Comments on Reproductive Isolation and Phylogeny of Mosquitoes of the Genus *Aedes*

W. Keith Hartberg
Institute of Arthropodology and Parasitology
Department of Biology
Georgia Southern College
Statesboro, Georgia 30458

This paper is intended to present a brief review of part of what we know concerning reproductive isolation and how it supports the ideas we have on the phylogenetic relationships in *Aedes*.

The mechanisms which reproductively isolate one species from another may be considered the most important attributes of a species since they are by definition the species criteria. Not only are there different kinds of reproductive isolation, but also the isolation of species in the same group of organisms is often achieved by different means. The isolation of a given pair of related species may result from several isolating mechanisms each reinforcing the actions of the others.

Figure 1 gives a classification of isolating mechanisms modified from McClelland (1967). The premating mechanisms are probably the most important. The effect of the different isolating mechanisms is genetically the same, that is, the limitation and prevention of gene exchange between populations of different species (possibly also subspecies, i. e., *Aedes aegypti aegypti* and *Aedes aegypti formosus*). We should remember that any reproductive isolation is caused by genetic differences between populations. It is essential that we emphasize population means and not individuals in any characteristics we intend to use to delineate species, since a great deal of polymorphism is evident in *Aedes* populations (Summers-Connal 1927, McClelland 1960, 1962, Craig et al. 1961, Craig and Hickey 1967, Hartberg and McClelland 1973).

Reproductive isolation is probably due to pleiotropic gene action rather than to the effects of a specific gene or genes. With the remoteness of the phenotype from the primary action of the gene, and the number of intervening steps which can be influenced by other genes and by environmental factors, secondary effects may give rise to a "genetic syndrome" consisting of a variety of phenotypic (morphological, behavioral, physiological) effects. Therefore, the measure of isolation separating two closely related species can be used to determine or infer the degree of behavioral, genetic and chromosomal homology between them.

Crossing experiments are one means of determining relationships. In crosses where fertile offspring can be obtained, even if only in one direction, it may be possible to determine the nature of the genetic mechanisms controlling the differences between the species, as well as to determine which of the mechanisms have been altered during speciation. Such investigations should provide a pattern showing evolutionary relationships.

It has only been in recent years that studies per se on reproductive isolation in mosquitoes have been undertaken. Most of the work has been with the genus *Aedes*. This paper will deal with the major works done with the subgenus *Stegomyia*, Groups A and C, and subgenus *Finlaya*. Figure 2 gives a suggested phylogeny of some of the *Stegomyia* to help the reader place the species that will be discussed.

In the *Finlaya* some studies have been conducted with both Group B and H. O'Meara and Craig (1970) attempted crosses between each of the four subspecies of *Aedes atropalpus (A. a. atropalpus, A. a. epactius, A. a. perichares, A. a. nielseni).* Their results indicate a high degree of interfertility between each of the four forms. Although additional quantitative experimentation is needed to determine the extent of this interfertility, it does not appear that *A. atropalpus* is a complex of species. Their findings make it clear that geographical isolation has played an important role in the development of subspecies in *A. atropalpus*.

The 16 diagonostic characters, based on adult and larval morphology to separate the 4 subspecies discussed by O'Meara and Craig (1970) provide raw material that should readily lend itself to genetic analysis. Such studies could provide some insight into genetic changes which have occurred during the formation of the subspecies. O'Meara (1972) has made a beginning with his analysis of polygenic regulation of autogenous fecundity in *A. atropalpus*.

Gwadz (1970) found that females of *A. a. atropalpus* could be inseminated one day after emergence, whereas *A. a. epactius* required four days. This indicates possible temporal or sexual isolation between these two. The genetic factors controlling this sexual receptivity appear to be closely linked to the gene which controls the penetrance of autogeny.

Brust (1974) suggests that the *A. atropalpus* group is made up of two species rather than four subspecies. He has shown a high genetic affinity between *A. a. epactius*, *A. a. perichares* and *A. a. nielseni*, while *A. a. atropalpus* has a low genetic affinity with them. SEM micrographs of the chorionic sculpturing of the eggs support the concept of two species. Brust supports the elevation of *A. a. atropalpus* and *A. a. epactius* to species status. Since *A. a. nielseni* and *A. a. perichares* do not differ biologically from *A. a. epactius*, they should be treated as one species (Brust 1974).

Table 1 shows the results obtained by Truman and Craig (1968) from crosses involving *Aedes hendersoni* and *A. triseriatus*. As can be seen, there is evidence for several isolating mechanisms separating these two mosquitoes, i. e., hybrid breakdown, mechanical isolation, and the almost complete absence of intermediates in field collections would indicate possible behavioral barriers. Several morphological differences between *A. hendersoni* and *A. triseriatus* appear to be controlled by single genes.

In the subgenus *Stegomyia*, most studies involving hybridization and reproductive isolation have been with species in Groups C and A. Table 2 summarizes some of the results obtained by Woodhill (1949, 1950) in crosses involving *Aedes scutellaris scutellaris* and *A. s. katherinensis*. He also found that in all cases eggs failed to hatch when *Aedes pseudoscutellaris* was crossed with *A. s. scutellaris* or *A. s. katherinensis*. It is obvious that isolating mechanisms are operative (probably postmating mechanisms), and Woodhill used his observations to support taxonomic considerations.

Perry (1950) conducted crossbreeding studies with *A. s. scutellaris* and *Aedes pernotatus*. In the cross between *A. s. scutellaris* females and *A. pernotatus* males he recovered only 18 female offspring, and no offspring were obtained from the reciprocal cross. In the backcross of the F_1 females from his first cross to *A. pernotatus* males he observed a pronounced reduction in number of offspring. His results would indicate several isolating mechanisms being operative, including hybrid breakdown, since several developmental abnormalities were noted in the hybrids.

No evidence of reproductive incompatibility was found in crosses between *A. s. scutellaris* and a Malaysian form designated as ssp. *malayensis* (Colless 1962). In the F_2 a variety of forms appeared; some in which the genitalia resembled *Aedes hensilli* which Colless (1962) considered the fourth subspecies of the "scutellaris" group. It would be interesting to pursue these findings with additional studies.

Aedes polynesiensis and *A. pseudoscutellaris* have been shown to be interfertile (Rozeboom and Gilford 1954, Woodhill 1954), although in some instances reduced percentages of hybrid eggs hatched. Thus a partial barrier exists, as well as indications of some mating (behavioral?) barriers. Rozeboom and Gilford (1954) obtained no viable eggs in a hybridization experiment between *A. polynesiensis* and *Aedes aegypti*.

Tesfa-Yohannes and Rozeboom (1974) present results of crossing experiments with *A. s. malayensis* and three populations *A. polynesiensis* which show that these two species are separated by both premating and postmating mechanisms. Viable eggs were only produced in a mating between *A. s. malayensis* females and Samoan *A. polynesiensis* males. It was not possible to obtain an F_2 generation, but some success was obtained in backcrosses to *A. s. malayensis*.

Gubler (1970) has shown that males of *Aedes albopictus* mate readily with females of *A. polynesiensis* with sperm transfer taking place, however, no fertile eggs are produced. He found no insemination of *A. albopictus* females by *A. polynesiensis* males and concludes the barrier is precopulatory (ethological isolation), whereas in the reciprocal cross it is apparently gametic. Gubler (1970) comments that his findings suggest the possibility of utilizing male *A. albopictus* to control *A. polynesiensis* on certain South Pacific islands. He also points out that the observed mating behavior and isolating mechanisms operative between *A. aegypti*, *A. albopictus*, and *A. polynesiensis* supports the taxonomic and phylogenetic status given them.

Leahy and Craig (1967) produced an excellent piece of work elucidating the barriers to hybridization between *A. aegypti* and *A. albopictus*. They used numerous strains from diverse origins in more than thirty combinations of species crosses. No hybrid offspring were hatched from more than 156,466 eggs. They demonstrated at least five barriers acting in sequence to isolate

A. aegypti and *A. albopictus*: (1) mating behavior, (2) structural incompatibility, (3) sperm inactivation, (4) reduced oviposition, (5) genetic incompatibility.

Evidence has been given by Nijhout and Craig (1971) for the presence of a sexual pheromone in the *Stegomyia*. Their data indicate that recognition of females is independent of flight sound or ultrasonic frequencies, but seems to be effected by contact chemoreception. Table 3 shows the results of their experiments on the ability of males of four species of *Stegomyia* to recognize their conspecific females. In every case the males preferentially seized conspecific females. It is of particular interest to note that *A. a. aegypti* males showed more discrimination against *A. simpsoni* than *A. mascarensis*, and the least towards *A. a. formosus* which certainly indicates a closer relationship. *Aedes mascarensis* males were also able to recognize their own females.

Interestingly *A. albopictus* showed 100% correct choice when tested with *A. mascarensis*. When observing mixed swarms of *A. albopictus* and *A. mascarensis* in the field in Mauritius, I observed many times *A. albopictus* males coming into contact with *A. mascarensis* females and "being repelled" without sexual union, and the same with *A. mascarensis* males and *A. albopictus* females. It is unfortunate that Nijhout and Craig (1971) didn't give the *A. mascarensis* males a choice between *A. mascarensis* and *A. albopictus* females.

Also, I would like to have seen *A. a. formosus* males given a choice between *A. mascarensis* and *A. albopictus* females. It is interesting to speculate on the probability of the *A. a. formosus* males showing more of a tendency for *A. mascarensis* females than the *A. a. aegypti* males showed, as *A. a. formosus* and *A. mascarensis* are more alike in their field behavior and possibly closer genetically.

At this point we can sum up by saying that it appears that the further removed genetically the two females are that are offered a male, the more often he will make the correct choice.

Gubler (1970) showed that *A. albopictus* males mate readily with *A. polynesiensis* females; however, Nijhout and Craig (1971) showed that there is strain variation in regards to the sexual acceptability of *A. polynesiensis* females to *A. albopictus* males. This again emphasizes the importance of considering the genetic makeup of particular populations whenever studying isolating mechanisms and/or phylogenetic relationships. Some genetic divergence and reproductive barriers have been shown to exist between strains of *A. polynesiensis* (Tesfa-Yohannes 1973).

It is intellectually stimulating to speculate on the possible applications of Nijhout and Craig's (1971) findings for mosquito control. Would it be possible to chemically isolate and manufacture these "contact sexual pheromones" and spray them over an area? Would the use of conspecific "pheromone" result in "overloading" of the males' receptors and cause enough confusion to interfer in normal mating patterns? Could the "pheromone" of a heterospecific type be produced and sprayed thus repelling target males from everything around them?

The results reported by Nijhout and Craig (1971) on mating preference are similar to those obtained earlier by Hartberg and Craig (1968). Sexual isolation was demonstrated by Hartberg and Craig (1968) between *A. aegypti* and *A. mascarensis* using three different experiments: (1) Male Choice, (2) "Hide-and-Seek", (3) Female Choice. The data obtained from the male choice experiment indicate that both *A. a. aegypti* and *A. a. formosus* males can readily discriminate between their own females and those of *A. mascarensis*. The "hide-and-seek" test further reinforces this observation by demonstrating that the *A. aegypti* male will seek out and mate with its own female even in a situation where the *A. mascarensis* females far outnumber the *A. aegypti* female..It should be noted that the *A. aegypti* male will mate readily with the *A. mascarensis* female when no choice of mates is given. The slight differences obtained with males of different strains of *A. aegyptic* may indicate strain differences in ability to discriminate mates.

Hartberg and Craig (1968) also showed that an interesting situation exists between *A. a. aegypti* and *formosus*. In the male choice experiment, both types of males showed a pronounced preference for their own females when given a choice with *A. mascarensis*. However, while type-form males preferred type-form females when given a choice with *formosus* females, the *formosus* males also preferred type-form females when given a similar choice. Perhaps the preference shown for type-form females by the *formosus* males may by due to a problem in experimental design. It is conceivable that the activity of the feral *formosus* females is modified when they are confined to cages. Perhaps the urban and domesticated type-form females are more adaptable to cages. If so, their activity would not be so greatly affected. This could possibly override the "sexual pheromone" (Nijhout and Craig 1971) and they would be more attractive to the males.

It is interesting to note that the *A. a. aegypti* males readily mate with *A. a. formosus* females when given only a choice between them and *A. mascarensis*. This would seem to indicate a much closer relationship between *A. a. aegypti* and *A. a. formosus* than between these two forms and *A. mascarensis*.

A. mascarensis males did not demonstrate a consistent strong choice as to mates. This may have been due to the experimental design and the behavior of *A. mascarensis* males under the experimental conditions. It seems probable that under field conditions sexual isolation would be more pronounced.

Hartberg and Craig (1970) were able to demonstrate hybrid breakdown (production of abnormal males) between *A. aegypti* and *A. mascarensis*. This was the first well-documented evidence of hybrid breakdown in Group A, although McClelland (1962) felt he had evidence for hybrid breakdown in crosses he did with *A. simpsoni* and *A. woodi*.

The hybrid breakdown between *A. aegypti* and *A. mascarensis* appears when the genetic contribution of *A. aegypti* is above 50% and the male-determining chromosome of *A. mascarensis* is present (Hartberg and Craig 1970). The data suggest a polygenic basis for this hybrid breakdown. The more the proportion of *A. aegypti* genetic material present in the progeny is above 50%, the greater the number of abnormal males produced.

The mode of inheritance and linkage of some of the factors determining morphological difference between *A. aegypti* and *A. mascarensis*, and the genetic basis of variations in *A. mascarensis* have been determined (McClelland 1962, Hartberg and McClelland 1973, Hartberg and Craig 1973, 1974). Evidence points to most differences being due to single genes.

McClelland (1962) presents data which indicates that hybrid breakdown is one of the isolating barriers between *A. woodi* and *A. simpsoni*. In a later study, Hartberg (1972) indicates that other isolating mechanisms separating these two species include behavior, mechanical isolation, and gametic isolation. The production of fertile hybrids between these two species, however, indicates a high degree of genetic and chromosomal homology. In the F_2 generation and in backcrosses to *A. woodi*, McClelland (1962) noted in some progeny that characters of *A. woodi* assorted independently. Hartberg (1972) in his study demonstrated that single factors form the genetic basis of some of the differences between the two species.

We know more about the genetics and reproductive isolation in *Stegomyia*, Group A, than we know about any other group. If we take all this information into consideration we can construct a phylogenetic tree for five of the 32 members of Group A (Fig. 3). Now that other species such as *A. heischi* and *A. metallicus* have been colonized, the tree could be enlarged and extended with a minimum of additional work.

The more knowledge we can accumulate of the formal genetics of species and of those reproductive barriers which keep them separate, the better will be our understanding of the evolution and phylogenetic relationships of the species. In addition, information accumulated in these types of studies could contribute directly to control programs through the uncovering of mechanisms that could be manipulated to our advantage and the mosquitoes' disadvantage.

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FIG. 1 -- CLASSIFICATION OF ISOLATING MECHANISMS

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1. PREMATING MECHANISMS: Barriers to gene flow preventing sexual contact between potential mates.

   (a) Ecological Isolation -- Populations are found in the same general territory (sympatric), but they occupy different "ecological niches."

   (b) Temporal Isolation -- Mating occurs at different seasons or at different times of day.

   (c) Sexual Isolation -- Mating discouraged through individual discrimination between potential mates.

2. POSTMATING MECHANISMS, Pre-zygotic: Barriers to gene flow preventing gametic union.

   (d) Mechanical Isolation -- Copulation attempted but no sperm transfer; physical non-correspondence of genitalia.

   (e) Gametic Isolation -- Sperm transfer takes place but eggs are not fertilized.

3. POSTMATING MECHANISM, Post-zygotic: Barriers to gene flow which eliminate or handicap hybrid zygotes.

   (f) Hybrid Inviability -- Hybrid zygotes are inviable, or adaptively inferior.

   (g) Hybrid Sterility -- F_1 hybrid zygote fully viable but partially or completely sterile.

   (h) Hybrid Breakdown -- Inviability, or adaptive inferiority, of all, or a part, of the F_2 or backcross hybrids.

Fig. 2 - A SUGGESTED PHYLOGENY OF STEGOMYIA

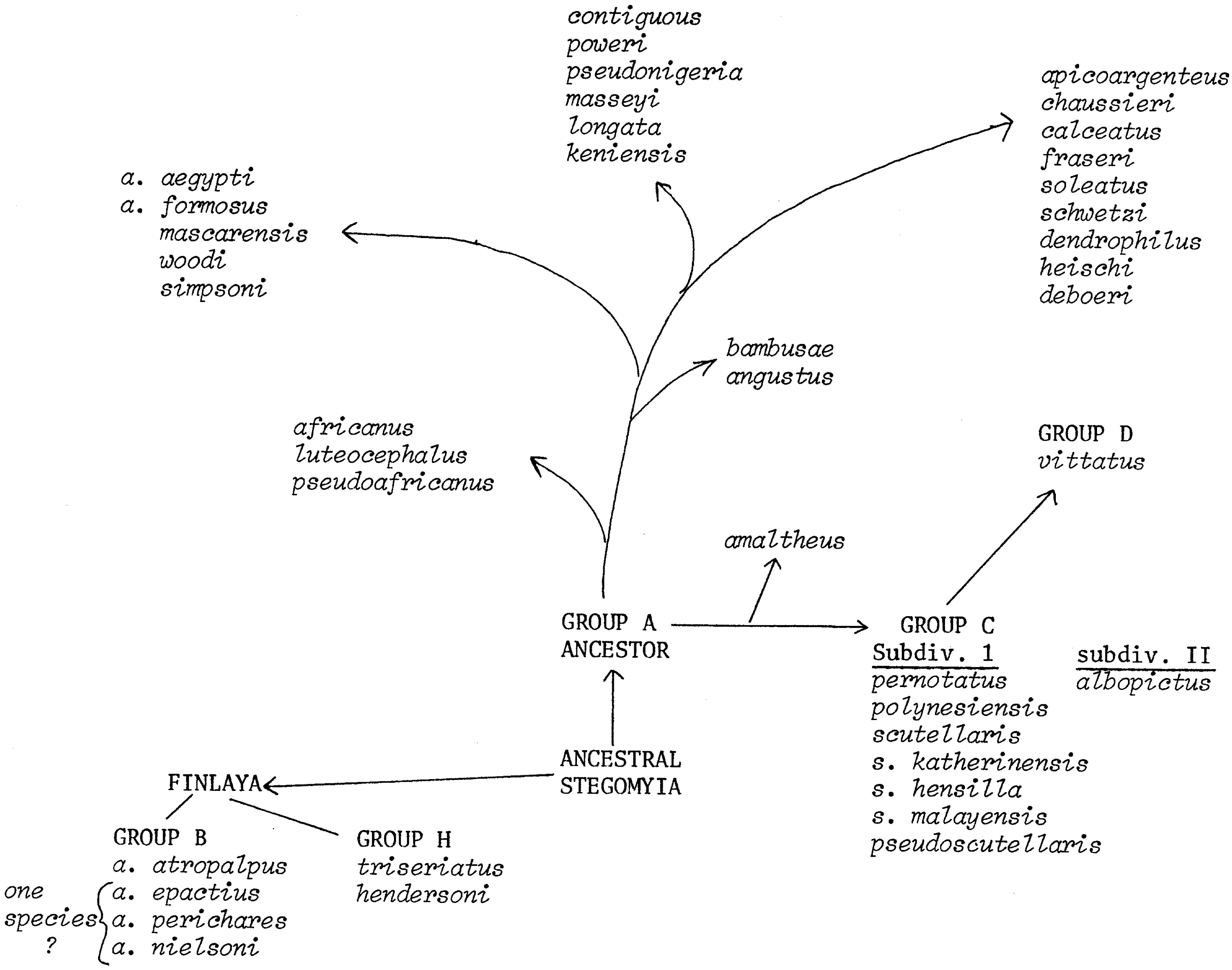

Fig. 3 - SUGGESTED PHYLOGENY OF STEGOMYIA GROUP A

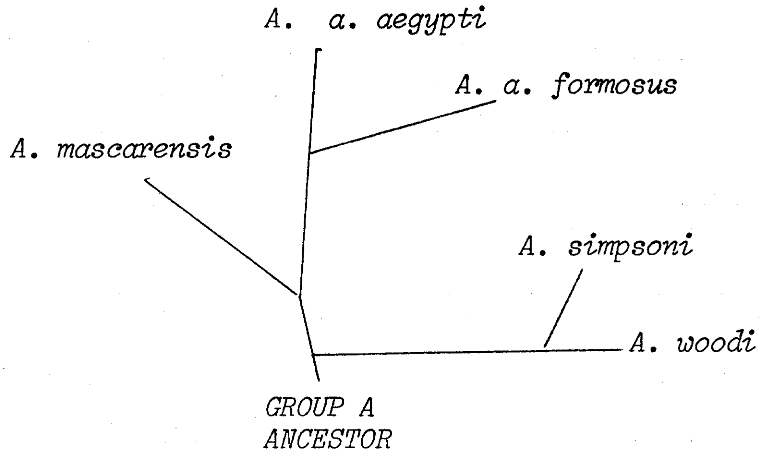

Table 1

Hybridization between *Aedes hendersoni* and *Aedes triseriatus*
(Truman and Craig, 1968)

Cross		Results
Female	Male	
A. hendersoni	*A. triseriatus*	F_1 females and F_1 males fertile & normal
A. triseriatus	*A. hendersoni*	F_1 females fertile; 51-71% F_1 males have abnormal genitalia
F_1 (h x t)	F_1 (h x t)	more difficult to mate; some "mechanical" problems (semen often deposited externally); some backcrosses gave few offspring.
Backcrosses except with F_1 male (t x h)		

Table 2

Results of experimental crossing with *Aedes scutellaris scutellaris*, *Aedes scutellaris katherinensis*, and F_1 hybrids (s x k) (Woodhill, 1949, 1950)

Crosses			% eggs hatched
scutellaris	*katherinensis*	*Hybrid*	
male	female		0
female	male		100
	female	male	0
	male	female	100
female		male	100
male		female	100

Table 3

Ability of males of four species of *Aedes (Stegomyia)* to recognize conspecific females (Nijhout and Craig, 1971)

Male Tested	Heterospecific* females	Correct choice, conspecific coupling (N = 40 trials) No.	%
A. a. aegypti	*A. a. formosus*	24	60
	A. mascarensis	29	72
	A. albopictus	31	78
	A. simpsoni	34	85
	A. mascarensis - *A. albopictus***	22	55
A. mascarensis	*A. a. aegypti*	26	65
A. albopictus	*A. a. aegypti*	40	100
	A. mascarensis	40	100
	A. polynesiensis	40	100
A. polynesiensis	*A. a. aegypti*	40	100
	A. mascarensis	40	100

*Males placed with 3 conspecific and 3 heterospecific females.

**In this trial there was no conspecific female; coupling with the closely related *A. mascarensis* was considered the correct choice.