

Variation in life table characteristics among populations of *Phlebotomus papatasi* at different altitudes

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ABSTRACT: Baseline biological growth data were obtained under laboratory conditions for four local populations of the phlebotomine sand fly *P. papatasi* (Scopoli, 1786) (Diptera : Psychodidae) in different eco-regions at altitudes between 368 and 1117 m in the Sanliurfa province of Turkey. The developmental time from egg to adult was found to be significantly different among the populations: 36 days for the AKL population (368 m), 43 days for the HHR population (488 m), 45 days for the HMD population (644 m), and 49 days for the ALT population (1117 m), respectively. Based on cohorts of adults in each population, horizontal life tables were constructed. The average longevity was determined to be in the range of 8.75 ± 2.39 to 11.60 ± 3.48 days for adult females, and it was found to be slightly longer for adult males. No significant difference was found in life expectancy at emergence, e_x when $x=1$, between females and males in general ($P>0.05$) in all the populations. While significant differences could be demonstrated among populations for predictive parameters such as net reproductive rate, R_o , and generation time, T_c , no significant differences among the populations were found in terms of intrinsic rate of increase, r_m , finite rate of increase, λ , birth (b) and death (d) rates ($P>0.05$). Populations that produced offspring earlier in life also produced more total female offspring, since T_c was negatively correlated with R_o among the populations ($r = -0.686$, $0.01 < P < 0.05$). Twenty-seven parameters in all life stages, both pre-adult and adult features of *P. papatasi*, were used as physiological variables and these operational taxonomic units were analyzed using Principal Component Analysis. Analyses confirmed results from the previous morphometric and molecular studies that the Alitas population orientated and clustered as a distinct group along the first two PCs. *Journal of Vector Ecology* 31 (1): 35-44. 2006.

Keyword Index: *Phlebotomus papatasi*, geographic variation, local population, altitude, life cycle, life table, predictive parameters, Sanliurfa, Turkey.

INTRODUCTION

Phlebotomus papatasi is an anthropophilic species with a broad geographical distribution in the Palearctic Region between the 50th and 10th latitudes (Artemiev and Nerenov 1984, Killick-Kendrick 1999). It is the vector of cutaneous leishmaniasis (CL) caused by *Leishmania major* in arid and semi-arid regions in North Africa, the Middle East, and Central Asia (Anonymous 1990, Killick-Kendrick 1990, Schlein and Jacobson 1999, Hepburn 2000, Wasserberg et al. 2002).

Turkey represents a crossroad between the two continents of Europe and Asia, showing various ecological and climatic differences which are important in the epidemiology of leishmaniasis. Visceral (VL) and canine leishmaniasis caused by *L. infantum* are sporadically determined in the Aegean, Mediterranean, and Central Anatolia Regions, while CL is highly endemic in south and southeast Anatolia (Uzun et al. 1999, Ok et al. 2002, Volf et al. 2002). Sanliurfa Province, located along the border of Syria in southeastern Anatolia, is the largest focus of typical CL (Volf et al. 2002, Svobodova et al. 2003). CL has become a greater threat in recent years in the province since economic opportunities of the Southeastern Anatolia Irrigation Project (GAP) have attracted human populations to Sanliurfa (Ok et al. 2002, Alten et al. 2003).

The dominant species were *P. papatasi* and *Phlebotomus*

sergenti Parrot, 1917 in the Sanliurfa province, which is a CL endemic area in Turkey (Volf et al. 2002, Toprak 2003). The latter species is the main vector of *Leishmania tropica*, the causative agent in Sanliurfa (Alptekin et al. 1999, Volf et al. 2002). Although *P. sergenti* was found to be the dominant species in the city center (Volf et al. 2002), Toprak (2003) found *P. papatasi*, the most important probable vector of CL, to be relatively more abundant in different sites and altitudes.

The life table is a statistical model which can be used in part to characterize a population. A considerable amount of information about a population can be derived from two sets of observations: age-specific mortality rates and age-specific fertility rates (Walter and Hacker 1974). The reproductive cycles of vector arthropods play a fundamental role in the epidemiology of the diseases they transmit. The parameters affecting transmission of disease include fecundity rate, mortality rate, density, distribution by ages, migration rate, and genetic variation of the vector arthropods (Black and Moore 1996).

Toprak, A. 2003. Investigations on sandflies (Diptera:Psychodidae) species occurring in Sanliurfa and the bio-ecology of cutaneous leishmaniasis vectors. Ph.D. Thesis, Hacettepe Universitesi Fen Bilimleri Enstitüsü, Ankara, 185 pp.

The aim of this study was to investigate the effects of altitude on populations of *P. papatasi*. It was hypothesized that the differences associated with altitude may affect physiological life-table attributes and reproductive parameters such as reproductive capacity, generation time, intrinsic rate of increase, and reproductive value. None of this information is available for *P. papatasi*. To better understand baseline parameters of the life cycle of *P. papatasi*, growth of this species was monitored under laboratory conditions. Horizontal life tables were constructed based on the developmental time of each instar in a cohort of females. Additionally, predictive population parameters were calculated and stage specific mortality data were collected.

MATERIALS AND METHODS

Sand fly collections

Adult sand flies were collected from four localities in Sanliurfa province (37° 09' N; 38° 47' E), SE Anatolia, Turkey. Based on results of previous studies (Alten et al. 2003, Belen et al. 2004), four localities at different elevations were selected for sampling purposes. The Akcakale (AKL) region (36°42'42"N, 36°56'31"S) is the lowest region with an altitude of 363 m. The Hayati Harrani (HHR) (37°07'22"N, 38°48'37"S) is situated in the city center and has an altitude of 488 m. They are dry, plain, and semi-urban areas. The Hamdun (HMD) region (37°29'44"N, 39°07'80"S) is at 644 m, mountainous, and is situated in a semi-urban area. The Alitas (ALT) region (37°48'56"N, 39°40'47"S) is the highest mountainous and rural area with an altitude of 1117 m. The incidence of cutaneous leishmaniasis ranged from 1% to 2.3% in these locations (Alten et al. 2003).

P. papatasi adults were collected by aspirating from houses and barns and from animal-bait traps with cows. Collections were also made using CDC miniature light-traps during the summer (August-September) (Alexander 2000). On each trapping night, four to six light traps were placed in each of the sampling localities. Houses and barns used as sampling stations varied from two-storey cement block enclosures to simple brick, stone, or cement houses with basements, cellars, caves, or barns for keeping poultry or livestock. There had been no periodic spatial spraying of insecticides in the study area since 1996 except in the HHR region.

Field-collected live females (fed or gravid) were transported to the laboratory in standard plaster-lined containers placed on ice (Volf et al. 2002). Taxonomic identification was made using the keys and descriptions of Perfiliew (1968), Artemiev (1991) and Killick-Kendrick et al. (1991).

Maintenance of cohorts in the laboratory

The rearing and feeding of adults and larvae of *P. papatasi* in the laboratory followed the methods of Endris et al. (1982), Modi and Tesh (1983), with modifications by Ferro et al. (1998), with a temperature of $27 \pm 1^\circ\text{C}$, a relative humidity between 65-75%, and a light:dark 14:10 h period.

Fed or gravid adults of *P. papatasi* collected from

different altitudes were kept in separate cloth cages, (35 cm x 35 cm) rearing chambers, and were provided with 30% sucrose solution as an energy source. Larvae were maintained on the diet from Young et al. (1981). All of the life-table experiments were set up with the adults emerged from the eggs laid by females collected from the field. Same-aged females were fed on hamster blood daily for 1 h within 12 h of emergence. Hamsters were anaesthetized with an intraperitoneal injection of 0.3 ml (10%) ketamine hydrochloride. After blood feeding, a cohort of 50 pairs (one female and one male) was taken from the first generation (F_1) of the colonies and individual pairs were separated into rearing pots 9 cm in diameter, containing approximately 2 cm Plaster of Paris on the bottom. Each day, data were collected on the days that males and females emerged, when females blood-fed, the number of females and males remaining alive, the time of oviposition, the number of oviposited eggs for each female, the date of emergence of larvae, the number of larvae, pupation time, number of pupae, and the date of emergence of adults and sex ratio. The life-cycle parameters were estimated by tracking the development of each cohort female progeny and averaging these data. This process was repeated for each altitudes.

Calculation of predictive population parameters based on horizontal life tables

Due to a lack of standardization among recent studies on the life table attributes of adult sand flies, the calculation procedures, formulae used, and their rationale in the present study are summarized below:

- Age specific survivorship, $l_x = y_x/y_0$ where y_x = the number of females or males on each day, x .

- Age specific life expectancy, $e_x = T/l_x$, where $\sum_{x=1}^w L_x$ and $L_x = (l_x + l_{x+1})/2$ with w = the day the last individual died.

- Net reproductive rate per cohort, $Ro = \sum_{x=1}^w l_x m_x$ where m_x is the mean number of female progeny produced by female of age x . The value of m_x was calculated using the following formula: $m_x = E_x s$ where E_x is the mean number of eggs produced per female per age x , and s = the proportion of the offspring (eggs) that were females.

- Age of mean cohort reproduction (generation time), $Tc = \sum_{x=1}^w x l_x m_x / Ro$ starting at $x=1$, the day of adult emergence.

- Intrinsic rate of increase, r_m , was calculated using the original Euler-Lotka equation (Krebs 1985) where $l = \sum_{x=1}^w l_x m_x e^{-r_m \cdot x}$ and where l_x, m_x, w are as above, e is the base

of natural logarithms, x is the age interval, $l_x m_x$ is the product of the survivorship of each cohort female by its fecundity at an age x .

- The finite rate of increase was calculated with the equation: $\lambda = e^{r_m}$ where e and r_m are as above.

- According to Fisher (cited by Pianka 1988), the reproductive value (V_x) of the cohort females at the age x was formerly expressed as the relative reproductive value at the moment of birth V_0 (in practice equal 1). V_x was calculated

by the following equation:
$$\frac{V_x}{V_0} = \frac{e^{r_m x}}{l_x} \sum_{x=1}^w l_x m_x e^{-r_m x}$$
 where

e , r_m , x , $l_x m_x$ are as above.

- Under optimum insectary conditions with the requisites of life constantly available, presumably the value of r_m for each population would be relatively constant, and thus birth rate (b) could be estimated from the stable age

distribution, where $b = \ln(1 + \beta)$ and $1/\beta = \sum_{x=1}^w L_x e^{-r_m(x+1)}$.

- Death rate (d) from the stable age distribution, $d = b - r_m$.

Vertical life tables and calculation of mortality

A vertical or temporal life table for arthropod populations is based on discrete developmental stages. Apparent mortality was expressed as percentage of dead individuals in a particular stage, relative to the survivors of the same age.

$$\% \text{ Apparent Mortality} = \frac{dj \times 100}{lj}$$

Where dj is the number of dead individuals in the j^{th} stage. The real mortality is presented as an additive percentage and serves to compare the role of different mortality factors in the same generation:

$$\% \text{ Real Mortality} = \frac{dj \times 100}{lj}$$

where dj is the number of dead individuals in the j^{th} stage, and lj is the number of eggs at the beginning of the generation (Cardenas et al. 1999).

Statistical comparison of the populations

Life tables were determined for each population. However, only some parameters derived from these life tables were used for statistical analysis. Differences in incubation period of eggs, total development time of larvae, oviposition period, and number of eggs per female for populations from different altitudes were compared using the non-parametric Kruskal-Wallis test. Pairwise differences between populations at different altitudes were analyzed using the non-parametric Mann-Whitney U Test. Chi-Square analyses were conducted to test differences in number of hatched eggs, larvae survival, development time of pupae, pupa survival, adult rate, and

number of ovipositing females among populations. To test for differences in male and female longevity among different altitudes, the Kaplan-Meier Test was utilized (Zar 1996). Physiological variations among the populations were determined by using the Principal Component Analysis (PCA) Syn-tax 2000 (Podani 2001) package. Mahalanobis Distance was used as the distance coefficient.

RESULTS

Because the aim of the study was to investigate the effects of altitude on the populations of *P. papatasi*, all life table experiments were started with 50 F_1 pairs. This also enabled us to avoid the convergent effects of laboratory conditions.

Life cycle

A comparison of immature and adult developmental attributes of *P. papatasi* populations from different altitudes is presented in Table 1. Cohort females from the ALT region produced the highest number of eggs (2,494) followed by HHR, HMD, and AKL regions. However, the differences among populations were not statistically significant. Similarly, the number of eggs per female, which is a very important attribute to predict the population size, was not significantly different among the populations ($P > 0.05$), but each ALT female laid slightly more eggs in its lifetime than did females from HHR, HMD, and AKL regions. This difference may be correlated with larger body size of ALT females (Belen et al. 2004). According to this correlation, it is likely that the larger *P. papatasi* females imbibe more blood per meal and produce more eggs. For all populations the number of eggs decreased as a linear function of female age. Among populations, significant differences were found in the incubation period of eggs ($P < 0.05$). ALT eggs had a significantly longer incubation period (7.20 ± 2.11 days) than the remaining populations as indicated by a Mann-Whitney Test ($P < 0.05$) (Table 1). After the incubation period, in HHR, HMD and AKL populations, hatching rate was found to be around 50%. Fertility, the number of larvae, was the highest for the ALT region. Hatch ratios (%) were lowest in the AKL and HMD regions exhibited among the lowest percentage hatch. Up to 50% of eggs obtained from these regions did not hatch because eggs exhibiting low hatch rates were unembryonated suggesting a lack of fertilization rather than sterility due to lethal developmental genes. In terms of hatching rates, significant differences were found among the populations ($P < 0.001$) and the pairwise Mann-Whitney test showed the ALT region to be significantly different from the remaining regions ($P < 0.001$).

The AKL population of *P. papatasi* at the lowest altitude (368 m) had significantly shorter larval developmental time (25.53 days in total) when compared with that of other populations ($P < 0.001$). The developmental time from 1st instar larva to pupa was found to be 26.48 days in HHR, 29.11 days in HMD, and 29.78 days in ALT ($P < 0.001$). Results showed that larval developmental time increased linearly with altitude and its effect on environmental temperature. The overall regression coefficient was negative and significantly differed

Table 1. Comparison of biological and life table characteristics of *Phlebotomus papatasi* populations under laboratory conditions.

	AKL (368 m)	HHR (488 m)	HMD (644 m)	ALT (1117 m)	
PRE-ADULT STAGES					
1	Number of laying eggs for 50 females	1749 (35)**	2097 (42)	1775 (36)	2494 (50)
2	Incubation period of eggs (days)*	6.57±1.46 (5-9)	6.57±1.94 (5-9)	5.91±1.12 (2-8)	7.20±2.11 (3-13)
3	Number of hatched (embryonated) eggs	820	1087	862	1652
4	Hatching rate(%)	46.88	51.83	48.56	66.23
5	Total development time of larvae (days)	25.53	26.48	29.11	29.78
6	Pupation rate (%)	16.34	81.63	65.89	67.79
7	Development time of pupae (days) *	3.88±1.08 (2-11)	9.63±1.21 (2-10)	9.47±1.69 (3-11)	11.63±2.31 (2-12)
ADULT STAGE					
8	Number of adults	102	791	538	1058
9	Adult rate (%) (from egg to adult)	5.83	37.72	30.30	42.42
10	Sex ratio- female (%)	41.17	48	47	53.11
11	Female : Male ratio	0.70	0.93	0.88	1.13
12	Number of blood feeding female (cohort)	50	50	50	50
13	Oviposition period (days) *	9.67±2.95 (5-16)	4.88±1.51 (4-10)	6.36±1.72 (4-12)	6.80±1.80 (4-12)
14	Number of oviposited female	39	41	36	44
15	Number of eggs per female	45.3	51.15	49.30	56.25
16	Female longevity (days) *	11.60±3.48 (5-19)	9.64±2.21 (6-15)	8.75±2.39 (3-17)	10.16±2.37 (6-17)
17	Male longevity (days) *	12.92±3.87 (5-25)	10.80±2.01 (7-15)	7.68±2.95 (3-19)	10.25±2.05 (6-15)
PREDICTIVE ATTRIBUTES**					
18	Ro-Ro'	14.47-6.87	21.35-10.22	16.68-8.10	26.41-17.49
19	Tc-Tc'	49.56-49.61	55.10-54.58	55.99-49.61	60.62-60.24
20	$r_m-r'_m$	0.11-0.08	0.11-0.09	0.10-0.08	0.11-0.10
21	$\Lambda-\lambda'$	1.11-1.08	1.12-1.09	1.11-1.08	1.11-1.10
22	B	0.29	0.31	0.32	0.31
23	D	0.19	0.2	0.22	0.2
24	Mean e_x for females	2.68	2.31	2.02	2.32
25	Mean e_x for males	2.80	2.51	1.97	2.34
26	Total Vx	200.61	100.37	323.34	196.35

* arithmetic mean ± standart deviation (minimum-maximum).** number of eggs per female at the beginning. *** terms defined in Materials and Methods.

Table 2 . Stage-specific mortalities of *Phlebotomus papatasi* populations collected from four different altitudes under laboratory conditions.

	Developmental stages	l_x	d_x	% apparent mortality	% actual mortality
AKL (368 m)	Total oviposited eggs	1,749	929	53.11	53.11
	larvae	820	686	83.65	39.22
	pupae	134	32	23.88	1.82
	adults	102			
HHR (488 m)	Total oviposited eggs	2,097	1,010	48.16	48.16
	larvae	1,087	189	17.38	9.01
	pupae	898	107	11.91	5.10
	adults	791			
HMD (644 m)	Total oviposited eggs	1,775	913	51.43	51.43
	larvae	862	297	34.45	16.73
	pupae	567	29	5.11	1.63
	adults	538			
ALT (1117 m)	Total oviposited eggs	2,494	842	33.76	33.76
	larvae	1,652	729	44.12	29.23
	pupae	1,120	62	5.53	2.48
	adults	1,058			

l_x , the number of individuals that enter a specific stage; d_x , the number of individuals that die within a specific stage

from 0 ($b = -0.064$, $P < 0.001$). The overall survival rate from eclosion to pupation was 16.34% in AKL, 81.63% in HHR, 65.89% in HMD, and 67.79% in ALT and time from pupation to adult was again found to increase as a linear function of altitude from AKL region to ALT. There was a highly significant difference among the populations in terms of larval survival rate, pupation rate, and developmental time of pupae ($P < 0.001$). While mean adult emergence time from pupa was found to be 3.88 ± 1.08 days in the lowest altitude (AKL), it was significantly longer (11.63 ± 2.31 days) in the highest altitude (ALT) ($P < 0.001$).

Developmental time from egg to adult was found to be significantly different between the lowest (36 days) and the highest (49 days) altitudes ($P < 0.001$). This finding was not unexpected according to "Hopkins's bio-climatic rule". Highly significant differences were found among populations in the lowest and the highest altitudes in terms of survivor rate from egg to adult ($P < 0.001$). ALT population of *P. papatasi* had a significantly higher survival rate (7-fold) than AKL, the lowest region ($P < 0.001$). Since all cohorts were examined under similar laboratory conditions, observed differences in survival rate led to the assumption that the ALT population was more "K-selected", according to McArthur and Wilson (1967).

The sex ratio of emerging adults was not significantly different from 1:1 in each population ($P > 0.05$). In agreement with our previous observations (Belen et al. 2004), increased altitude significantly delayed development but did not significantly alter the sex ratio at emergence (Table 1). However, female ratio at emergence of *P. papatasi* at the lowest altitude (AKL) was found to be significantly lower (41.17%) than that of the highest altitude (ALT). This ratio increased gradually to 53.11% in ALT depending on increasing altitude. This finding was assumed that in contrast to ALT, the AKL population was more "r-selected".

Horizontal life tables and predictive population parameters

Separate life tables were constructed for each of the four populations and predictive population parameters calculated from data of these life tables are compared in Table 1. Age specific survivorship curves (l_x) for males and females, the reproductive value (V_x), and fecundity (m_x) are depicted graphically in Figure 1, while life expectation curves for both sexes are plotted in Figure 2.

Under the assumption that the cohort parental females from four altitudinal populations of *P. papatasi* were reared in similar laboratory conditions at the same time, their pre-reproductive ages will approximate those of their respective progeny (Pianka 1988). The pre-reproductive ages of cohort females were estimated as the average pre-reproductive ages of their respective progeny: 36 days for AKL, 43 days for HHR, 45 days for HMD, and 49 days for ALT. With these data, together with the average longevity data of cohort females in the range of 8.75 ± 2.39 to 11.60 ± 3.48 days, the class ages column (x) of the horizontal life tables was constructed.

Although no significant differences were found ($P > 0.05$) in terms of adult longevity among either the populations or between females and males in three populations, the males

demonstrated a slightly longer adult survivorship than females except in the HMD population (Figure 1). In general, survivorship patterns of both sexes approximated Slobodkin's (1961) Type II curve with little increase in mortality during early age intervals in all the populations. According to l_x data, the longest female and male longevity was determined in AKL population, 19 days and 25 days, respectively (Table 1, Figure 1). Similarly, there were no significant differences among populations ($P > 0.05$) in female life expectancy at emergence, e_x when $x=1$, even though the AKL females lived, on the average, 3 days longer than HMD females (Table 1). Differences in female life expectancy were not significant due to within-group variability.

Adult females of all the populations began taking a blood meal approximately 48 h after emerging with the first egg being laid 9.67 ± 2.95 days after adult emergence for AKL females, 4.88 ± 1.51 days for HHR, 6.36 ± 1.72 days for HMD, and 6.80 ± 1.80 days for ALT females (Table 1). Oviposition period of females among the populations was found to be significantly different ($P < 0.001$). The shortest oviposition period was detected in HHR females. This situation hints towards some ideas like differential insecticide applications which might result in bottlenecks as a possible explanation for time variation. The total reproduction period of the populations was found to be in the range of 7-11 days. The reproductive values (V_x) together with fecundity, m_x , were plotted in Figure 2. No significant differences were obtained between reproductive values of the populations ($P > 0.05$). For the AKL population, the increase in reproductive value was exponential (despite a fecundity of zero between 1-6 days) until the females reached an age of 19 days. For the HHR and HMD, the increases in reproductive values were also exponential (note zero fecundity between 1 to 6 days and 1 to 4 days, respectively) until the females reached an age of 15 and 17 days, respectively. In ALT, the highest reproductive value was found when the females reached an age of 17 days. These maximum peaks of V_x coincided with the days in which oviposition was maximum. V_x then abruptly decreased in the following days when the majority of the cohort females died. This population parameter also provided a clear estimate of the increase potential of *P. papatasi* in laboratory conditions.

The net reproductive rate, R_0 , was highest for the ALT (26.41) and HHR (21.35) and lowest for the AKL (14.47) and HMD (16.68) populations (Table 1). The ALT and HHR populations were found to be significantly different from the AKL population producing the fewest progeny ($P < 0.01$). The age of mean cohort reproduction, T_c , occurred latest in life for the HHR, HMD, and ALT populations which live at relatively high altitudes and earliest in life for the AKL population, which live at the lowest altitude (368 m). Populations that produced offspring earlier in life also produced more total female offspring, since T_c was negatively correlated with R_0 among the populations ($r = -0.686$, $0.01 < P < 0.05$). T_c was also correlated among the populations with female wing length (Belen et al. 2004) ($r = 0.851$, $P < 0.01$), i.e., populations having larger females produced their offspring later in life. Surprisingly, no significant differences among the populations were found in terms of intrinsic rate

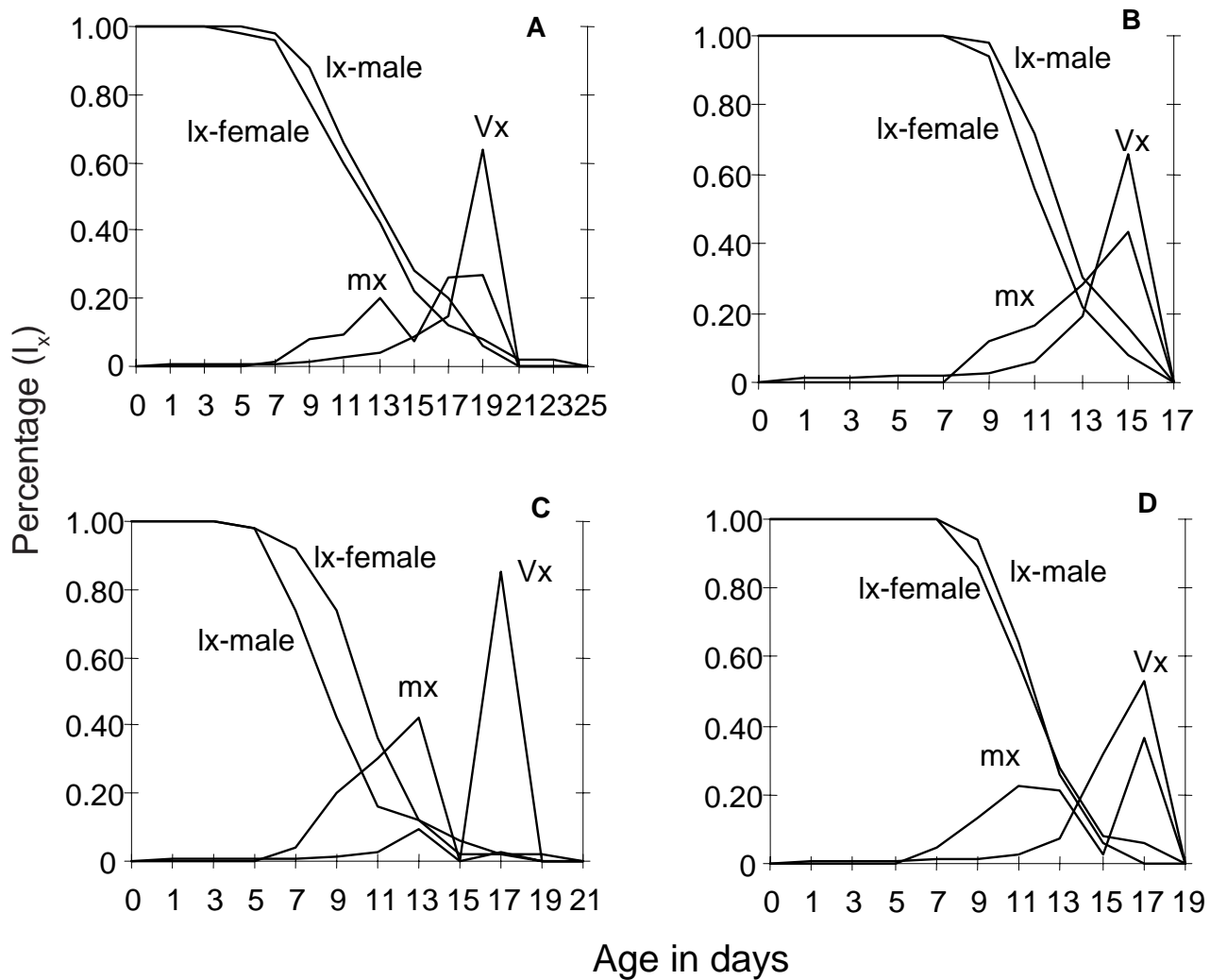


Figure 1. Age-specific survivorship (l_x) for females and males, age-specific fecundity (m_x), and reproductive value (V_x) plotted as a function of age in days for four *Phlebotomus papatasi* populations collected from different altitudes [A: Akçakale (AKL) 368 m; B: Hayati Harrani (HHR) 488 m; C: Hamdun (HMD) 644 m; D: Alitas (ALT) 1,117 m], Sanliurfa, southeastern Turkey.

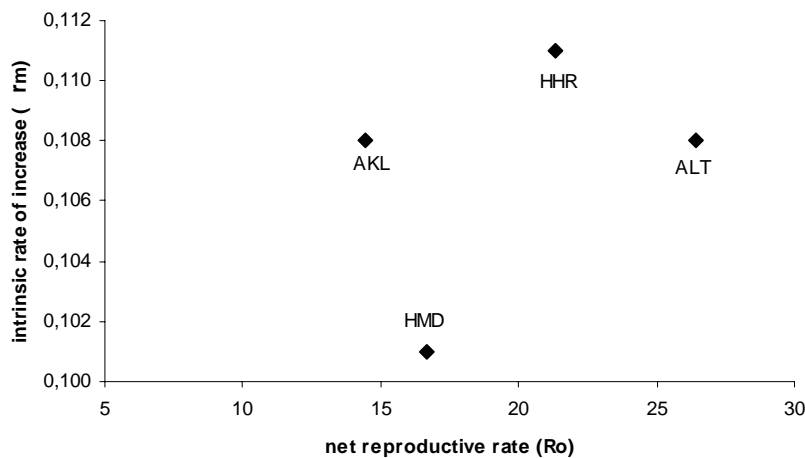


Figure 2. Relationship of intrinsic rate of increase, r_m , and net reproductive rate, R_o , based on observed sex ratios and hatching rate of eggs showing the differences among altitudinal populations of *Phlebotomus papatasi* from Sanliurfa in terms of maximal rate of population increase and the multiplication rate per generation under laboratory conditions. Full marks indicate the local populations given in Table 1. [AKL: Akçakale 368 m; HHR: Hayati Harrani 488 m; HMD: Hamdun 644 m, ALT: Alitas 1,117 m]

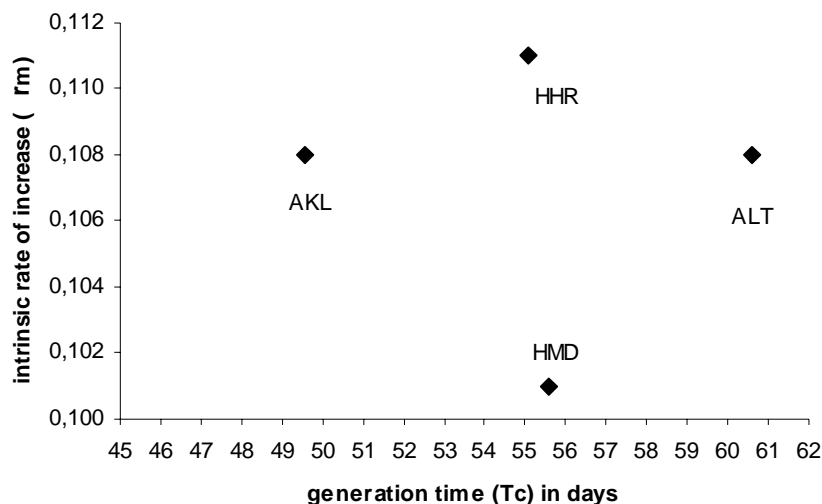


Figure 3. The intrinsic rate of increase, r_m , plotted as a function of the mean generation time, T_c , showing the differences among altitudinal populations of *Phlebotomus papatasi* from Sanliurfa in terms of maximal rate of population increase and the mean period elapsing between the birth of parents and the birth of offspring. Full marks indicate the local populations given in Table 1. [AKL: Akçakale 368 m; HHR: Hayati Harrani 488 m; HMD: Hamdun 644 m, ALT: Alitas 1,117 m]

of increase, r_m , finite rate of increase, λ , and birth (b) and death (d) rates ($P > 0.05$) (Table 1).

The heterogeneity among the life table strategies of the population studied was further exemplified by the clustering patterns when r_m was plotted as a function of R_o and T_c (Figures 2 and 3). The ALT population clustered, while HHR and HMD populations, which live at almost the same altitude, seemed close to each other according to r_m and T_c . However, the results

obtained from Figure 2 support the original contention that HHR population was somewhat closer to ALT. According to final clustering patterns, we determined that although the differentiation was not exact, the HHR and HMD constituted one group while AKL and ALT roughly constituted other two separate groups.

Variation among the parameters in Table 1 obtained from life tables of the different populations was also evaluated using Principal Component Analysis (PCA) (Figure 4). Maholonobis distance (D^2) was used as the distance constant in the analysis and each observation was treated as a single character. When life table data were taken into consideration it was determined that the ALT formed a distinct group from the other three, AKL, HMD, and HHR, along the first two PCs. In addition, HHR was found to be somewhat closer to ALT population in terms of all parameters. Differentiation among the populations showed an exact gradient line from the lowest altitude to the highest.

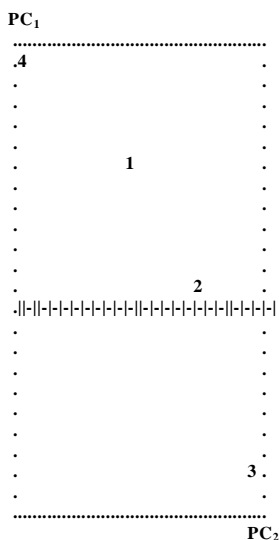


Figure 4. Summation of the characters obtained from the life table on the first two principal components by PCA . The first two PCs summarize the differences among local populations of *Phlebotomus papatasi* collected from four different altitudes [AKL: Akçakale 368 m; HHR: Hayati Harrani 488 m; HMD: Hamdun 644 m, ALT: Alitas 1,117 m], Sanliurfa, southeastern Turkey, which together describe 99.91% of the total variance. 1. HMD 2. HHR 3. ALT 4. AKL

Analysis of stage-specific mortality

Vertical life tables were constructed based on the l_x (live individuals) and d_x (mortality) at age stage. Table 2 shows analysis of the stage-specific mortality based on data in the vertical life tables for the populations. The highest real mortality was found in the egg stage of four populations, while very little mortality occurred at the pupal stage. The percentage overall real mortalities in AKL, HHR, HMD, and ALT populations were found to be 92%, 62.27%, 69.79%, and 65.47%, respectively. Highly significant differences were found between AKL population and the others in terms of overall real mortality values.

DISCUSSION

Distribution of the phlebotomine sand flies are highly disjunctive within its range, depending on local environmental factors such as precipitation and temperature, physical factors such as geographical barriers and habitat availability, and biotic factors such as the distribution and abundance of vertebrate hosts (Cross et al. 1996, Ferro et al. 1998, Ghosh et al. 1999). Although altitude per se is not a selective factor, biotic and abiotic properties of the environment are highly correlated with altitudinal gradients, most obvious of which is climate (Karan et al. 2000). Like most ectotherms, life-history traits of phlebotomine sand flies are heavily dependent on temperature, therefore species situated along altitudinal gradients have to adapt to a variety of climatic conditions (Masaki 1967, 1978, Ayres and Scriber 1994, Telfer and Hassal 1999).

In general, populations with excellent dispersal ability have similar gene frequencies, whereas with poor vagility, differences between geographically disjunctive populations increase (Ghosh et al. 1999). Sand fly populations are usually localized, the individuals rarely migrate more than 100 m (Alexander 1987, Morrison et al. 1993, Munstermann et al. 1998). The hopping behavior has given rise to the assumption that they do not disperse far from their breeding sites (Killick-Kendrick 1999). However, *P. ariasi* has been shown to move further than 2 km, although maximum dispersal of phlebotomine sand flies seldom exceeds 1 km (Alexander and Young 1992, Killick-Kendrick 1999). Thus, genetic differences among not only geographical strains from widely separated origins, but also among local populations of *P. papatasi*, can be expected.

Previous studies showed significant molecular and morphometric differences between different altitudinal populations of *P. papatasi* from similar eco-regions (Belen et al., 2004). When allozyme data indicated the level of heterozygosity was low, especially in the ALT population, no heterozygotes were observed for the alleles. The main reason for this was assumed to be long-term isolation in that study, but other factors such as behavior, mating choice, and genetic drift via bottlenecks may have played a role. In addition, the populations were also found to be significantly different for 17 morphological characters. When populations were grouped according to size differences, UPGMA results showed the HHR and ALT populations were two distinct groups, while AKL and HMD populations clustered together. These size and genetic approaches indicate that geographical variations exist among local populations of *P. papatasi* (Belen et al. 2004).

We have also found differences in several life history parameters among the populations of *P. papatasi* in this study, which is the first work that has focused on the details of reproductive biology of *P. papatasi*. This species is widely distributed in the southern part of Turkey and is known to vary morphologically between populations (Belen et al. 2004). Therefore, the variation we observed in life history parameters is not unexpected. With supporting data previously obtained from morphometric and molecular studies, results of the

present study also showed that four local populations of *P. papatasi* were significantly different in terms of life table attributes, and the ALT population at the highest altitude was distinct from the other populations.

Differences among populations in terms of r - and K -characteristics indicate that these populations live under different ecological conditions and geographical variations may reflect different ecological strategies (McArthur and Wilson 1967, Capy et al. 1993). Sampling stations of the AKL eco-region were in a semi-urban environment at the lowest altitude (368 m), those of HHR were in an urban environment at the intermediate altitude (488 m), while the ALT eco-region was located in a rural area at the highest altitude (1,117 m). From the results presented in this study, it was evident that both AKL and HHR populations were found to be more r -selected while, in contrast, the adult cohort of ALT showed K -selective behavior during the experiments. The lifespan of ALT was found to be longer than the others, there was a longer generation time, and the mean wing length of females in ALT was larger than those of AKL ($F_{3,005}=5.869$) according to Belen et al. (2004). AKL and HHR, on the other hand, had a rapid development at least in the juvenile stages and the adults had a slightly shorter life span. Furthermore, AKL and HHR had a major egg-laying thrust from day 6 to day 10 of the adult life while ALT had two major egg-laying thrusts extending over days 6 to 20 of the adult life. Differences in egg-laying strategy resulted in a larger net reproduction rate- R_o (26.41) for ALT but an almost similar intrinsic rate of increase- r_m with AKL and HHR (0.11).

Different reproductive strategies have been selected for the populations. We hypothesize that ALT as a rural population devotes less energy to the production of offspring and its eggs are produced over a longer period of time than those of the urban populations. The difference in R_o between the populations suggested that the risk of an individual of *P. papatasi* surviving to reproduction is higher in the urban environment than in the rural. This can probably be explained in terms of the relative patchiness or coarseness of grain in the environment. The rural populations might maintain themselves better if they had a higher R_o except that some other aspect of their overall fitness would suffer. Walter and Hacker (1974) analyzed subspecies variation of *Aedes aegypti* (Diptera: Culicidae) as an attempt to find a genetic basis for many morphological, physiological, and demographic characters. By controlled crosses and backcrosses of inbred strains they found that mean lifetime, r_m , and also R_o were genetically controlled, with the F_1 showing heterotic effects. They also found that the populations living in rural environments at high altitudes were significantly less variable morphologically than the urban populations.

In conclusion, the results presented here indicate that geographic variation associated with altitude in life table characteristics exist among even local populations of *P. papatasi*. As with other attributes, these variations can best be explained as responses to natural and also artificial selection. Our concern has been to stimulate discussion about the use of not only morphometric and molecular methods but also life tables to determine the variations and to examine

evolutionary strategies. With the increased pressure to use biological control methods, especially those which rely upon altering the genetic structure of a population, greater attention must be given to understanding the life history characteristics of sand fly populations in the field.

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