considered an unimportant malaria vector in that State (LOURENÇO-DE-OLIVEIRA *et al.*, 1989; OLIVEIRA-FERREIRA *et al.*, 1990; KLEIN *et al.*, 1991a, b).

Thus, further investigations were required to determine whether biological differences could exist between *An. oswaldoi* collected in Acre and those from Rondônia. In order to clarify these points, experimental infections using laboratory-raised mosquito families were conducted. Analysis of male genitalia showed that all progeny from Rondônia, originating from anophelines identified as *An. oswaldoi s.l.*, were actually *An. konderi*. In Acre, although *An. oswaldoi s.s.* and *An. konderi* were sympatric, 85·0% of the families were *An. oswaldoi s.s.*, showing that there are differences in the distribution and prevalence of these species in these 2 States. These findings suggest that the specimens identified as *An. oswaldoi s.l.* by KLEIN *et al.* (1991a, b) and used in their experiments were possibly different from those used by BRANQUINHO *et al.* (1993, 1996).

The infection rates found for oocysts and sporozoites in *An. darlingi* controls of both *An. oswaldoi* and *An. konderi* were very similar (Figs 1 and 2). Therefore, we feel justified in inferring that the higher positivity rate of oocyst infection in the midguts of *An. oswaldoi s.s.* as compared with *An. konderi* is indeed a plausible conclusion. The same can be considered when sporozoite infection rates in the salivary glands are compared, since only *An. oswaldoi s.s.* specimens were positive.

The number of mosquitoes available for examination of midguts and salivary glands depended on the seasonal availability of the species of the *An. oswaldoi* complex and on the proportion of mosquitoes that ingested blood and survived until the end of sporogony. Such problems limited the number of feeds examined. The results presented represent a total of 7 and 8 feeds for *An. konderi* and *An. oswaldoi s.s.*, respectively. However, 3 of the 7 feeds of *An. konderi* mosquitoes and of the control, *An. darlingi*, were negative, while 4 of the 8 trials were negative for both *An. oswaldoi s.s.* and the control.

Some explanations for these results are: (1) mosquitoes could have failed to ingest viable male and female gametocytes, (2) the blood taken contained no viable gametocytes, (3) the ookinetes failed to cross the midgut wall, or (4) the oocysts failed to develop into sporozoite forms. The inability of oocysts to rupture and produce viable sporozoites, or the failure of sporozoites to migrate to and/or penetrate the salivary glands, the destruction of released sporozoites, are alternative mechanisms of mosquito resistance to infection by *Plasmodium* parasites (ROSENBERG, 1985; PONNUDURAI *et al.*, 1988). Other factors in the blood meal may also interfere with anopheline infection rates (CARTER *et al.*, 1988; NAOTUNNE *et al.*, 1991).

Our findings indicate that *An. oswaldoi s.s.* can transmit *P. vivax* and suggest that this species is more susceptible than *An. konderi*. Although *An. oswaldoi s.s.* is an exophilic and zoophilic species, it may be involved in malaria transmission. In areas where human outdoor activities at dusk, when mosquito activity is intense, are the rule, exophily does not seem to impede malaria transmission. This applies especially when there is a high density of the anopheline population, as occurred in previous studies in Acre, at a time when more than 85·0% of captured mosquitoes were *An. oswaldoi* and in 1 locality this was the only species which tested positive for *Plasmodium* (NATAL *et al.*, 1992; BRANQUINHO *et al.*, 1993). In spite of being considered to be mainly zoophilic, all *Plasmodium*-positive *An. oswaldoi* specimens reported by BRANQUINHO *et al.* (1993) had been collected on human bait (M. S. Branquinho, personal communication). Therefore, despite presenting a low infection rate in the salivary glands in relation to the controls (*An. darlingi*), there are strong indications that *An. oswaldoi* may have importance in local malaria transmission in Acre.

Owing to problems in the morphological distinction of members of the *An. oswaldoi* complex, we have been conducting molecular characterization of these mosquitoes based on ribosomal DNA sequences. Our preliminary results indicate that a molecular distinction will be possible between *An. oswaldoi* and *An. konderi*, thus giving another indication that these are actually 2 distinct species.

Acknowledgements

We thank José dos S. Lemos, Fátima dos Santos and all the staff of Laboratório de Entomologia, Fundação Nacional de Saúde (FNS-RO), for technical help and for local support in Rondônia, Luiz H. S. Gil, CEPEM-RO, for providing some F1 *An. darlingi* and Renan S. Santos (FNS-AC) for mosquitoes from Acre. This research was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), FNS-RO, FIOCRUZ-RJ, and LIM49-HCFMUSP. M. T. M. is a PhD student, supported by FAPESP.

References

Arruda, M. de, Carvalho, M. B., Nussenzweig, R. S., Maracic, M., Ferreira, A. W. & Cochrane, A. H. (1986). Potential vectors of malaria and their different susceptibility to *Plasmodium falciparum* and *Plasmodium vivax* in northern Brazil identified by immunoassay. *American Journal of Tropical Medicine and Hygiene*, **35**, 873–881.

Branquinho, M. S., Lagos, C. B. T., Rocha, M. R., Natal, D., Barata, J. M. S., Cochrane, A. H., Nardin, E., Nussenzweig, R. S. & Kloetzel, J. K. (1993). Anophelines in the state of Acre, Brazil, infected with *Plasmodium falciparum, P. vivax*, the variant *P. vivax* VK247 and *P. malariae*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**, 391–394.

Branquinho, M. S., Araújo. M. S., Natal, D., Marrelli, M. T., Rocha, M. R., Taveira, F. A. L. & Kloetzel, J. K. (1996). *Anopheles oswaldoi* a potential malaria vector in Acre, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **90**, 233.

Carter, R., Kumar, N., Quakyi, I., Good, M., Mendis, K., Graves, P. & Miller, L. (1988). Immunity to sexual stages of malaria parasites. *Progress in Allergy*, **41**, 193–214.

Causey, O. R., Deane, L. M. & Deane, M. P. (1946). Studies on Brazilian anophelines from the Northeast and Amazon Regions II. An illustrated key by male genitalia characteristics of the Anophelini, with a note on dissection technique. *American Journal of Hygiene Monograph Series*, **18**, 21–34.

Collins, F. H., Zavala, F., Graves, P. M., Cochrane, A. H., Gwadz, R. W., Akoh, J. & Nussenzweig, R. S. (1984). First field trial of immunoradiometric assay for the detection of malaria sporozoites in mosquitoes. *American Journal of Tropical Medicine and Hygiene*, **33**, 538–543.

Deane, L. M. (1986). Malaria vectors in Brazil. *Memórias do Instituto Oswaldo Cruz*, **81**, 5–14.

Faran, M. E. (1980). Mosquito studies (Diptera, Culicidae) XXXIV. A revision of the *albimanus* section of the subgenus *Nyssorhynchus of Anopheles. Contributions of the American Entomological Institute*, **15**, 61–62.

Faran, M. E. & Linthicum, K. J. (1981). A handbook of the Amazonian species of *Anopheles* (Diptera: Culicidae). *Mosquito Systematics*, **13**, 1–81.

Galvão, A. L. A. & Damasceno, R. G. (1942). *Anopheles (Nyssorhynchus) konderi* nova espécie de *Anopheles* do vale do Amazonas e considerações sobre as espécies do complexo *tarsimaculatus* (Diptera, Culicidae). *Folia Clínica et Biológica*, **14**, 115–135.

Hayes, J., Calderon, G., Falcon, R. & Zambrano, V. (1987). Newly incriminated anopheline vectors of human malaria parasites in Junin Department, Peru. *Journal of the American Mosquito Control Association*, **3**, 418–422.

Klein, T. A. & Lima, J. B. (1990). Seasonal distribution and biting patterns of *Anopheles* mosquitoes in Costa Marques, Rondônia, Brazil. *Journal of the American Mosquito Control Association*, **6**, 700–707.

Klein, T. A., Lima, J. B. P. & Tada, M .S. (1991a). Comparative susceptibility of anopheline mosquitoes to *Plasmodium falciparum* in Rondonia. *American Journal of Tropical Medicine and Hygiene*, **44**, 598–603.

Klein, T. A., Lima, J. B. P., Tada, M. S. & Miller, R. (1991b). Comparative susceptibility of anopheline mosquitoes in Rondonia, Brazil to infection by *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene*, **45**, 463–470.

Lounibos, L. P., Duzak, D. & Linley, J. R. (1997). Comparative egg morphology of six species of the *albimanus* section of *Anopheles (Nyssorhynchus)* (Diptera: Culicidae). *Journal of Medical Entomology*, **34**, 136–155.