Speciation in the *Aedes (Stegomyia) scutellaris* group (Diptera: Culicidae): Contributing factors

by Vas Dev[1]

ABSTRACT. A multifaceted approach was used to study the mechanisms of speciation in the *Aedes scutellaris* group of species. A genetic evidence is provided in support of the hybrid origin of species. It appears that hybridization has been a potent factor in speciation together with geographical isolation and cytoplasmic differentiation. An East to West migration pattern is suggested.

The *Aedes scutellaris* group provides an ideal species complex for the study of mechanisms of speciation because of the number of species within the group and their insular pattern of distribution in the South Pacific (Belkin 1962, Huang 1972, Huang and Hitchcock 1980). Most species are endemic to single islands and can easily be brought into the laboratory culture and crossed without forced copulation. Considerable information has been accumulated on species relationships of this group based on experimental hybridization (see, Macdonald 1976, Rai et al. 1982), and a few have been examined cytologically (Dev and Rai 1984, Sherron and Rai 1984). A multidisciplinary approach is necessary to study the genetics and evolution of reproductive isolating mechanisms (Bush 1975). This approach has revealed a multitude of factors which might have been important in speciation in 5 species of this group based on data obtained for experimental hybridization (Dev and Rai 1982a, 1985a) and cytological analyses of species and their hybrids (Dev and Rai 1984). These included *Ae. polynesiensis* Marks, *Ae. pseudoscutellaris* (Theobald), *Ae. kesseli* Huang and Hitchcock, *Ae. malayensis* Colless and *Ae. alcasidi* Huang. Of these, *Ae. polynesiensis* is widespread on a number of islands in the South Pacific, and occurs sympatrically with *Ae. pseudoscutellaris* in Fiji. *Aedes kesseli* is endemic to the Tonga islands. *Aedes malayensis* and *Ae. alcasidi* are species found in the western range of species group in Southeast Asia.

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[1] Department of Biology, University of Notre Dame, Notre Dame, IN 46556, USA. Present address: Department of Zoology, Panjab University, Chandigarh-160014, India.

Reciprocal crosses, backcrosses and F_2 crosses revealed that there were no complete reproductive barriers between certain species, e.g., *Ae. polynesiensis* and *Ae. pseudoscutellaris*. Many species, especially of the western range, were only unidirectionally incompatible. This phenomenon was first documented by Woodhill (1949) between *Ae. scutellaris scutellaris* Walker and *Ae. scutellaris katherinensis* Woodhill and since then has been found in many species (Dev and Rai 1985a, Sherron and Rai 1983). The basis of this unidirectional incompatibility appeared to be of cytoplasmic origin (Dev and Rai 1982b, Dev in prep.); this is similar to conclusions reached by Smith-White and Woodhill (1955) for species of this group, and Laven (1958) for the *Culex pipiens* complex. Thus, cytoplasmic differentiation is one of the important factors which restricts gene flow between species of this group. Kitzmiller and Laven (1958) have emphasized the utilization of cytoplasmic differentiation as a mechanism of speciation in mosquitoes, especially in the *Culex pipiens* complex. Cytoplasmic differentiation was observed by the differential fertility of interspecific hybrids when backcrossed to either parent (Dev and Rai 1982a, 1985a). Hybrid males from the cross involving *Ae. kesseli* females and *Ae. polynesiensis* males always produced lower egg hatch when backcrossed to either parent. However, the hybrid males from the reciprocal cross resulted in lower egg hatch when backcrossed to *Ae. polynesiensis* females as opposed to *Ae. kesseli* females. Such differential fertility must ensue from some interaction between female cytoplasm and male genome.

Overall, the data from experimental hybridization supports the hypothesis of allopatric speciation through geographical isolation. Species in close geographic proximity showed relatively higher genetic affinities than species separated by wide geographical distances. for example, the species of Polynesia were more closely related to each other but were individually or as a group distantly related to other species of the western range (Dev and Rai 1982a). Similar conclusions have been reached by other workers based on electrophoretic data, morphological similarity and distribution patterns (Hilburn and Rai 1981, Rai et a., 1982, Pashley and Rai 1983). It seems that geographical isolation has been a very important factor in speciation in this species group due to their insular pattern of distribution; this hypothesis was strongly favored by Rai et al. (1982). Perhaps due to an allopatric mode of speciation there has been no selection for the development of reproductive isolating mechanisms and only low levels of morphological differentiation because habitats occupied by these species are similar. It is interesting that hybridization between several species of this group is evidently determined cytoplasmically and is exactly comparable to the situations found in the *Culex pipiens* complex except that the latter is a single species. Members of both species groups have been reported to be infected by rickettsia-like organisms, *Wolbachia* species determining the crossing relationships (Yen and Barr 1971, 1973, Wright and Wang 1980, Meek 1984). This type of speciation mechanism involving infection by a microorganism has been termed as nongenetic (Powell 1982).

Cytological analyses of species and their hybrids revealed fixed inversion and chromosome size differences between some species; however these differences do not account for lower fertility of interspecific hybrids (Dev and Rai 1984). Nevertheless, it was apparent that the greater the morphological similarity between two species, the smaller the chromosomal divergence and vice versa. Thus, chromosomal repatterning seemed to be associated with speciation. The role of cytoplasmic differentiation in the *Aedes scutellaris* group of species was observed by studies of the cytology of reciprocal species hybrids. The chromosome cytology of male hybrids obtained by crossing *Ae. kesseli* females and *Ae. polynesiensis* males was strongly associated with chromosomal breakage when compared with hybrid males from the reciprocal cross (Dev and Rai 1984). Similar observations have been made between other interspecific hybrids of this species group (Sherron and Rai 1984).

Darlington (1958) suggested that the frequency and distribution of crossing over must have an important effect on the genetic structure of the species. Garber (1958) showed that chiasma frequency comparisons have cytotaxonomic value in studying relationships among plant species of the genus *Collinsia*. An analysis of chiasma frequencies in species and hybrids of the *Aedes scutellaris* group of species supports these statements (Dev and Rai 1984). The mean chiasma frequency for each species differed significantly and was species specific. Chiasma frequency analysis has provided the cytological basis for species status assigned by Belkin (1962) to members of the *scutellaris* group based on morphological differences. Furthermore, analysis of chiasma frequencies of species hybrids provided a measure of genetic relatedness between species. Belkin (1962) argued for the hybrid origin of several species of this group based on morphological comparisons. Analysis of chiasma frequencies seemed to provide cytological evidence for a hybrid origin of species. While the mean chiasma frequency of each species was significantly different from all others, the mean chiasma frequency of hybrids between two species was not significantly different from 3rd species (Dev and Rai 1984). For example, the mean chiasma frequency of *Ae. pseudoscutellaris* was not significantly different from those of reciprocal hybrids between *Ae. polynesiensis* and *Ae. kesseli*. Since chiasma frequency is a function of the genetic structure of the species (Darlington 1958), this indicates that the genetic structure of *Ae. pseudoscutellaris* is very similar to reciprocal hybrids between *Ae. polynesiensis* and *Ae. kesseli*. Therefore, *Ae. pseudoscutellaris* appear to be of hybrid origin between the latter two species or these two species originated from *Ae. pseudoscutellaris;* the former being more likely the case. *Aedes pseudoscutellaris* has more resemblance to *Ae. polynesiensis* than *Ae. kesseli* (Huang 1975); perhaps this is due to introgression by continuous backcrossing to *Ae. polynesiensis* parents. Introgression between these two species would have been likely because they coexist in Fiji and there being no reproductive isolation between these two species (Dev and Rai 1982a). Similarly, *Ae. malayensis* appeared to be of hybrid origin between *Ae. polynesiensis* females and *Ae. pseudoscutellaris* males. *Aedes alcasidi* seems to have originated from *Ae. pseudoscutellaris* females and *Ae. polynesiensis* males. The proposed common ancestry of *Ae. malayensis* and *Ae. alcasidi* further demonstrates the role of cytoplasmic differentiation in speciation. The common ancestry of these two species is further supported by their very close genetic affinities revealed by their mating compatibility (Dev and Rai 1982a), electrophoretic analysis (Hilburn and Rai 1981) and morphological similarity (Huang 1972). Thus out of 5 species examined, 3 appear to be of hybrid origin (Fig. 1). Hybridization between the proposed parental species has been demonstrated under laboratory conditions and no hybrid breakdown was evidenced in F_2 and backcross progenies (Dev and Rai 1982a); thus making hybrid origin of species more likely a process in this species group. In fact, hybrid origin has been considered to be the most important among transilience modes of speciation based on a population genetic approach model (Templeton 1981). Many more species within this group need to be examined in this way in order to construct a phylogenetic tree. A hybrid origin of species has also been proposed by Mattingly (1954) for other *Stegomyia* mosquitoes; however, it has also been seriously questioned by others (McClelland 1967, Rai 1980).

In addition to conventional procedures for studying species relationships, genetic analysis of the morphological differences among *Ae. polynesiensis*, *Ae. pseudoscutellaris* and *Ae. kesseli* revealed major gene substitutions with several modifiers for two characters, white scaling on the hind femur and a larval gill pattern. The trait anal saddle phenotype was polygenic (Dev and Rai 1985b). Thus, concurrent with the development of reproductive isolation mechanisms, individuals of a population are also subject to ordinary pressures of variation and/or selection.

In summary, *Ae. polynesiensis* appears to be the major ancestral species, and there has been a gradual westward movement aided by man of founders from two species giving rise to a new species of hybrid origin. Belkin (1962) held the same opinion based on morphological similarities, i.e., East to West migration pattern. However, Marks (1954), Hoyer and Rozeboom (1977) and Sherron and Rai (1984) have suggested West to East migration pattern. In fact the latter authors even suggested *Ae. malayensis* and *Ae. alcasidi* as the ancestral species based on data on chromosome lengths. In either case, hybridization has been a potent factor in speciation together with geographical isolation and cytoplasmic differentiation.

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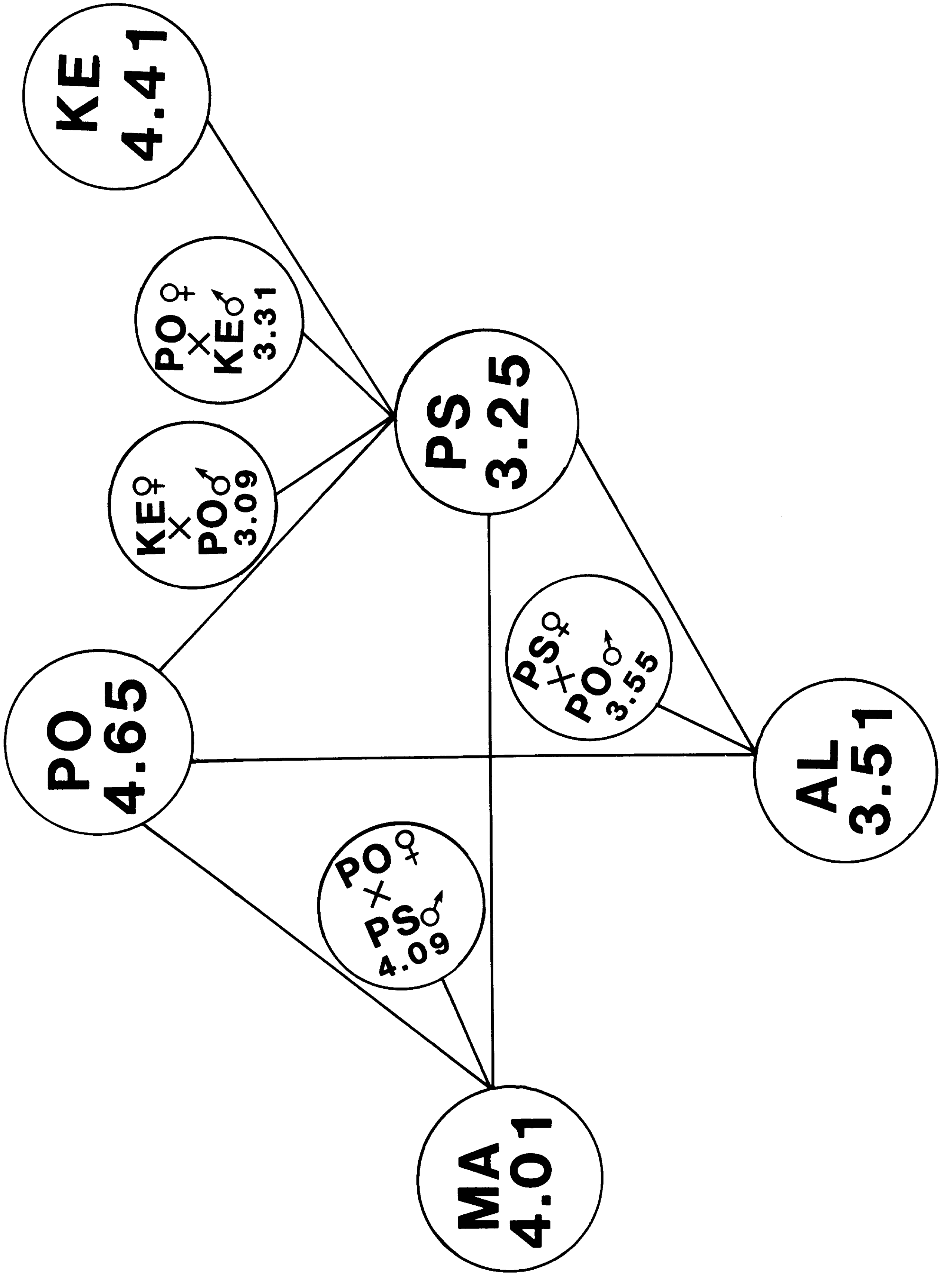
Explanation to Figure 1.

Hybrid origin in 5 species of the *Aedes (Stegomyia) scutellaris* group. Numbers are mean chiasmata frequencies of species and hybrids taken from Dev and Rai (1984).

Abbreviations:

PO = *Ae. polynesiensis*
PS = *Ae. pseudoscutellaris*
KE = *Ae. kesseli*
MA = *Ae. malayensis*
AL = *Ae. alcasidi*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PO♀ × KE♂ 3.31
KE♀ × PO♂ 3.09
PS 3.25
PO 4.65
PS♀ × PO♂ 3.55
AL 3.51
PO♀ × PS♂ 4.09
MA 4.01