

## Isolation of a *Plasmodium vivax* refractory *Anopheles culicifacies* strain from India

T. Adak<sup>1</sup>, O. P. Singh<sup>1</sup>, Nutan Nanda<sup>1</sup>, V. P. Sharma<sup>1,2</sup> and Sarala K. Subbarao<sup>1,3</sup>

<sup>1</sup> National Institute of Malaria Research, Delhi, India

<sup>2</sup> Former Director, National Institute of Malaria Research, Delhi, India

<sup>3</sup> Indian Council of Medical Research, New Delhi, India

### Summary

*Anopheles culicifacies sensu lato* comprises five sibling species. We report the isolation of an *An. culicifacies* species B strain which is completely refractory to *Plasmodium vivax* sporogonic development and partially refractory to *P. falciparum*. Parasite development in this strain is arrested by a melanotic encapsulation mechanism in the mid-gut. We compare the infectivity of this refractory strain and four other species B strains from different epidemiological zones of India with *P. vivax* in the laboratory.

**keywords** Malaria, *Anopheles culicifacies*, *Plasmodium vivax*, malaria refractory

### Introduction

*Anopheles culicifacies* Giles 1901 is the most important vector of malaria and is responsible for nearly 65% of the 2–3 million malaria cases reported annually from India (Sharma 1999). This mosquito transmits almost all rural malaria in India and is considered an important vector in the countries west of India and Sri Lanka. *Anopheles culicifacies* has been recognized as a complex of five genetically distinct species, provisionally designated as species A and B (Green & Miles 1980), C (Subbarao *et al.* 1983), D (Vasanthan *et al.* 1991) and E (Kar *et al.* 1999). Distinct biological variations exist among different members of the complex with respect to their distribution pattern, seasonal prevalence, feeding preferences and response to insecticides (Subbarao 1988). Members of the complex also vary in disease-transmission potential (Subbarao *et al.* 1980, 1988a, 1992) and susceptibility to *Plasmodium* (Adak *et al.* 1999; Kaur *et al.* 2000). While assessing natural susceptibility of *An. culicifacies sensu lato* from different geographical areas against *Plasmodium vivax* infection, we came across an iso-female line, which was 100% refractory to *P. vivax* infection. This iso-female line was later identified as species B. Subsequently, we screened many other field populations collected from different epidemiological zones of the country and established four more species B strains, which are being maintained as cyclic colonies in the insectary. We assessed

the susceptibility of these five species B strains against different *P. vivax* isolates in the laboratory.

### Materials and methods

Indoor resting wild *An. culicifacies sensu lato* adult females were collected from human dwellings by hand catch method and transported to the laboratory in Delhi. Iso-female progenies obtained from wild female mosquitoes were held separately in 30 × 30 × 30 cm cloth cages in the insectary at 27 ± 2 °C, with 75 ± 5% relative humidity (RH) and photoperiods of 14 h light and 10 h darkness. Adult mosquitoes were offered water soaked raisins and 1% glucose soaked cotton pads as a source of energy. A few adult female mosquitoes from each *F*<sub>1</sub> iso-female progeny were identified to sibling species using species-specific diagnostic inversion genotypes as described by Subbarao *et al.* (1988b). At least 50–60 iso-female lines from a particular geographical locality showing species-specific diagnostic inversion genotype of species B were pooled together to establish a strain. Following this method four *An. culicifacies* species B strains were established from Rameshwaram, Tamil Nadu state, 9° 17' N, 79° 22' E; Nadiad, Gujarat state, 22° 45' N, 72° 45' E; Rourkela, Orissa state, 22° 06' N, 84° 00' E; and Ladpur, Haryana state, 28° 48' N, 76° 28' E. Furthermore, progenies of a single iso-female line originated from Haldwani, Uttaranchal state, 29° 23' N, 79° 30' E 100% refractory against

T. Adak *et al.* **Malaria-refractory *Anopheles culicifacies***

*P. vivax* infection, were selected. This iso-female line was later identified as sibling species B and designated as a *P. vivax* refractory strain. All five strains are being maintained as cyclic colonies in the insectary since 2001. We assessed susceptibility of all these species B strains in the laboratory against *P. vivax*, the predominant human malaria parasite species in India. In each feeding experiment, we used cohorts of 50–75 mosquitoes (4–6-day-old) starved for 12–16 h from each of the five species B strains. 2–3 ml of *P. vivax* infected blood were drawn from consenting volunteer patients (aged  $\geq 16$  years) with a mature *P. vivax* gametocytes density between 0.05% and 0.5%. We followed the protocol approved by the Human Ethical Committee of the Centre as described by Adak *et al.* (1999). All *P. vivax* positive patients were treated with 600 mg chloroquine once on day 0 and 15 mg of primaquine for 5 consecutive days (adult dose) following the national drug policy of National Vector Borne Disease Control Programme (NVBDCP), Government of India.

Infectivity of species B Haldwani strain which was 100% refractory to *P. vivax* was also assessed against *P. falciparum* blood isolates, selected from consenting volunteer patients with mature *P. falciparum* gametocytes following above mentioned *P. vivax*-protocol. All *P. falciparum* positive patients were treated with 1500 mg chloroquine (600 mg on day 0, 600 mg on day 1 and 300 mg on day 3) and a single dose of 45 mg primaquine.

The mosquitoes were fed on *P. vivax* blood isolates through membrane essentially following the method as of Adak *et al.* (1999). In all feeding experiments, laboratory reared *An. stephensi*, which are highly susceptible to *P. vivax* infection, were fed in parallel with all species B strains on the same blood isolate, to assess the comparative infectiousness to mosquitoes. After 30 min of feeding, unfed and partially fed mosquitoes from each cohort were removed. Only fully engorged mosquitoes were kept securely in 30 × 30 × 30 cm cloth cages in the insectary for subsequent examination of sporogonic development. A minimum of 50% of the surviving *An. culicifacies* and *An. stephensi* mosquitoes from each feeding experiment fed on the same blood isolate were dissected on day 6 and 7 in normal saline (0.65% NaCl) and stained in a drop of 0.5% mercurochrome (Eyles 1950). Subsequently, midguts were placed under a small piece of cover glass and examined for the presence of infection in the midgut under a ×10 interference phase contrast lens of an Axiophot Zeiss microscope. From the remaining surviving mosquitoes, sporozoites were harvested from salivary glands dissected in normal saline on day 12 or 13 and stored in liquid nitrogen for future studies. Differential infection among all these strains was assessed by comparing two indicators; the per cent gut positivity for oocysts/encapsulated parasites

(oocyst rate) and geometric mean (GM) number of oocysts/encapsulated parasites per gut (oocyst density) among all the infected mosquitoes. The oocyst densities in both mosquito species were not normally distributed as evidenced by the test of skewness and kurtosis. However, the log transformed data,  $\log(x + 1)$  was found to be very close to the normal distribution. The transformed data was further used for the analysis of variance (ANOVA) and Student's *t*-test, to test the statistical differences between oocyst densities estimated from different localities. Replicates in which reference mosquito *An. stephensi* did not acquire any infection were excluded from the analysis.

### Results and discussion

The results from various paired feeding experiments involving each of the five species B strains and *An. stephensi* are summarized in Table 1. The results of statistical analysis, analysis of variance (ANOVA) and Student's *t*-test are given in Table 2.

Examination of midguts of Haldwani strain on day 6 after an infectious *P. vivax* blood meal revealed only encapsulated parasites which varied in shape and size, and were covered with melanin-like dark brown pigment (Figure 1c–e). The other four strains of species B had both encapsulated parasites and normal oocysts in the gut. The gut infections of all infected mosquitoes in all strains were categorized in three groups: guts with encapsulated parasites, guts with normal developing oocysts and guts with both. The mean number of encapsulated parasites ( $8.05 \pm 0.06$ ) in Haldwani strain was significantly lower than that of normal oocysts ( $27.75 \pm 0.07$ ) in *An. stephensi*. The significantly higher mean number of normal oocysts in *An. stephensi* than lower numbers of encapsulated parasites in the Haldwani strain was probably because of the involvement of some other immune defence mechanism in reducing the infectivity in addition to melanotic encapsulation. The amount of blood engorged by *An. culicifacies* is always smaller than that engorged by *An. stephensi*, which is bigger in size, resulting in low intake of blood containing infective gametocytes by *An. culicifacies*. The proportion of mosquito guts with normal oocysts, encapsulated parasites or both in each of these five species B strains of infected *An. culicifacies* is shown in Figure 2.

The GM number of normal oocysts in *An. stephensi* was consistently higher than in different strains of *An. culicifacies* representing different localities:  $18.69 \pm 0.11$ – $48.36 \pm 0.35$  in *An. stephensi* against  $0.36 \pm 0.06$ – $12.95 \pm 1.01$  in *An. culicifacies*. This observation is significant and could be due to variation in inherent infectivity potential of these two species.

T. Adak *et al.* Malaria-refractory *Anopheles culicifacies***Table 1** Comparative susceptibility of five *Anopheles culicifacies* species B strains and *Anopheles stephensi* to *Plasmodium vivax*

Mosquito species/locality	Parasite species (number of isolates)*	Number of infected/dissected (%)	Oocyst rate (%)			Mean (GM $\ddagger$ ) of oocysts		
			Normal oocysts $\pm$ SE (fiducial limit) $\ddagger$	Encapsulated parasites $\pm$ SE (fiducial limit) $\ddagger$	Mixed (normal + encapsulated) $\pm$ SE (fiducial limit) $\ddagger$	Normal oocysts $\pm$ SE (fiducial limit) $\ddagger$	Encapsulated parasites $\pm$ SE (fiducial limit) $\ddagger$	
<i>An. culicifacies</i> Rameshwaram	<i>P. vivax</i> (51)	115/317 (36.28)	56.52 $\pm$ 2.29 (52.03-61.01)	21.74 $\pm$ 1.59 (18.63-24.85)	21.74 $\pm$ 1.59 (18.63-24.85)	4.09 $\pm$ 0.13 (2.97-5.51)	1.13 $\pm$ 0.1 (0.75-1.59)	
<i>An. stephensi</i>		194/305 (63.61)	100	0	0	18.69 $\pm$ 0.11 (15.06-23.15)	0	
<i>An. culicifacies</i> Nadiad	<i>P. vivax</i> (12)	13/67 (19.40)	38.46 $\pm$ 6.56 (25.60-51.33)	61.54 $\pm$ 6.56 (48.67-74.40)	0	0.9 $\pm$ 0.38 (0.012-2.59)	3.84 $\pm$ 0.73 (0.64-13.27)	
<i>An. stephensi</i>		51/65 (78.46)	100	0	0	34.97 $\pm$ 0.2 (24-50.6)	0	
<i>An. culicifacies</i> Rourkela	<i>P. vivax</i> (5)	9/17 (52.94)	66.67 $\pm$ 7.41 (52.15-81.19)	33.33 $\pm$ 7.41 (18.81-47.85)	0	12.95 $\pm$ 1.01 (2.53-54.09)	0.73 $\pm$ 0.42 (-0.13-2.43)	
<i>An. stephensi</i>		23/25 (92.00)	100	0	0	48.36 $\pm$ 0.35 (26.55-87.47)	0	
<i>An. culicifacies</i> Ladpur	<i>P. vivax</i> (47)	178/420 (42.38)	12.92 $\pm$ 0.84 (11.27-14.57)	84.27 $\pm$ 0.99 (82.32-86.22)	2.81 $\pm$ 0.20 (2.41-3.21)	0.36 $\pm$ 0.06 (0.21-0.54)	6.47 $\pm$ 0.10 (5.14-8.08)	
<i>An. stephensi</i>		202/296 (68.24)	100	0	0	19.76 $\pm$ 0.10 (16.04-24.29)	0	
<i>An. culicifacies</i> (Haldwani)	<i>P. vivax</i> (92)	371/708 (52.40)	0	100.00	0	0	8.05 $\pm$ 0.06 (7.06-9.16)	
<i>An. stephensi</i>		411/536 (76.68)	100	0	0	27.75 $\pm$ 0.07 (23.99-32.06)	0	
<i>An. culicifacies</i> (Haldwani)	<i>P. falciparum</i> (9)	30/82 (36.58)	60.00 $\pm$ 4.38 (51.41-68.59)	46.67 $\pm$ 4.54 (37.76-55.57)	6.67 $\pm$ 1.14 (4.44-8.89)	1.19 $\pm$ 0.19 (0.56-2.08)	0.45 $\pm$ 0.09 (0.22-0.72)	
<i>An. stephensi</i>		38/50 (76.00)	100	0	0	23.89 $\pm$ 0.26 (14.70-38.45)	0	

\* Number of infected blood isolates used for membrane feeding.

 $\ddagger$  Figure in parenthesis are 95% fiducial limits. $\ddagger$  Geometric mean.

T. Adak *et al.* Malaria-refractory *Anopheles culicifacies***Table 2** (a) Analysis of variance (ANOVA) of gut infection with normal and encapsulated parasites in *An. culicifacies* species B from different localities. (b) Test of significance (*t*-test) of geometric means of normal and encapsulated parasites in different strains of *An. culicifacies*

Source of variation	SS	d.f.	MS	F	P-value
(a)					
Normal oocyst					
Between localities	28.41	3	9.47	41.31	<0.0001
Within localities	71.52	312	0.23		
Total	99.93	315			
Encapsulated parasite					
Between localities	22.38	3	7.46	24.56	<0.0001
Within localities	94.78	312	0.31		
Total	117.16	315			
(b)					
		P-value			
Comparison groups		Normal oocysts	Encapsulated parasites		
(b)					
Ladpur vs. Nadiad		0.3293	0.4534		
Ladpur vs. Rourkela		0.0106	0.0028		
Ladpur vs. Rameshwaram		0.0000	0.0000		
Nadiad vs. Rourkela		0.0249	0.1313		
Nadiad vs. Rameshwaram		0.0121	0.1674		
Rourkela vs. Rameshwaram		0.1919	0.5781		

In the Haldwani strain, 52.40% of the guts were positive among all the dissected mosquitoes and only for encapsulated parasites. The encapsulation also occurred in four other species B strains, but the proportion of guts with encapsulated parasites among strains varied widely, ranging between  $21.74 \pm 1.59$  and  $84.27 \pm 0.99$ , being significantly higher in the Ladpur strain (84.27%) than in the Nadiad, Rourkela and Rameshwaram strains in decreasing order. The proportion of gut containing encapsulated parasites was significantly different in all comparison groups except between Rourkela and Rameshwaram. Furthermore,  $2.81 \pm 0.20\%$  of guts of the Ladpur strain and  $21.74 \pm 1.59\%$  guts of the Rameshwaram strain contained both encapsulated parasites and normal oocysts, unlike the other two species B strains. Correspondingly, the percentage of guts containing normal oocysts varied widely, ranging between  $12.92 \pm 0.84$  and  $66.67 \pm 7.41$ .

The analysis of variance (ANOVA) shows that the GM number of normal oocysts as well as the GM number of encapsulated parasites were significantly different between the localities ( $P < 0.001$  Table 2a).

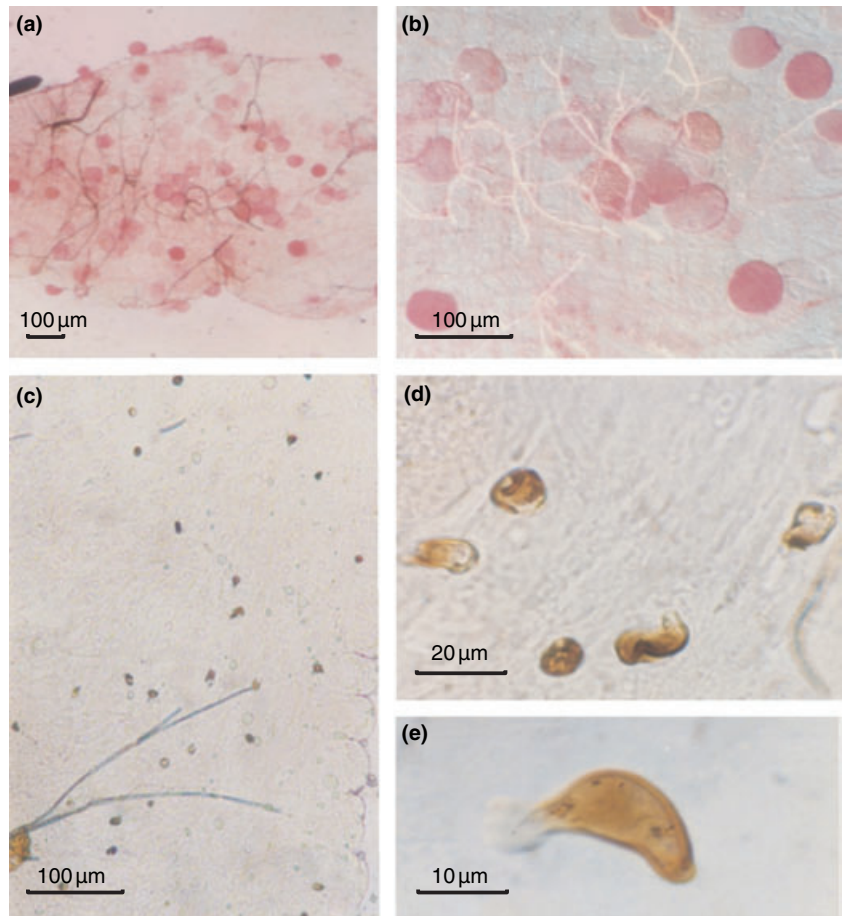
Comparison of GM numbers of normal oocysts between localities revealed that it was significantly higher in Ladpur than in the Rourkela ( $P < 0.01$ ) and Rameshwaram strains

( $P < 0.001$ ). In Nadiad, the GM number of normal oocysts was significantly lower than in Rameshwaram ( $P < 0.02$ ) and Rourkela ( $P < 0.05$ ). However, there was no significant difference in GM number of oocysts between Ladpur and Nadiad and Rourkela and Rameshwaram strains. The GM numbers of encapsulated parasites were significantly higher in Ladpur than in the Rourkela ( $P < 0.01$ ) and Rameshwaram strains ( $P < 0.0001$ ). However, there was no significant difference in mean number of oocysts between other comparison groups (Table 2b).

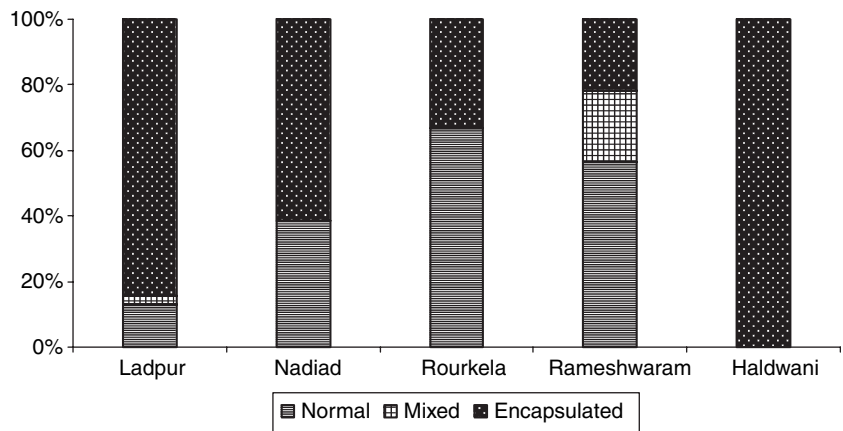
Most of the few studies on malaria refractory mosquitoes are restricted to animal parasite models, except that a strain of *An. stephensi* was found refractory to *P. falciparum* (Feldmann & Ponnudurai 1989) and *An. gambiae* originally selected against *P. cynomolgi* but was also refractory to human malaria parasites (Collins *et al.* 1986). However, none of these strains is completely refractory to any of the human *Plasmodium*. This is the first report of a malaria mosquito strain in *An. culicifacies* completely refractory to *P. vivax* infection. This strain has been maintained in the laboratory (originated from a single female progeny) as a cyclic colony without any selection for the last 5 years. Representatives from this cyclic colony have been checked periodically for infectivity with *P. vivax*, consistently producing only encapsulated parasites without any deviation/exception. However, the refractoriness of this strain has so far been tested only against *P. vivax* isolates of Delhi origin and remains to be tested against isolates from other malaria endemic zones of the country.

Interestingly, the *An. culicifacies* Haldwani strain, which is completely refractory to human malaria parasite *P. vivax*, showed only partial refractoriness against *P. falciparum*. Results of artificial feeding experiments with nine *P. falciparum* isolates against *An. culicifacies* Haldwani strain are given in Table 1. Dissection and examination of guts revealed that these were either positive for only normal oocysts or encapsulated parasites or for both. The GM numbers of normal and encapsulated parasites estimated per gut among the infected mosquitoes were  $1.19 \pm 0.19$  and  $0.45 \pm 0.09$ , respectively.

Complete refractoriness against *P. vivax* and partial refractoriness against *P. falciparum* could be correlated to different structural patterns of *Plasmodium* surface protein and the pattern recognition mechanism by the mosquito host. The phenoloxidase system, which is an integral part of the cellular and humoral defence system in Diptera (Gotz 1986) in response to foreign organisms, and is well known to synthesize melanin, which mediates the process of melanotic encapsulation, has been observed in different wild *An. culicifacies* strains in this study, which is a rare natural event.



**Figure 1** (a) and (b) Normal oocysts of *Plasmodium vivax* on the gut of a susceptible *An. stephensi* female on day 6 after the infectious blood meal (stained with 0.5% mercurochrome); (c, d) and (e) Encapsulated parasites of *Plasmodium vivax* on the gut of a refractory *An. culicifacies* on day 6 after the infectious blood meal.



**Figure 2** Proportion (%) of infected guts with normal oocysts, encapsulated parasites and mixed infection in *An. culicifacies* strains from different geographical areas.

To date, the molecular basis of refractoriness and more generally parasite recognition and killing are not well understood. Studies carried out to understand the molecular mechanism of refractoriness in refractory L3-5 strain of *An. gambiae* (Collins *et al.* 1986) revealed not only the

involvement of several quantitative trait loci (QTL) but also the relative contribution of each locus to parasite encapsulation by melanization, which varies with the species of parasites (Zheng *et al.* 1997, 2003). A different refractory mechanism resulting in complete lysis of *P. gallinaceum*

T. Adak *et al.* **Malaria-refractory *Anopheles culicifacies***

ookinetes in the midgut of this strain was reported by Vernick *et al.* (1995). The most recent studies in this direction in *An. gambiae* have demonstrated knockdown of thioester containing protein, TEP1 gene (Blandin *et al.* 2004); and Leucine-rich immune proteins LRIM1 gene (Osta *et al.* 2004). Thus major parasite losses occur by TEP1 and LRIM1 gene mediated parasite killings during the midgut invasion of parasite. The knock down of these two genes in susceptible mosquitoes results in a three- to fivefold increase in parasite survival in the midgut, while in refractory mosquitoes knock down of TEP1 gene increases parasite numbers and completely abolishes their melanization (Blandin *et al.* 2004). In this context, it may be appropriate to mention that in the present host parasite interaction study, we have also suggested the involvement of other mosquito immune mechanism beside melanization.

The isolation of a wild *An. culicifacies* strain which is 100% refractory to *P. vivax* infection gives us an opportunity to use this laboratory model of infection for future research related to the understanding the molecular basis of refractoriness. Our finding is relevant in view of the current research interest in driving the refractory genes into vector populations as a means of interrupting malaria transmission.

**Acknowledgements**

We thank Mr Pritam Singh, Mr Pratap Singh, Mr Uday Prakash, Mr O.P. Verma and Mr Satish Kumar for their excellent technical assistance. We are grateful to Dr N.N. Singh for his critical and constructive suggestion and Mr Ballabh Kumar Sharma for typing the manuscript.

**References**

- Adak T, Kaur S & Singh OP (1999) Comparative susceptibility of different members of the *Anopheles culicifacies* complex to *Plasmodium vivax*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 573–577.
- Blandin S, Shiao S-H, Moita LF *et al.* (2004) Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. *Cell* **116**, 661–670.
- Collins FH, Sakai RK, Vernick KD *et al.* (1986) Genetic selection of a *Plasmodium*-refractory strain of the malaria vector *Anopheles gambiae*. *Science* **234**, 607–610.
- Eyles DE, (1950) A stain for malaria oocysts in temporary preparations. *Journal of Parasitology* **36**, 501.
- Feldmann AM & Ponnudurai T (1989) Selection of *Anopheles stephensi* for refractoriness and susceptibility to *Plasmodium falciparum*. *Medical and Veterinary Entomology* **3**, 41–52.
- Gotz P (1986) Encapsulation in arthropods. In: *Immunity in Invertebrates: Cells, Molecules and Defence Reactions* (ed M Brehelin) Springer-Verlag, Berlin, pp. 153–170.
- Green CA & Miles SJ (1980) Chromosomal evidence for sibling species of the malaria vector *Anopheles (Cellia) culicifacies* Giles. *Journal of Tropical Medicine and Hygiene* **83**, 75–78.
- Kar I, Subbarao SK, Eapen A *et al.* (1999) Evidence of new malaria vector species, species E, within the *Anopheles culicifacies* complex (Diptera: Culicidae). *Journal of Medical Entomology* **36**, 95–100.
- Kaur S, Adak T & Singh OP (2000) Susceptibility of species A, B and C of *Anopheles culicifacies* complex to *Plasmodium yoelii yoelii* and *Plasmodium vinckei petteri* infections. *Journal of Parasitology* **86**, 1345–1348.
- Osta MA, Christophides GK & Kafatos FC (2004) Effects of mosquito genes on *Plasmodium* development. *Science* **303**, 2030–2032.
- Sharma VP (1999) Current scenario of malaria in India. *Parassitologia* **41**, 349–353.
- Subbarao SK (1988) The *Anopheles culicifacies* complex and control of malaria. *Parasitology Today* **4**, 72–75.
- Subbarao SK, Adak T & Sharma VP (1980) *Anopheles culicifacies* sibling species distribution and vector incrimination studies. *Journal of Communicable Diseases* **12**, 102–104.
- Subbarao SK, Vasantha K, Adak T & Sharma VP (1983) *Anopheles culicifacies* complex: evidence for a new sibling species, species C. *Annals of Entomological Society of America* **76**, 985–988.
- Subbarao SK, Adak T, Vasantha K *et al.* (1988a) Susceptibility of *Anopheles culicifacies* species A and B to *Plasmodium vivax* and *Plasmodium falciparum* as determined by immunoradiometric assay. *Transaction of the Royal Society of Tropical Medicine and Hygiene* **82**, 394–397.
- Subbarao SK, Vasantha K & Sharma VP (1988b) Cytotaxonomy of malaria vectors in India. In: *Biosystematics of Haematophagous Insects* (ed MW Service) Oxford University Press, Oxford, pp. 25–37.
- Subbarao SK, Vasantha K, Joshi H *et al.* (1992) Role of *Anopheles culicifacies* sibling species in malaria transmission in Madhya Pradesh. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 613–614.
- Vasantha K, Subbarao SK & Sharma VP (1991) *Anopheles culicifacies* complex: population cytogenetic evidence for species D (Diptera: Culicidae). *Annals of Entomological Society of America* **78**, 531–536.
- Vernick KD, Fujioka H, Seeley DC, Tandler B, Aikawa M & Miller LH (1995) *Plasmodium gallinaceum*: a refractory mechanism of ookinete killing in the mosquito, *Anopheles gambiae*. *Experimental Parasitology* **80**, 583–595.
- Zheng L, Cornel AJ, Wang R *et al.* (1997) Quantitative trait loci for refractoriness of *Anopheles gambiae* to *Plasmodium cynomolgi*. *Science* **276**, 425–428.
- Zheng L, Wang S, Romans P, Zhao H, Luna C & Benedict MQ (2003) Quantitative trait loci in *Anopheles gambiae* controlling the encapsulation response against *Plasmodium cynomolgi* Ceylon. *BMC Genetics* **4**, 16.

T. Adak *et al.* **Malaria-refractory *Anopheles culicifacies***

**Corresponding Author** T. Adak, Malaria Research Centre (ICMR), 2 Nanak Enclave, Delhi-110009, India. Tel/Fax: +91-11-27234234, 27411737; E-mail: adak.mrc@gmail.com

**Isolement en Inde d'une souche d'*Anopheles culicifacies*, réfractaire au *Plasmodium vivax***

*Anopheles culicifacies sensu* comprend 5 espèces parentées. Nous rapportons ici, l'isolement d'une souche d'*Anopheles culicifacies* espèce B, complètement réfractaire au développement sporogonique du *Plasmodium vivax* et partiellement réfractaire à celui du *Plasmodium falciparum*. Le développement du parasite dans cette souche est bloqué par un mécanisme d'encapsulation mélanotique à mi-hauteur de l'intestin. Nous avons comparé au laboratoire, l'infectivité de cette souche réfractaire ainsi que celle de 4 autres souches de l'espèce B, provenant de différentes zones épidémiologiques de l'Inde à celle du *Plasmodium vivax*.

**mots clés** Malaria, *Anopheles culicifacies*, *Plasmodium vivax*, malaria réfractaire

**Aislamiento de una cepa de *Anopheles culicifacies* refractaria a *Plasmodium vivax* proveniente de la India**

*Anopheles culicifacies sensu lato* comprende cinco especies hermanas. Reportamos el aislamiento de una especie de la cepa B de *An. Culicifacies* completamente refractario al desarrollo de esporogonios de *Plasmodium vivax* parcialmente refractario a *Plasmodium falciparum*. El desarrollo parasitario de estas cepas es detenido por un mecanismo de encapsulamiento melanótico en el intestino medio. Comparamos la infectividad de esta cepa refractaria y de otras 4 cepas de especie B de diferentes zonas epidemiológicas de la India con *Plasmodium vivax* en el laboratorio.

**palabras clave** Malaria, *Anopheles culicifacies*, *Plasmodium vivax*, malaria refractaria