gill, ovary and notochord. To further confirm tissue distribution, the more sensitive RT–PCR technique was performed. An amplification product of the expected size (558 bp; see Figure 1 for primer position) was observed in all tissues examined, but signals for gill, ovary and notochord were comparatively weak (Figure 5). The relative abundance of *AmphiCB* transcript in the hind-gut and hepatic caecum suggests a possible digestive role of *AmphiCB* in the amphioxus alimentary canal. However, the ubiquitous expression of *AmphiCB* in amphioxus implies it may also play a housekeeping role. This merits further study at the protein level.

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Insecticide susceptibility status of malaria vectors in some hyperendemic tribal districts of Orissa

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Insecticide susceptibility status of adult, wild-caught *Anopheles culicifacies* and *An. fluviatilis* against diagnostic dosages of DDT (4%), malathion (5%) and deltamethrin (0.05%) was determined according to standard WHO procedure in some districts of Orissa. All these districts are predominantly inhabited by the tribal population and are hyperendemic for malaria. The results showed that *An. culicifacies* is resistant to DDT in all the eight districts, to malathion in Mayurbhanj, Bolangir, Nuapada and Kalahandi districts and is showing signs of development of multiple resistance to DDT, malathion and deltamethrin in Bolangir, Nuapada and Kalahandi districts. *An. fluviatilis* was found susceptible to DDT, malathion and deltamethrin in all the districts except Mayurbhanj, where 95 and 87.5% mortality was observed against DDT and malathion respectively. The delayed knock-down effect of deltamethrin in *An. culicifacies* (KDT_{50}: 11.78–25.31 min; KDT_{90}: 24.20–65.22 min) and *An. fluviatilis* (KDT_{50}: 20.87–25.19 min; KDT_{90}: 45.81–54.11 min) was observed in all the districts, which is an indication of incipient resistance. Based on these findings, appropriate changes in the indoor residual spray strategy have been suggested to achieve effective vector control.

VECTOR control programmes in India rely mostly on indoor residual spraying by DDT[1]. The spectacular success achieved

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in malaria control between 1958 and 1965 was mainly attributed to DDT. However, this achievement was short-lived and soon after malaria resurgence took place. One of the technical reasons for malaria resurgence was development of DDT resistance in primary malaria vector, *Anopheles culicifacies*[2–4], which is responsible for the transmission of 60–70% of new cases of malaria in India[5]. Malathion, an organophosphate insecticide, was introduced in the programme during 1969 and within a span of 4 years, malathion resistance in *An. culicifacies* was reported from Gujarat and Maharashtra[6,7]. Synthetic pyrethroids were introduced in the public health programme during 1990s in some parts of India, for selective control of the multiple resistant malaria vectors in high-risk malarious areas.

Orissa is one of the worst malaria-affected states in India, where this disease is a major public health problem. Orissa alone contributes 23% of the total malaria cases, 43% of all *Plasmodium falciparum* cases and more than 50% of total malaria-related deaths in the country, although it represent only about 4% of the population of India. There are 30 districts, out of which 21 (158 PHCs) are high-risk areas and are under World Bank-assisted programme of Enhanced Malaria Control Project (EMCP) since 1998. Under this project, indoor residual spraying of DDT is supplemented with synthetic pyrethroids and also insecticide-treated mosquito nets have been distributed in some districts. The high-risk malaria areas of Orissa are under the influence of two primary vectors, viz. *An. culicifacies* and *An. fluviatilis*. DDT resistance in Orissa was first reported[8] during 1966. Subsequently, there were reports of DDT and HCH resistance in *An. culicifacies* from Koraput and Sundargarh districts during 1990 and 1991 respectively[9,10]. Since then, there are no reports of insecticide resistance. Moreover, there is no information on the susceptibility status of malaria vectors in other highly malarious districts. Therefore, a study was undertaken to know the current insecticide susceptibility status of *An. culicifacies* and *An. fluviatilis* to DDT, malathion and deltamethrin in some hard-core malaria districts of Orissa. The results of this study are reported here.

Insecticide susceptibility tests were conducted during 2002–03 in eight districts of Orissa reporting maximum number of malaria cases and deaths in the State. These districts are mainly located in the northern and western part of the state (Figure 1) and are predominantly inhabited by different ethnic tribal communities. All these eight districts are under EMCP of the National Vector-Borne Disease Control Programme, Government of India since 1998. The tests were performed on wild-caught blood-fed, indoor-resting *An. culicifacies* and *An. fluviatilis* females collected through suction-tube method in the morning hours between 6 and 8 a.m. from human dwellings and cattle sheds. All the field-collected mosquitoes were brought to the base laboratory in a $1' \times 1'$ cloth cage wrapped in a wet towel. The mosquitoes were exposed to the diagnostic doses of 4% DDT, 5% malathion and 0.05% deltamethrin impregnated papers, as well as corresponding control papers for 1 h according to standard procedure recommended by WHO[11]. The mosquitoes were kept in the recovery tubes for 24 h and mortality was recorded. In test replicates, where mortality in the control tubes was more than 5%, the mortality of the exposed mosquitoes was corrected using Abbott's formula.

To determine the knock-down susceptibility status of *An. culicifacies* and *An. fluviatilis* to synthetic pyrethroids, the field collected mosquitoes were exposed to 0.05% deltamethrin-impregnated papers, a revised diagnostic dose recommended by WHO using WHO adult susceptibility kit according to standard procedure (WHO, 1998, unpublished document). The number of mosquitoes knocked down after 5, 10, 15, 30, 45 and 60 min till the last mosquito was knocked down, were recorded. The exposed knocked-down mosquitoes were kept in the recovery tube with access to cotton pad soaked with 5% glucose solution in water. Mortality was recorded after 24 h recovery period. Mortality in the control tests was also recorded after 24 h and the per cent mortality of exposed mosquitoes was corrected using Abbott's formula. The knock-down times (KDT_{50} and KDT_{90}) were calculated using log-time and probit-mortality regression model[12]. Data on temperature and relative humidity at the time of experimentation were also recorded.

The insecticide susceptibility status of *An. culicifacies* to diagnostic doses of DDT and malathion in different districts is given in Table 1. The data show that *An. culicifacies* is resistant to DDT in all the study districts. The per cent mortality in *An. culicifacies* against DDT (4%) in all the study districts ranges between 8 and 62.5. The susceptibility of *An. culicifacies* to malathion was 100% in Sundargarh, Rayagada and Phulbani, whereas it was 50% in Mayurbhanj, 68.3% in Bolangir, 75% in Nuapada and 88.3% in Kalahandi districts. The test could not be performed in Keonjhar district because of less number of mosquitoes.

Table 2 shows the susceptibility status of *An. fluviatilis* to DDT and malathion in five districts of Orissa, where the species is prevalent in large numbers. The data show that *An. fluviatilis* is 100% susceptible to DDT and malathion except in Mayurbhanj district, where 95% and 87.5% mortality was observed respectively. It may be mentioned here that *An. fluviatilis* is a major vector of malaria in these districts and is responsible for high transmission during post-monsoon months. However, with the onset of summer, the species starts diminishing and remains at low density during most part of the year. The species predominantly is endophilic and endophagic, but a small proportion does rest outdoors (MRC, unpublished data). Logically, a good insecticide coverage should be able to bring down malaria transmission in areas under the influence of *An. fluviatilis*. However, malaria continues to persist in all these districts mainly because the spray operations are inadequate and their time of application does not coincide with the period of high prevalence of this species.

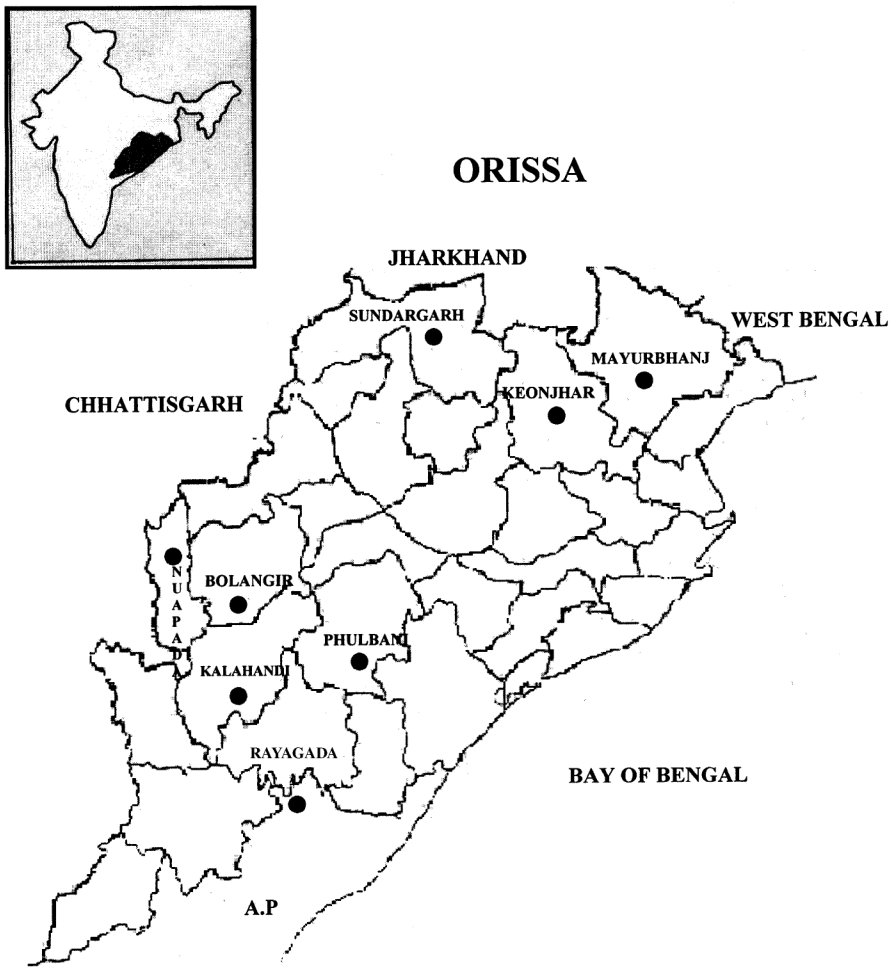

Figure 1. Map of Orissa showing location of districts where insecticide resistance studies were conducted.

The corrected per cent mortalities in *An. culicifacies* after 1 h exposure to 0.05% deltamethrin followed by 24 h recovery time in different districts of Orissa are shown in Table 1. The species was found to be 100% susceptible to deltamethrin in Sundargarh, Keonjhar, Rayagada, Phulbani and Mayurbhanj districts; and KDT_{50} and KDT_{90} values varied between 11.78 and 25.31 min and 24.20 and 44.45 min respectively. In Nuapada, Bolangir and Kalahandi, the per cent mortalities were 81.7, 95.0 and 96.7 respectively, and KDT_{50} and KDT_{90} ranged between 13.91 and 23.28 min and 32.14 and 65.22 min respectively. The knock-down data indicate further precipitation of pyrethroid resistance in this species, particularly in Bolangir and Nuapada districts.

The susceptibility data of *An. fluviatilis* to 0.05% deltamethrin in five districts of Orissa revealed that the species is fully susceptible to synthetic pyrethroids (Table 2). KDT_{50} and KDT_{90} values in different districts ranged between 20.87 and 25.19 min and 45.81 and 54.11 min respectively. The data show that although the species is 100% susceptible, the knock-down times for this species are comparatively higher than *An.culicifacies* in all the districts.

It is obvious from the study that *An. culicifacies* is resistant to DDT in all the eight districts, whereas it shows signs of development of multiple resistance to malathion and deltamethrin in Bolangir, Nuapada and Kalahandi districts. The delayed knock-down effect of deltamethrin in the remaining five districts of the present study is also an indication of development of incipient resistance. Earlier studies have shown *An. culicifacies* to be susceptible to malathion (5%) and deltamethrin (0.025%) in Koraput district, Orissa at a time when these insecticides were not used in that area[9]. Malathion has never been used in public health programmes in Orissa. However, it is widely used in agriculture. Synthetic pyrethroids were introduced in Orissa during 1999, both as indoor residual insecticide and as a chemical for the treatment of mosquito nets in districts under EMCP. Mayurbhanj district was under indoor residual spray with synthetic pyrethroids during 1999–2001 and subsequently, there was a switch-over to DDT spray. This may be one of the factors for dilution of DDT resistance in Mayurbhanj compared to other districts. Interaction with the villagers in the study districts revealed that they were

Table 1. Susceptibility status of *An. culicifacies* to DDT, malathion and deltamethrin and knock-down times against 0.05% deltamethrin in different districts of Orissa

District	Percentage corrected mortality (*n*)			Knock-down times (0.05% deltamethrin)			
	DDT 4%	Malathion 5%	Deltamethrin 0.05%	KDT_{50}	KDT_{90}	χ^2 (df)	Slope (*b*)
Sundargarh	12.0 (100)	100.0 (100)	100.0 (100)	16.91	39.73	9.2074 (4)	1.5004
Keonjhar	14.0 (50)	NP	100.0 (80)	15.15	34.41	6.7935 (4)	1.5621
Bolangir	23.3 (60)	68.3 (60)	95.0 (60)	23.28	65.22	5.9986 (4)	1.2437
Nuapada	8.3 (60)	75.0 (60)	81.7 (60)	19.04	59.05	12.9313 (4)	1.1321
Kalahandi	12 (60)	88.3 (60)	96.7 (60)	13.91	32.14	6.4411 (4)	1.5305
Rayagada	15 (60)	100.0 (60)	100.0 (60)	11.78	26.98	5.4881 (4)	1.5470
Phulbani	20 (60)	100.0 (60)	100.0 (60)	12.75	24.20	3.0963 (4)	2.0001
Mayurbhanj	62.5 (40)	50.0 (40)	100.0 (60)	25.31	44.45	13.1259 (4)	2.2757

n, Number of mosquitoes exposed; NP, Test not performed; KDT, Knock-down time; df, Degree of freedom; Slope (*b*) derived from regression equation of knock-down values.

Table 2. Susceptibility status of *An. fluviatilis* to DDT, malathion and deltamethrin and knock-down times against 0.05% deltamethrin in different districts of Orissa

District	Percentage corrected mortality (*n*)			Knock-down times (0.05% deltamethrin)			
	DDT 4%	Malathion 5%	Deltamethrin 0.05%	KDT_{50}	KDT_{90}	χ^2 (df)	Slope (*b*)
Sundargarh	100.0 (100)	100.0 (60)	100.0 (100)	20.87	49.00	23.4158 (4)	1.5016
Keonjhar	100.0 (100)	100.0 (40)	100.0 (120)	24.11	54.11	33.7544 (4)	1.5856
Kalahandi	100.0 (60)	100.0 (60)	100.0 (60)	21.37	52.77	12.1651 (4)	1.4175
Phulbani	100.0 (60)	100.0 (40)	100.0 (40)	21.80	45.81	6.0913 (4)	1.7252
Mayurbhanj	95.0 (40)	87.5 (40)	100.0 (40)	25.19	46.21	8.7680 (4)	2.1123

n, Number of mosquitoes exposed; KDT, Knock-down time; df, Degree of freedom; Slope (*b*) derived from regression equation of knock-down values.

extremely happy with the impact of synthetic pyrethroids on mosquitoes as well as other household pests when it was used for the first time, but subsequent applications of synthetic pyrethroids did not produce the same impact. There are reports of *An. culicifacies* showing resistance to synthetic pyrethroids in Tamil Nadu and Gujarat[13,14], which are disturbing trends because there is possibility of widespread resistance to other related compounds of this group.

An. fluviatilis was found susceptible to DDT and malathion in all the districts except Mayurbhanj. Earlier studies have shown that this species is susceptible to DDT, malathion and deltamethrin in Mayurbhanj and Koraput districts of Orissa[9]. However, resistance to DDT has been reported from Puri and Balasore districts of Orissa[15]. In the present study, *An. fluviatilis* was found susceptible to deltamethrin in all the districts, although the insecticide has been in use in public health programme in these districts either for indoor residual spray or focal spray or as a chemical for the treatment of mosquito nets.

Effective vector control can only be achieved by proper management of insecticide resistance in the field populations. There are several methods on the strategic use of available insecticides to delay the onset of resistance such as to avoid indiscriminate use, avoid use of insecticides that simultaneously select resistance to other chemically related insecticides and rotation of insecticides[16]. Malaria is the most important public health problem in the tribal districts of Orissa, especially those located in the forest ecotypes and under the influence of *An. fluviatilis*, where the disease causes high levels of morbidity[17]. Therefore, based on the present study, it is suggested that areas under the influence of *An. fluviatilis* should be covered under DDT residual spray because the species is fully susceptible to DDT, whereas areas under the influence of *An. culicifacies* should be covered under spraying of synthetic pyrethroids. Also the time of spray should coincide with the peak prevalence time of the vector species. Both these strategies may produce good epidemiological results.

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Vertebrate fauna from the subsurface Cambay Shale (Lower Eocene), Vastan Lignite Mine, Gujarat, India

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A rich collection of vertebrate fossils comprising mainly fish and some mammals has recently been recovered from subsurface beds of the Cambay Shale (Lower Eocene) exposed in the Vastan Lignite Mine, Surat District, Gujarat. Here we record a diverse assemblage of elasmobranch and teleostean fish, including the selachians *Triakis*, *Eogaleus*, *Abdounia*, *Rhizoprionodon* and *Eutrichiurides*, which are new to the subcontinent. The Vastan ichthyofauna has general affinities with the Palaeocene–Eocene fish assemblages from northern Africa, Europe and Uzbekistan, but it is most closely similar to faunas known from Rajasthan and the northwest sub-Himalaya.

THE Cambay Basin situated on the western margin of the Indian shield embodies Palaeogene and younger sediments that have been known to yield varied but sparse faunal and floral remains[1–7]. Recent prospecting by the two of us (R.S.R. and H.S.) in the subsurface beds of the Early Eocene Cambay Shale exposed in an open cast lignite mine at Vastan in the southern part of the basin has revealed multiple fossiliferous horizons containing foraminifers, ostracodes, molluscs, mammals and fish. Mammals are being treated separately and here we document the fish remains representing 23 taxa mainly of sharks, batoids and tetraodontoids. This record has threefold significance: (i) it adds to the knowledge of the Lower Eocene vertebrate faunas of the subcontinent, which are inadequately known but important for palaeobiogeographic inferences in the context of India–Asia collision; (ii) it includes *Triakis*, *Eogaleus*, *Abdounia*, *Rhizoprionodon* and *Eutrichiurides* that are new to the subcontinent, and (iii) vertebrate fossils from the Cambay Basin were earlier known only by otoliths[7], whereas now we have well-preserved dentitions as well as some postcranials. Previous reports of Eocene elasmobranch and teleostean fish in India are from Gujarat[7–10], Rajasthan[11,12] and the northwest sub-Himalayan region[13–15].

In the Cambay Basin, the Palaeogene beds are exposed as thin strips along the Saurashtra coast and to the east of the Gulf of Cambay (Figure 1). The Vagadkhol/Olpad Formation deposited over the Deccan Traps comprises the oldest sediments (Palaeocene–Lower Eocene) in the basin. It is overlain by 75–1500 m thick Cambay Shale comprising greenish and whitish-grey and black clay/shales with lignite seams[16].

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