EXPERIMENTS IN CROSSING TWO STRAINS OF *ANOPHELES BARBIROSTRIS* VAN DER WULP 1884 (DIPTERA : CULICIDAE) IN THAILAND

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INTRODUCTION

Anopheles (Anopheles) barbirostris species group consists of two subgroups, viz., *barbirostris* subgroup which includes *barbirostris*, *campestris*, *donaldi*, *franciscoi*, *hodgkini* and *pollicaris* ; and *vanus* subgroup which includes *ahomi*, *barbumbrosus*, *reidi*, *manalangi* and *vanus* (Reid, 1962). The distribution of these two subgroups is limited to the Oriental region, except for *vanus*, which has been recorded from the western tip of New Guinea (Reid, 1968). In Thailand at least six species, i.e., *barbirostris*, *barbumbrosus*, *campestris*, *donaldi*, *hodgkini* and *pollicaris* are found, but *donaldi* and *pollicaris* are found only in extreme southern Thailand. The medical importance of this species group, so far only *barbirostris*, *campestris* and *donaldi* have been incriminated as vectors of malarial and/or filarial parasites in many Southeast Asian countries. These three species have also been recorded as the suspected vectors of malaria and Brugian filariasis in southern Thailand (Reid, 1968 ; Harinasuta *et al.*, 1976).

As early as 1942, two types of *barbirostris* were recognized in Malaysia (Reid, 1942). The dark-winged type with abundance of pale sternal scales was recognized by Reid (1962) as a distinct species, *campestris*, not only on morphological difference, but also on behaviour traits and vector capabilities which were quite distinct from those of *barbirostris*. Harrison and Scanlon (1975) suggested that the characters used by Reid to separate Malayan *campestris* and *barbirostris* are not valid in Thailand. They also reported that not only do many Thai *campestris* have light wings, but in areas where *campestris* does not occur some specimens of *barbirostris* exhibit dark wings. Moreover, *barbirostris* usually exhibits few pale sternal scales in areas where it is found with or near *campestris* and it often exhibits extensive pale sternal scaling in areas where *campestris* does not occur. They also recommended that the pupal skins of these two species could be used to separate them at about 95 - 97% level. In view of *barbirostris* having a high morphological variation in adults, the relationship among variable forms of the adult has to be defined more vigorously, especially the genetic relationships among these variations. Several crossing experiments have been done with anopheline mosquitoes which are not easily identified morphologically, such as within the *An. gambiae* Giles complex (Davidson *et al.*, 1967), the *An. maculipennis* Meigen complex (Kitzmiller *et al.*, 1967), the *An. punctulatus* Doenitz complex (Bryan, 1973), *An. hyrcanus* Pallas complex (Oguma, 1978), and *An. balabacensis* complex (Kanda *et al.*, 1981 ; Baimai *et al.*, 1981). The studies reported herein describe the experiments in crossing two strains of *An. barbirostris* in Thailand.

MATERIALS AND METHODS

Adult engorged females of *An. barbirostris* were collected from baited water buffaloes in

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Bang Luke Canton, Chumphon Province and Kao Mai Kaew, Chon Buri Province of Southern and Central Thailand respectively. These two strains were successfully reared in the laboratory, Chumphon strain for 49 generations and Chon Buri strain for 36 generations, by the induced mating technique (Ow Yang *et al.*, 1963). The characteristic differences between these two strains were the darkness of the wing at the stem of vein 5 and the pronounce distribution of abdominal pale scales on the sternite which were very similar to that of *campestris*, Chumphon strain, whereas in Chon Buri strain, all particular characters belonged to *barbirostris* with light wing and few pale scales present on the sternite. The other distinct points in our observations were the body sizes (4 day-old unfed adults fasted for 12 hours at 26 ± 1°C, 70-80% R.H.) which were determined by body weight in mg and number of egg deposition per gravid female. For Chumphon strain, the average body weight in mg of female, male and egg deposition per gravid female were 1.64 ± 0.49 mg, 1.19 ± 0.23 mg and 143 ± 47.54, while in Chon Buri strain the above weights and egg deposition were 0.97 ± 0.23 mg, 0.82 ± 0.19 mg and 83.33 ± 18.95 respectively (Table 1). The statistical analysis of these distinct points between both strains revealed significant difference.

Adult females and males of both strains taken from pupae that had been placed individually in test tubes were used for the experiments in parent and hybrid colonies

Table 1

The comparison between two strains of *An. barbirostris*, Chumphon (CHP) and Chon Buri (CHB), and F_1 hybrids.

Mosquito strains	sex	No. mosquitoes tested	Average body weight in mg per mosquito (range)*	Average egg deposition per gravid female (range)**
CHP	female	20	1.64 ± 0.49 (0.81 – 2.60)	–
	male	20	1.19 ± 0.23 (0.62 – 1.60)	–
	gravid female	13	–	143 ± 47.54 (41 – 214)
CHB	female	20	0.97 ± 0.23 (0.63 – 1.35)	–
	male	20	0.82 ± 0.19 (0.58 – 1.19)	–
	gravid female	15	–	83.33 ± 18.95 (62 – 110)
(CHP × CHB) F_1	female	20	1.45 ± 0.32 (0.89 – 1.86)	–

* $t = 9, P_1 < 0.01, F_{2,57} = 18.78, P_2 < 0.01$

** $t = 4.1, P_3 < 0.01$

P_1, P_3 for CHP and CHB; P_2 for CHP, CHB and (CHP × CHB) F_1

Tabe 2

Cross mating between two strains of *An. barbirostris*, Chumphon (CHP) and Chon Buri (CHB) in the laboratory.

Cross female × male	No. cross	No. oviposition (%)	No. insemination (%)	Total eggs (range)	Embryonation rate (No.)	No. hatch (%)	No. pupation (%)	No. emergence (%)	No. Average percentage from total emergence Female	Male
CHP × CHP	20	13 (65)	16 (80)	1859 (41-214)	85.7 (84/98)	1559 (83.9)	1331 (85.4)	1117 (71.6)	532 (47.6)	585 (52.4)
CHB × CHB	20	15 (75)	18 (90)	1250 (62-110)	91.9 (79/86)	1134 (90.7)	961 (84.7)	836 (73.7)	461 (55.1)	375 (44.9)
CHB × CHP	48	17 (35.4)	35 (72.9)	1335 (54-103)	69.8 (90/129)	–	–	–	–	–
CHP × CHB	36	11 (30.6)	24 (66.7)	1509 (130-185)	78.5 (51/65)	917 (60.8)	432 (47.1)	153 (16.7)	132 (86.3)	21 (13.7)
(CHP × CHB)F_1 × CHP	54	–	38 (70.4)	–	–	–	–	–	–	–

Reciprocal crosses between two strains and backcrosses of F_1 hybrids were done by induced mating soon after mosquitoes fed on golden hamster blood. Following individual oviposition by mated females, eggs were counted and placed in hatching trays. Spermathecae of mated females were examined for evidence of insemination. After hatching, larvae were counted and reared in the laboratory. The techniques for preparation of the chromosome were similar to those described by French *et al.* (1962) and Kanda (1971). The number of chromosomes observed was 20 for each of the arms in both parents and F_1 hybrids. The preparations were compared with the standard chromosome of Chowdaiah *et al.* (1970).

RESULTS

Chon Buri (CHB) female X Chumphon (CHP) male : From 48 crosses, 17 batches totaling 1335 eggs (which was fewer than of the parental strains) were laid. Egg numbers ranged from 54 to 103 per mosquito. Dissection of all ovipositing females showed the presence of sperms. Embryonation rate was 69.8% whereas the hatchability rate was 0% (Table 2). Dissection of deposited eggs showed the dead larvae inside which had failed to hatch.

Chumphon (CHP) female X Chon Buri (CHB) male : From 36 crosses, 11 batches totaling 1509 eggs were obtained. The number of eggs ranged from 130 to 185 per mosquito. Dissection of all ovipositing females showed the presence of sperms. The percentage of embryonation and hatchability of eggs were 78.5% and 60.8%, a lower rate than obtained in the parental crosses (Table 2). Only 153 larvae could successfully develop to adults of which 132 (86.3%) were females and 21 (13.7 %) were males.

The average body weight of F_1 hybrid females was 1.45 ± 0.32 mg which showed statistical difference when compared to parental Chumphon strain and Chon Buri strains (Table 1). In morphological studies all adult females resembled the Chumphon strain in all characters. All F_1 hybrid males had abnormal genitaliae with very short claspers, degeneration of accessory gland and testes which were very easy to break during the dissection (Fig. 1-A and B). The salivary chromosome of both strains from parental colonies showed homosequential banding patterns without any rearrangement when compared to standard chromosome described by Chowdaiah *et al.* (1970), while the F_1 hybrids' chromosome showed incomplete asynapsis along the whole length of all autosomes, except for X-chromosome which showed complete synapsis (Fig. 1 - C).

(CHP X CHB) F_1 female X Chumphon (CHP) males : From 54 crosses, none of them successfully laid eggs. Dissection of all mated females showed the atrophy of the ovaries without the development of follicles (Fig. 1 - D).

DISCUSSION

An. barbirostris was selected for this study because of the marked variation in the external morphological characters of adults (Reid, 1968 ; Harrison and Peyton, 1975). Therefore, one might expect some degree of genetic incompatibility even though there are variations in only the adults characters. However, the crosses do indicate that they are genetically isolated and are similar in other respects to the results of reported anopheline crossing studies within the species complex. The existence of morphological variations and reproductive isolation between the strain from Chumphon and Chon Buri are evident by the present results. The significant differences in the darkness of the wing at the stem of vein 5, the degree of distribution of abdominal pale scale on the sternite, the average

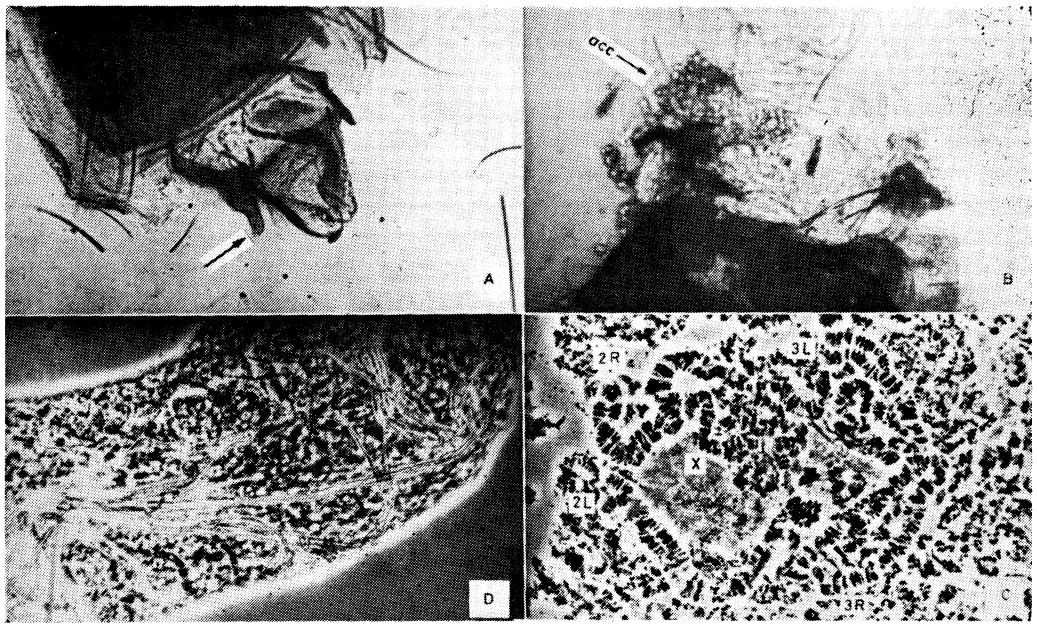

Fig. 1—The results of (CHP × CHB) F_1 hybrids with poor development of reproductive organs with very short clasper (A), degeneration of accessory glands (acc) and testes (B), incomplete synapsis along the whole lengths of autosome (C) and part of an ovary showing absence of follicular development (D).

egg deposition per gravid female of the two strains and the intermediate form of the average body weight of the F_1 hybrids were observed. Secondly, viabilitity disturbance in the F_1 hybrids with extreme reduction in the number and fertility of the F_1 hybrid females and males, and thirdly, the asynapsis of the salivary chromosome of the F_1 hybrids between these two strains were evident. Even though these are preliminary results of laboratory crossing studies between the strains within *An. barbirostris*, the present findings furnish impetus for a study of further relationships particularly in the natural populations also by the identification of karyotype and by biochemical methods.

SUMMARY

Crossing experiments between two strains of *Anopheles (Anopheles) barbirostris* from Chumphon and Chon Buri provinces in Thailand were done by induced copulation in order to determine the genetic relationship. On comparison of the F_1 hybrids and those of their parent species as the control, there was a difference in the number of eggs laid, hatchability and viability. The low viability of the F_1 hybrids with high larval and pupal mortalities, producing only a few F_1 hybrids, and the fact that the F_1 hybrids' salivary chromosome showed asynapsis suggest there exists reproductive isolation between both strains. The data presented suggest that these two strains exhibit possible presence of a species complex in *An. barbirostris*.

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