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Mini-Review

Non-reciprocal fertility among species of the *Aedes (Stegomyia) scutellaris* group (Diptera: Culicidae)

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Summary. The genetic basis of non-reciprocal fertility in the *Aedes scutellaris* group has been viewed in relation to cytoplasmic incompatibility in the *Culex pipiens* complex and related to the presence or absence of rickettsia-like microorganisms *Wolbachia* spp. A crossing scheme is proposed to explore the genetic basis of non-reciprocal fertility between two species by crosses involving a third mutually compatible species.

Key words. Cytoplasmic incompatibility; *Aedes scutellaris; Wolbachia;* speciation.

There has been considerable interest in the phenomenon of non-reciprocal fertility in mosquitoes because of its potential use in population suppression programs. Also, the mechanism(s) underlying non-reciprocal fertility may be important for an understanding of speciation. This phenomenon is widespread in the family Culicidae; an increasing number of species complexes are being recognized and crossing relationships between sibling species are at present being studied. Non-reciprocal fertility in the *Aedes scutellaris* group was first reported to occur between *Ae. scutellaris scutellaris* Walker and *Ae. scutellaris katherinensis* Woodhill[1]. Fertile hybrids were obtained by crossing *Ae. s. scutellaris* females with *Ae. s. katherinensis* males; in the reciprocal cross *Ae. s. katherinensis* females were inseminated by *Ae. s. scutellaris* males but all eggs laid were inviable. Woodhill[2] further showed that the *Ae. s. katherinensis* females when crossed with hybrid males derived from the cross *scutellaris* females × *katherinensis* males laid inviable eggs; the other three backcrosses were successful. The origin and nature of such non-reciprocal fertility is of genetic interest and can be analysed using egg hatch as a marker since viable hybrids can be obtained in one direction and the hybrids can be backcrossed to parental species.

Smith-White[3] drew attention to possible genetic mechanisms underlying this phenomenon of non-reciprocal fertility and suggested a sequential backcrossing scheme which was later exploited by Laven[4] studying the *Culex pipiens* complex. Smith-White and Woodhill[5], studying the *Ae. scutellaris* complex, used this backcrossing scheme to determine the possible genetic bases for non-reciprocal fertility. The latter authors concluded that non-reciprocal fertility must depend either on anomalous meiosis in oogenesis or on nucleus-independent cytoplasmic factors. The second mechanism paralleled the findings of Laven[4,6], based on his extensive sequential backcrossing experiments with the *C. pipiens* complex. Since this phenomenon of non-reciprocal fertility or unidirectional incompatibility appears to have a cytoplasmic basis, this is often referred to as cytoplasmic incompatibility, and these terms are used interchangeably. McClelland[7] has proposed an alternative to the hypothesis of cytoplasmic inheritance to account for the phenomenon of non-reciprocal fertility, reciprocal fertility and reciprocal incompatibility, which involves cytoplasmic conditioning alleles and preferential segregation.

Yen and Barr[8] attributed this cytoplasmic incompatibility in the *C. pipiens* complex to the presence of rickettsia-like organisms, *Wolbachia* spp. Later they demonstrated that the incompatibility barrier could be broken by removing these microorganisms by a treatment of larvae with antibiotics, e.g. tetracycline hydrochloride[9]. Further, Barr[10] offered a hypothesis to account for strain differences affecting fertility and multiplicity of crossing types in *C. pipiens* based on the presence of different kinds of symbionts and their ability to evolve with their hosts. These microorganisms have also been reported in species of the *Ae. scutellaris* group[11–14]; further, incompatible crosses have been known to become compatible after heat or antibiotic treatment[15]. However, several questions raised by Trpis et al.[15], and Subbarao[16] remain unanswered and the mechanism of non-reciprocal fertility remains unsettled. Furthermore, for some crosses both antibiotic and heat treatments failed to produce compatibility[17,18]; in other crosses the presence or absence of *Wolbachia* seemed to be in conflict with the crossing relationships observed[19]. Thus, *Wolbachia* may not be the sole cause of incompatibility. This is in agreement with conclusions drawn from studies on segregation for cytoplasmic properties and cytoplasmic types in the *C. pipiens* complex[16,20,21]. Barr[10,22] has also presented evidence for segregation in crossing type with common maternal ancestor in *C. pipiens*.

While both species complexes, i.e. *Ae. scutellaris* and *C. pipiens*, provide systems in which to study the genetic basis of non-reciprocal fertility or cytoplasmic incompatibility there are some similarities and differences which should be noted. In the *Ae. scutellaris* complex incompatibility is interspecific as opposed to intraspecific and interspecific incompatibility in the *C. pipiens* complex. Both species complexes are involved in transmitting Bancroftian filariasis, and yet two different mechanisms may operate. Therefore it would be interesting to know whether similar mechanisms are involved in determining crossing relationships in these two species complexes.

The *Ae. scutellaris* group comprises over 30 closely related species distributed in Southeast Asia and the South Pacific[23–25]. Since the early report of Woodhill[1], many more species groups have been reported to show similar compatibility relationships[18,26–28]. The table summarizes the crossing relationships among 7 species of this group. Crosses involving *Ae. malayensis* × *Ae. alcasidi*, *Ae. malayensis* × *Ae. hebrideus*, *Ae. malayensis* × *Ae. s. katherinensis*, *Ae. hebrideus* × *Ae. alcasidi*, and *Ae. hebrideus* × *Ae. s. katherinensis* were unidirectionally incompatible, whereas those involving *Ae. s. katherinensis* × *Ae. alcasidi* were bidirectionally compatible[26,27]. Species from the western range of distribution seem to form a subgrouping distinct from species of the Polynesia region including *Ae. polynesiensis*, *Ae. pseudoscutellaris* and *Ae. kesseli* which were bidirectionally compatible. These species have also been reported to differ with respect to filarial susceptibility[15,17,19]. Unidirectional incompatibility in the above mentioned cases was complete and showed a strictly maternal mode of inheritance. No parthenogenetic female was recorded in any of these interspecific crosses, whereas parthenogenesis occurred frequently in the *C. pipiens* complex[29]. Sherron and Rai[18] recorded partial compatibility among certain species of this group involving *Ae. cooki* females which were similar to reports for the *C. pipiens* complex; this mechanism of partial compatibility is more poorly understood than incompatibility[30].

Wolbachia are reported to be present in *Ae. malayensis*, *Ae. hebrideus* and the three species from Polynesia; none were found in *Ae. alcasidi* and *Ae. s. katherinensis*[12]. The theory proposed by Yen and Barr[9] that *Wolbachia* are responsible for cytoplasmic incompatibility appeared to contradict some of the crossing relationships in the *scutellaris* group of species as noted by Meek[12]. In addition, results from interspecific crosses made by different workers and even from some made by the same worker at different times have not always been in agreement. For example, a cross of *Ae. kesseli* females × *Ae. pseudoscutellaris* males was incompatible when attempted in February 1981; however, a few viable hybrids were obtained during June 1981[31]. A similar occurrence was recorded from the interspecific cross between *Ae. kesseli* females and *Ae. alcasidi* males. In addition, conflicting results were reported by different laboratories, e.g. Trpis et al.[15] reported non-reciprocal fertility between *Ae. polynesiensis* and *Ae. malayensis*, whereas these two species were recorded to be bidirectionally incompatible by Dev and Rai[26]. Both Trpis et al.[15], and Meek and Macdonald[19] reported non-reciprocal fertility between *Ae. polynesiensis* and *Ae. alcasidi*, but in the opposite direction; these species were found to be bidirectionally incompatible by Dev and Rai[26]. Other such examples have been recorded by Dev[31]. To account for these variations and conflicts observed in the crossing relationships based on the presence or absence of *Wolbachia*, it is necessary that the genetic bases of such phenomena be explored further prior to field release experiments.

Let us consider the *Ae. malayensis* × *Ae. alcasidi* cross in detail: when *Ae. malayensis* females were crossed with *Ae. alcasidi* males viable and fertile hybrids were produced; in the reciprocal cross *Ae. alcasidi* females were inseminated by *Ae. malayensis* males but inviable eggs were laid[26]. These two species have similar karyotypes ($2n = 6$) and meiosis was normal in parental species and hybrids[32]. When F_1 females from *Ae. malayensis* × *Ae. alcasidi* crosses are backcrossed to *Ae. alcasidi* males, a proportion of non-crossover female progeny would be expected to possess a chromosomal constitution identical to that of *Ae. alcasidi* (fig. 1). If unidirectional incompatibility is dependent upon the nuclear genome, such females should produce inviable eggs when further crossed to *Ae. malayensis* males. Thirty-five such females from the above crossing scheme were tested but all laid viable eggs and the sex-ratio was normal (i.e. 1:1), thus

Summary of interspecific crossing relationships among species of the *Aedes (Stegomyia) scutellaris* group* (data included in this table are from Dev and Rai[26,27])

♀ ♂	*malayensis*	*alcasidi*	*hebrideus*	*katherinensis*	*polynesiensis*	*pseudoscutellaris*	*kesseli*
malayensis		+	+	+	–	–	–
alcasidi	–		–	+	–	–	–
hebrideus	–	+		+	–	–	–
katherinensis	–	+	–		–	–	–
polynesiensis	–	–	–	–		+	+
pseudoscutellaris	–	–	–	–	+		+
kesseli	–	±	–	–	+	±	

+ = compatible; – = incompatible; ± = variable; * line indicates two subgroupings (cf. text)

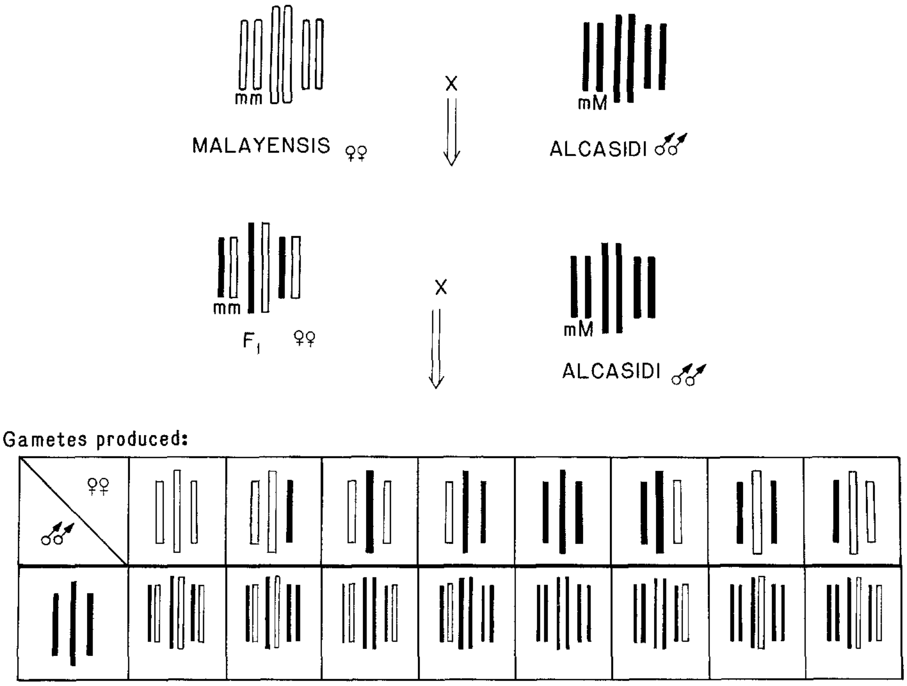

Figure 1. Diagrammatic representation of chromosomal constitution of non-crossover gametes and progeny when F_1 (*Ae. malayensis* ♀♀ × *Ae. alcasidi* ♂♂) hybrid females are crossed with *Ae. alcasidi* males. The diploid number of chromosomes is six ($2n = 6$) in both species, and sex is determined by a single pair of alleles, females being *(mm)* and males *(mM)*. This diagram is adapted from Motara[38].

Aedes alcasidi	♀♀	×	*Ae. s. katherinensis*	♂♂
		↓		
F_1	♀♀	×	*katherinensis*	♂♂
		↓		
Bc_1	♀♀	×	*katherinensis*	♂♂
		↓		
Bc_2	♀♀	×	*katherinensis*	♂♂
		↓		
Bc_3	♀♀	×	*katherinensis*	♂♂
		↓		
$Bc_{(n-1)}$	♀♀	×	*katherinensis*	♂♂
		↓		
Bc_n	♀♀	×	*malayensis*	♂♂

Figure 2. Proposed crossing scheme for genomic replacement of *Ae. alcasidi* by that of *Ae. s. katherinensis* through sequential backcrossing. The end cross would provide additional insight into the nature of cytoplasmic incompatibility (cf. text).

the female mating type remained that of *Ae. malayensis*, which suggested a maternal mode of inheritance[33].

For these species, it should be possible to elucidate the nature of the cytoplasmic factor determining crossability, and also to determine the degree of dependence of this factor on the nuclear genome, through utilization of a third mutually compatible species as proposed by Smith-White[3]. A suitable third species would be *Ae. s. katherinensis*, the males of which are compatible with *Ae. alcasidi* females as well as *Ae. malayensis*. The crossing scheme for these three species is given in figure 2.

Crosses of *Ae. alcasidi* with *Ae. s. katherinensis*, and continuous backcrosses to *Ae. s. katherinensis* always using the latter as the male parent, would bring about a gradual replacement of the *alcasidi* female genome by that of *katherinensis* male genome. Assuming that determination of incompatibility resides in the nuclear genes, replacement of the female genome should bring about a change in the mating compatibility relationship. In this example, *Ae. alcasidi* females (with *katherinensis* genome) should become compatible with *Ae. malayensis* males, which normally are not compatible, while *katherinensis* males are compatible with *Ae. alcasidi* and *Ae. malayensis* species females. The outcome of such a cross would then indicate whether the cytoplasmic factor was dependent on the *Ae. alcasidi* nucleus or independent and permanent. These crosses should be performed to give additional insights as to the nature of the cytoplasmic factor. In principle, this crossing scheme is very similar to that used by Laven[4,6], and Smith-White and Woodhill[5], except that it involves a third mutually compatible species. It is likely that many such combinations with a third mutually compatible species will be found in the future; crosses with these species would further test this hypothesis for this species complex.

The *Ae. scutellaris* complex provides an ideal opportunity to demonstrate genetic control through cytoplasmic incompatibility[34]. An understanding of the mechanism of unidirectional incompatibility in this species complex would provide a framework for large scale field release operations for population suppression in the South Pacific, similar to that provided by studies of the *C. pipiens* complex[21,35]. In addition, this understanding would provide insight into a mechanism which has been considered a potent factor in speciation[6,36,37]. Thus far, the phenomenon of unidirectional incompatibility appears to be similar in both *Ae. scutellaris* and *C. pipiens*, except that the lethal effect at the egg stage operates earlier and more severely in *Aedes* than in *Culex*, as noted by Smith-White and Woodhill[5]. In summary, much remains to be done to clarify the underlying mechanism of unidirectional incompatibility in the *Ae. scutellaris* complex.

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Short Communications

Rate-compensated synaptic events in antarctic fish: Consequences of homeoviscous cold-adaptation[1]

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Summary. At ambient sub-zero temperatures, muscles from antarctic fish produce spontaneous postsynaptic currents (MEPCs) of significantly shorter duration than those of temperate fishes. Fast decay of antarctic MEPCs is a predictable consequence of the increased membrane fluidity attributable to fatty acid unsaturation in cold-adapted animals.

Key words. Antarctic fish; synaptic currents; decay rates; cold adaptation.

A widespread feature of adaptation to low temperatures in living organisms is an increase in the proportion of unsaturated fatty acids, preserving optimum membrane fluidity (the Homeoviscous Hypothesis of Temperature Adaptation)[2–5]. Increased membrane fluidity is also thought to underly the action of many general anesthetics, which substantially shorten the duration of synaptic events, and reduce the mean lifetime of chemically-opened synaptic channels (the Lipid Fluidity Hypothesis of Anaesthesia)[6–8]. These two hypotheses can be combined to predict that low-temperature synaptic events in cold-adapted poikilotherms should be of relatively short duration compared with similar events in warm-adapted animals. This prediction may be tested by comparing miniature end plate currents (MEPCs) of an antarctic teleost, *Pagothenia borchgrevinki* (Family Nototheniidae), with those already reported for a temperate fish, *Trachurus novaezelandiae* (Family Carangidae)[9]. The results are consistent with the membrane fluidity hypothesis.

Specimens of *P. borchgrevinki* were taken from under the sea ice of McMurdo Sound, Antarctica, close to New Zealand's Scott Base (77° 51′ S, 166° 48′ E), where the mean annual seawater temperature is $-1.87 \pm 0.1\,^{\circ}\mathrm{C}$[10]; fish used for neuromuscular recordings were either freshly caught, or were held in cages under the ice until required. Lipids extracted from brain and retina of *P. borchgrevinki* show the increased fatty acid unsaturation characteristic of cold-adapted poikilotherms[11,12]. *T. novaezelandiae* were collected in Port Jackson (Sydney), Australia (33° 51′ S, 151° 12′ E) from ambient summer water temperatures of 23 °C, and acclimated to 12–15 °C in the laboratory. Like *T. no-*