Emergence periodicity of *Phlebotomus argentipes* annandale and brunetti (Diptera: psychodidae): A laboratory study

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Abstract *Phlebotomus argentipes* Annandale and Brunetti (Diptera : Psychodidae) is the vector for visceral leishmaniasis in India. The aspects of its biology such as feeding and man vector contact are associated with emergence periodicity of the adult. Hence, the present study was made to find out the actual emergence period of *P. argentipes*. Wild caught *P. argentipes* were confined in the rearing pots inside laboratory. The newly emerged adults were collected at hourly intervals and released in to separate polythene bags and were held at 4°C till death. Sand flies were segregated sex-wise after the death under a microscope. The emergence of adult was observed throughout the day. However, the male preferred dawn emergence and the female the dusk. Two peaks of emergence were found in a day; first one in the morning (0900h) and the second one in the evening (1800h). The ratio of both sexes was found to be about equal. The emergence of adult was found to be 77% out of total eggs laid, which was completed within 7–10 days from the 1st day of emergence under laboratory conditions (25°C to 31°C and 70% to 75% relative humidity). This study has important bearings to find out the actual time for personal protection against biting of sand flies to prevent the transmission of Kala-azar.

Keywords Sand fly, *Phlebotomus argentipes*, Emergence, Visceral Leishmaniasis

Introduction

Phlebotomus argentipes Annandale and Brunetti (Diptera: Psychodidae) is the established vector of *Leishmania donovani* causing visceral leishmaniasis (VL) in India (Swaminath *et al.*, 1942). The biological activities of *P. argentipes* like, preference of blood meal, landing and biting rhythm on hosts, etc. are associated with its emergence periodicity. After emergence the insects tend to mate and feed for survival and oviposition. The optimal emergence time may depend upon the mean and distribution of emergence of males and females in the population as studied in pitcher-plant mosquito, *Wyeomyia smithii*. In addition, male cohorts achieve greater reproductive success when their emergence was spread over several days rather than occurring in a single day. However, reproductive success did not differ between normal and super dispersed emergence distributions, suggesting that, under more variable, natural conditions, the specific pattern of male emergence may be far less important than variation in emergence time (Cristina *et al.*, 2002). The feeding behavior of *P. argentipes* is preferentially *zoophilic* than *anthropophilic* in nature. The feeding starts with mating from the evening with the out number of males than females. The peak landing/biting period of *P. argentipes* is the mid night in nature. The male and female ratio was found to be 30:1 as maximum in the early evening, which was found to be decreased subsequently in later night period (Dinesh *et al.*, 2001). The period of midnight to early morning was reported

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as maximum landing and biting period in different parts of India such as Pondicherry and West Bengal (Srinivasan *et al.*, 1992; Hati *et al.* 1981; Palit *et al.*, 1988). *P. argentipes* was found as opportunistic feeder-taking blood meal from available hosts, either human or cattle (Palit *et al.*, 1988) but preferred animal blood seven times more than the human blood if equal opportunity was found available (Dinesh *et al.*, 2001). The contact period of host and vector is directly associated with the emergence period of the adult from pupae. In natural populations, male and female emergences are not restricted to points in time. Rather, for both sexes, there is a temporal distribution in emergence and for a given distribution of female emergence there should be an optimal distribution of male emergence (Bulmer 1983; Iwasa *et al.*, 1983; Parker and Courtney, 1983; Thornhill and Alcock, 1983; Sawada *et al.*, 1997).

Population of the same species may differ in their life cycle, fecundity, fertility, adult longevity and emergence from different geographical regions and among different morpho - species. It is expected that further investigations on morphometry, cuticular hydrocarbon, isoenzyme, may confirm the variations as studied in *Lutzomyia umbratilis* in Brazil (Justiniano *et al.*, 2004). Morphological variations in the populations of *P. argentipes* have been reported from different parts of India (Dinesh *et al.*, 2005 A,B; Illango *et al.*, 1994; Illango, 2000).

The study on emergence periodicity will help in implementing the personal protection measures against landing/ biting of sand flies in a particular time period of

the day. This may also help in finding out sibling species of sandflies having different emergence period in the different geographical regions.

Materials and Methods

Indoor resting sand flies were collected with the help of flashlight and aspirator from human dwellings and cattle sheds at Gulmehiya bagh village of Patna district in Bihar, India during February and March 2006. *P. argentipes* was scrutinized based on the external morphology. Gravid female *P. argentipes* were confined in 4 different pools (n = 5–9) in separate rearing pot. These were kept on wetted cloths lying over enamel tray under laboratory conditions. Each pot was covered with black paper. Eggs were laid by the female flies on folds of wet filter papers already kept inside the pots for providing suitable resting and egg laying surface. The eggs laid on filter paper were transferred on to the bottom surface of the rearing pots by washing with water. Larval food; made of rabbit faeces and slit was sprinkled on the bottom surface of the pot after hatching. Proper attention was given to the maintenance of larvae and pupae. After two days of reaching the pupal instar the rearing pots were observed closely for the emergence of the adult. The sand flies emerged from the individual rearing pot were released into a separate polythene bag, at hourly intervals and dry preserved in vials with labeling at 4°C for further study. The dead sand flies were segregated sex-wise under dissecting microscope.

Fig.1 Periodicity of the emergence of *P. argentipes*

Fig. 2 Sexwise periodicity of the emergence of *P. argentipes*

Results

The wild caught gravid *P. argentipes* could be maintained in rearing pots that provided suitable environmental conditions under laboratory (25°C to 31°C and relative humidity 70%–75%). The total number of eggs laid was 1045 with an average of 40.25 eggs per *P. argentipes*. Out of which 913 (87%) eggs hatched into first instars 799 (76%) attained adulthood. The emergence of adult was observed during each hour of day. The male and female flies were found emerging in almost equal numbers; 49 and 51 per cent respectively, but their emergence periodicity was different. There were two peaks during the emergence; one in the morning at 0900h and another in the evening at 1800h (Fig. 1). The emergence of the male flies was found to be more in the morning than the females, where as females emerged in more numbers than the males in the evening (Fig. 2). The emergence continued till 7–10 days.

Discussion

The seasonal emergence of male sand flies before females (protrandy), is a form of sexual selection that maximizes mating opportunities for males while minimizing the pre-reproductive period for females (Wiklund and Fagerstrom, 1977; Fagerstrom and Wiklund, 1982; Bulmer, 1983; Iwasa *et al.*, 1983; Parker and Courtney, 1983; Zonneveld and

Metz, 1991; Taylor *et al.*, 1998). Both theoretical models and empirical studies state or assume implicitly that there is a stabilizing selection on male emergence time because late-emerging males miss opportunities to sire offspring and males that emerge too early risk death before they have the opportunity to mate. However, for any given male, the emergence time that maximizes his contribution to the next generation may depend on the timing and pattern of emergence of other males in the population (Iwasa *et al.*, 1983; Parker and Courtney 1983; Thornhill and Alcock 1983; Zonneveld 1996). Because evaluating the lifetime number of offspring sired by males is time consuming or difficult, empirical studies use correlates of male reproductive success such as access to females or opportunities to mate (Botterweg 1982; Michener 1983; Parker and Courtney 1983; Elgar and Pierce 1988; Hastings 1989; Wang *et al.*, 1990; Zonneveld 1992; Wiklund *et al.*, 1996; Carvalho *et al.*, 1998), mating success (Wiklund and Fagerstrom 1977). Diel periodicities of adult emergence for three species of Chironomidae and one Caenidae from a Perth wetland were recorded during spring and summer. All chironomid species emerged mainly at dusk, regardless of sex, with the peak rate of emergence occurring during a 30 to 60 min interval beginning within 30 min of the time of light extinction. Light intensity appeared to be the major factor regulating the time of emergence. The emergence of mayfly occurred at dawn during both spring and summer and may be controlled by an endogenous circadian rhythm. (Pinder *et al.*, 1993). For

many aquatic insects adult emergence occurs towards the end of the life cycle. In many species mating and oviposition occur immediately after emergence, and death occurs soon afterwards. Since the survival of the adults is determined, at least in part, by predation and ambient environmental conditions (Jackson 1988), the timing of emergence may be crucial to overall reproductive success and dispersal ability.

The emergence periodicity is an important biological phenomenon among sand flies which is associated with the feeding on the host. In the present study we observed prior appearance of male flies than females. This may be to strengthen genitalia of male sandflies in order to get ready for courtship with the female. Early emergence of male has been observed for many insects, male genitalia of some insects undergo a 180° rotation after emergence in culicinae (Clements 1992). The 180° rotation of male genitalia and late female emergence seems to be a linked phenomenon. Genitalia rotation is necessary for male to become sexually mature. The emergence of both sexes had shown different pattern in the same species of sandfly *Lutzomyia umbratilis*, the major vector of cutaneous leishmaniasis in Northern South America. The difference was noticed in biological behaviors including pattern of emergence of adults from the populations of two different regions east (Manaus) and west (Manacapuru) of the Rio Negro at Brazil. The larval stage of females of Manacapuru was shorter than that of males. Hence, the male starts to emerge six days later than the females. The percentage of adult emerged from Manacapuru and Manaus was found 50% and 65% respectively (Justiniano *et al.*, 2004). The emergence of male prior to females is normally observed in Nematocera. This may be due to intrinsic phenomena (Frottini 1973). Males of *L. longipalpis* emerged two days before the females in Brazil (Jose Francisco Luitgards-Moura *et al.*, 2000). In general when the females emerge, males are ready for copulation. We found two peaks of emergence during 24 hours; first in the morning (0700h to 1200h) at 0900h and second in the evening (1500h–2200h) at 1800h with almost equal number of both the sexes. Maximum males were found emerging in the morning than the evening. This indicates the equal larval period of both the sexes of *P. argentipes*. The dawn is long known to be the time of choice for emergence of most insects due to cool and moist environment (Pittendrigh 1957). In *L. flaviscutellata*, a neotropical species; 37% emergence of adult was found with respect to number of eggs confined for rearing (Ward 1977). In the case of *L. longipalpis* it was found to be 23% (Killick-Kendrick *et al.*, 1977). The emergence of adult *L. umbratilis* continued till 7–9 days at Manaus and

37 days at Manacapuru at Brazil (Justiniano *et al.*, 2004). In the present study 77% emergence of adult was found with respect to total number of eggs laid and the emergence of adult continued till 7–10 days with peak on third day with males outnumbering females. Female emergence gradually dominated male emergence. The females use to take blood meal during night time and the male which does not take blood meal mate before or during taking blood meal by females. After blood meal the female lays eggs to continue their generations.

The study had shown the actual emergence period of *P. argentipes* in the laboratory conditions that might be similar to the field. This information will be of help in taking measures to prevent the bite of sandfly in a particular period of the day and hence to interrupt the transmission of the disease.

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