decolonization) and be temporally unstable.

Our findings indicate that microsatellite markers can be successfully used for characterizing and establishing genotyping signatures of widely separated *P. papatasi* populations. Genetic information coupled with GIS-based approaches will be helpful for predicting gene flow in *P. papatasi* and will reveal differences in vector density and associations with land use patterns, the understanding of which is crucial for controlling cutaneous leishmaniasis caused by *L. major*.

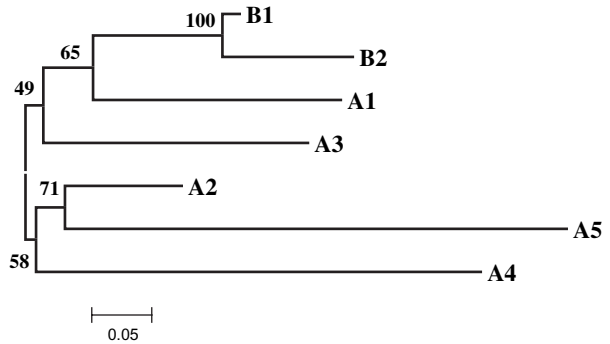


Fig. 4. Genetic distances between subpopulations pre-defined by STRUCTURE. Individuals belonging to subpopulations as inferred by STRUCTURE are shown in Fig. 2.

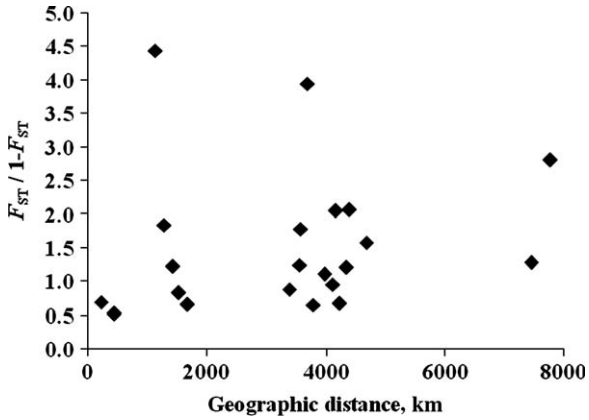


Fig. 5. Relationship between pairwise F_{ST} and geographic distance among subpopulations of *Phlebotomus papatasi.*

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