SEGWEGATION OF CYTOPLASMIC INCOMPATIBILITY PROPERTIES IN *CULEX PIPENS FATIGANS*

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ABSTRACT

Maternally inherited variants, which arose within a laboratory colony of *Culex pipiens fatigans*, have been studied by rearing cultures from single egg rafts. Segregation, i.e., variation of cytoplasmic incompatibility properties between the male progeny of individual females, was demonstrated. Also, from the daughters of individual females, sub-lines were derived within which all the males showed the same incompatibility or compatibility properties. Among the descendants of tetracycline-treated individuals were lines which superficially simulated these phenomena, but these lines ultimately reverted to the cytoplasmic compatibility type of the strain which was submitted to the treatment. The types of variations in cytoplasmic incompatibility properties that have been studied are discussed.

CYTOPLASMIC incompatibility is a naturally occurring phenomenon in the *Culex pipiens* complex, whereby crosses between many strains of different geographical origin are sterile. It has been shown by backcrossing that the crossing type is a maternally inherited character without any involvement of chromosomal genes (LAVEN 1957). Field release experiments (e.g., LAVEN 1967) suggest that cytoplasmic incompatibility could be used in the control of the main mosquito vector of bancroftian filariasis. The Paris strain was reported to be bidirectionally incompatible with the Delhi strain (KRISHNAMURTHY and LAVEN 1972). However, SUBBARAO et al. (1974, 1977) found polymorphism in cytoplasmic types in *Culex pipiens fatigans* populations from various parts of the Indian subcontinent. Males of the minority type (De 19) were found to be compatible with all the cytoplasmic types tested. The majority type (De 2), whose males are incompatible with Paris females, could be separated from the De 19 type by rearing single egg raft cultures. All the males from any one of the cultures showed the same compatibility properties.

In addition to the polymorphism observed in natural populations, SUBBARAO et al. (1977) reported the variants ISB 20 and ISB 49, which arose in a laboratory colony of the IS31B strain that has Paris cytoplasm (KRISHNAMURTHY and

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This paper reports studies on ISB 20 and ISB 49, which revealed the occurrence of variation among the progeny of individual females. A similar phenomenon has been briefly reported by French (1970).

Yen and Barr (1973) treated larvae with tetracycline and derived a line whose males showed universal compatibility and whose females showed sterility in matings with all untreated mosquitoes. It is not known to what extent the mechanism of the sterility in these matings is similar to the incompatibility between different wild-type strains. Electron microscopy revealed that the treated mosquitoes were free from rickettsiae, while the untreated mosquitoes contained large numbers of rickettsiae (Yen and Barr 1973, 1974), and the progeny of treated female mosquitoes were referred to as aposymbiotic lines. The authors proposed that these rickettsial organisms are responsible for the cytoplasmic incompatibility phenomenon. The present paper also reports studies on tetracycline treatment of both larval and adults mosquitoes.

### MATERIALS AND METHODS

#### Stocks

The following stocks were used in the experiments:

- **De**: a laboratory colonized strain of Delhi origin (Singh et al. 1975).
- **De 2**: Single egg raft isolate of the majority type from De.
- **De 19**: Single egg raft isolate of the minority type from De.
- **Pa**: Paris cytoplasm with Delhi genome (Krishnamurthy and Laven 1976).
- **IS31B**: Paris cytoplasm with Delhi genome, including a male-linked translocation (Krishnamurthy and Laven 1976).
- **Ha**: Hamburg cytoplasm with Delhi genome (Krishnamurthy and Laven 1976).
- **Ba**: Bangkok cytoplasm (type A) with Delhi genome (Krishnamurthy and Laven 1976).
- **Pr**: Prague cytoplasm with Delhi genome (Subbarao et al. 1977).

#### Experimental procedure

To avoid the effects of aging in causing partial compatibility (Singh, Curtis and Krishnamurthy 1976), all mating were carried out with two- to three-day-old males. Chicks were provided as a blood source on the fourth day after mating, and egg rafts (which are clumps of about 150 eggs laid by individual females) were collected on the ninth day. The hatchability of the egg rafts was scored after two days. In the great majority of cases incompatible and compatible egg rafts were qualitatively distinct with no egg hatch in the former and a high percentage of egg hatch in the latter. However, in a few cases rafts gave a hatch of 1-5 larvae and these were classified as incompatible, since a small percentage of eggs are known to hatch after incompatible matings as a result of parthenogenetic development (Laven 1957). From compatible matings, in which the male parent had inherited the male-linked translocation of the IS31B stock, the egg hatch was reduced to about 50%. This nuclear genetic marker provided a check against contamination of the experimental stocks by untranslocated material maintained in the same laboratory or from the local wild population, and it did not reduce the egg hatchability into the range where it could have been confused with that of incompatible egg rafts.

#### Single raft cultures

In order to study samples of progeny from individual females, egg rafts were reared in individual bowls. Some of the males and females from each culture were inbred and the remainder were test-crossed with various cytoplasmic types.
Tetracycline treatment

(a) Larval treatment: larvae of the Pa strain were reared in concentrations of 0.025 and 0.05 mg/ml tetracycline, the solution being changed on alternate days. Treated males and females were inbred and males were testcrossed with Delhi females. Progeny of treated inbred lines were testcrossed with all the available cytoplasmic types.

(b) Adult treatment: in the normal rearing procedure, cotton pads with 1% glucose solution are provided for feeding adults, and for experimental purposes 0.1 or 1 mg/ml tetracycline was added to the solution for the first four days of adult life.

RESULTS

Variation in IS31B colony

As reported by Subbarao et al. (1977), the males from 50 single egg raft cultures from the IS31B colony were testcrossed with Delhi females to determine their crossing type. Males from two of the rafts, designated ISB 20 and ISB 49, showed compatibility, while all males from the other 48 cultures showed incompatibility with Delhi females (Figure 1).

All males of ISB 49 showed compatibility with females of all the available cytoplasmic types, but females of ISB 49 retained the Paris compatibility and incompatibility properties (Figure 1).

ISB 20 females, like ISB 49 females, retained Paris compatibility/incompatibility properties, but among males of ISB 20, unlike ISB 49, some individuals showed compatible and others incompatible properties with respect to De 2, De 19, Ba and Ha females (Figure 2). We refer to such variation among the progeny of a single female as “segregation,” as was done by French (1970).

Figure 1.—Effect of isolation of single egg rafts from the IS 31B colony.
Fifteen single-raft cultures were reared from ISB 20 to investigate the genetic constitution of the ISB 20 line. These were designated ISB 20(1)–(15). Females and males were mated and males were also testcrossed with De 2 females (Figure 2). Six lines (with a total of 111 rafts) gave males, all of which were compatible, two lines (with a total of 30 rafts) gave all incompatible males, while the remaining seven lines showed a segregation among the males similar to the original ISB 20 lines.

Two of these segregating sublines ISB 20(7) and ISB 20(9) were tested further by rearing single raft cultures, ISB 20(7)a-m and ISB 20(9)a-o, and test-crossing the males (Figure 3). In sublines ISB 20(7)g and ISB 20(9)f, it was found that male progeny were, respectively, all compatible and all incompatible with De 2 females but, in the other sublines, segregation continued.

To test whether the properties of ISB 20 and ISB 49 were cytoplasmically or chromosomally inherited, males and females of each type were outcrossed to the normal Pa stock. When the males of the ISB 20 and ISB 49 stocks were outcrossed, the male progeny showed the properties of the Pa stock, i.e., bidirectional incompatibility with De 2. Conversely, when females of ISB 49 and of several of
the sublines of ISB 20 were outcrossed, the male progeny retained the properties of the line to which their maternal parent belonged. This remained true when four successive backcrosses of females to Pa males were carried out.

Studies with tetracycline

(a) Larval treatment of the Pa strain: There was extension of larval development time and larval and adult mortality following tetracycline treatment, and from the 0.05 mg/ml treatment the few rafts obtained from the surviving adults failed to hatch.

In the matings between males and females treated with 0.025 mg/ml there were abnormally small rafts but a small number of larvae were obtained. Treated males were also testcrossed with De females, and the rafts showed a mean hatch of 20% (range 0.7–67%), i.e., the incompatibility barrier had been partially broken. The F₁ male progeny from treated females × treated males were compatible with both De and Pa females (Figure 4). The F₁ female progeny were incompatible with De 2 males, but a majority of females were compatible with Pa males. Thus, the majority of individuals appeared to belong to the cytoplasmic type that has been designated ISB 49, while the minority resembled Yen and Barr's aposymbiotic lines in that the females gave sterile rafts when mated with the Pa type, i.e., the strain from which they were derived.

In the F₂ generation, males varied in their compatibility with De, and females again showed either fertility or sterility when mated with Paris males (Figure 4). To study this variation further, 15 single raft cultures, TT1–15, from the F₂ generation were reared, and the males and females from each were testcrossed with De females and Pa males, respectively (Figure 4). Twelve cultures were
found to regain Paris cytoplasmic characters, i.e., the males were incompatible with De and the females were compatible with Pa. In two lines, TT5 and TT7, males were compatible with De females, but females produced sterile rafts when mated with Pa males. At the next generation, males and females from these two lines were testcrossed with females and males of all the available cytoplasmic types (Figure 4). Males from both lines were found to be compatible with all cytoplasmic types, but females produced sterile rafts when mated with the Pa type (from which they were derived) and with several other types. However, the TT5 and TT7 females were partially compatible with De 19 males: the mean hatchability was 8.4% with a range from 0.0–25.3% hatch. Eggs from
unhatched and partially hatched egg rafts from these crosses were predominantly unembryonated. The fertility in matings within the TT5 and TT7 lines was almost normal (mean egg hatchability of 88.8%).

As shown in Figure 4, the single-raft culture TT9 showed a mixture of female types, and further single-raft isolations from it produced lines that had reverted to Paris properties, and other lines that showed segregation among males similar to that of the ISB 20 line described above. However, subsequent results (not shown in Figure 4) indicated complete reversion of the TT9 line to the Pa type. (b) Adult treatment of Pa strain: Following 1 mg/ml tetracycline treatment, only one of the rafts laid by the treated females hatched, but following 0.1 mg/ml tetracycline treatment, all the rafts hatched.

Progeny from both the treatments were reared, and the males and females were then testcrossed with De females and Pa males, respectively. Males derived from parents treated with 1 mg/ml showed compatibility with De females and the females were compatible with Pa males. These properties resemble the ISB 49 isolate described above. Some of the males from the 0.1 mg/ml treatment showed compatibility with De females, while others showed incompatibility, but all females were compatible with Pa males, i.e., the results resembled those with ISB 20. However, after two generations of breeding (without any further treatment) of both the 1.0 mg/ml and 0.1 mg/ml treated lines, it was found that the compatibility of the males with De females had reverted to the Pa cytoplasmic type, i.e., to the properties of the stock from which they were derived.

DISCUSSION

Subbaro et al. (1977) showed that the origin of the ISB 20 and ISB 49 types was a mutation-like process from a previously cytoplasmically invariable colony. The present paper demonstrates that the properties of the two new variants ISB 20 and ISB 49 were maternally inherited. ISB 49 provides an interesting analogy with the naturally occurring De 19 variant of Indian populations (Subbarao et al. 1974, 1977). The males of both of these types were compatible with all the strains available for testing, and the females show typical compatibility/incompatibility properties. In the case of ISB 49 the properties of the females resemble those of Pa, and in the case of De 19 they resemble the majority Delhi type, De 2.

In the ISB 20 line there was variation in the compatibility properties of sons of an individual mother. In addition, the line gave rise to pure sublines, within which all males showed the same properties with respect to females of several other cytoplasmic types. It seems probable that the segregations between the sons of individual females and between the lines derived from the daughters of individual females are related phenomena. We suggest that, between the oocytes produced by individual ISB 20 females, there is an unequal assortment of the cytoplasmic determinants of incompatibility. It might be that, in females of the ISB 20 line, two different types of rickettsia co-exist, and that some of the progeny inherit rickettsiae all or predominantly of one type. Alternatively, one might
postulate only one type of rickettsia and that some individuals inherit reduced numbers of them. However, the numbers of rickettsiae in normal \textit{C. pipiens} oocytes are very large (Yen and Barr 1974), and the segregations we found were clear-cut, and individual males with an intermediate level of compatibility were not found. It appears, therefore, that either of the above two hypotheses would require the additional postulate of threshold densities of rickettsiae, above which a male produces all incompatible sperms and below which it produces all compatible ones. The existence of such sharp thresholds seems to us rather difficult to imagine, and we suggest as another alternative hypothesis that the process of unequal assortment may not act at the level of the rickettsiae themselves, but rather may involve other cytoplasmic factors that control the rickettsiae. It is suggested that these controlling particles may be present in small numbers so that they could be entirely absent from some oocytes due to "sampling error." Sager and Ramanis (1968, 1970) have mathematically analyzed their data on cytoplasmic variants in Chlamydomonas. Though there is some quantitative information in our \textit{Culex p. fatigans} data (Figures 1, 2 and 3), we consider it premature to attempt to frame any detailed hypothesis on the segregation process.

The occurrence of several sublines of ISB 20 all of whose males were compatible with De 2 females suggests that the ISB 49 variant is probably a secondary derivative of the original ISB 20 "mutation".

As shown in Figure 4, from treatment of larvae with 0.025 mg/ml tetracycline, two lines (TT5 and TT7) were derived whose properties resembled those of the aposymbiotic lines of Yen and Barr (1973), i.e., the males showed no incompatibilities and the females were compatible with males of their own lines, but produced sterile rafts when mated with males of the Pa strain from which they were derived. Electron microscope studies by C. M. S. Dass, University of Delhi, India (personal communication) revealed no rickettsiae in oocytes of the TT5 and TT7 lines, whereas rickettsiae were readily demonstrable in the De 2, De 19, IS31B, ISB 20 and ISB 49 strains. Thus, we regard TT5 and TT7 as aposymbiotic. It was found that these females were partially compatible with the naturally occurring De 19 type, and there therefore appears to be a limited degree of resemblance between the De 19 type and the aposymbiotic type. It should be recalled, however, that De 19 females show conventional compatibility/incompatibility properties, unlike aposymbiotic females. The partial resemblance of De 19 males to the aposymbiotic type suggests that it may not be possible to find a cytoplasmic type whose females are incompatible with it. This would have important practical consequences for the application of cytoplasmic incompatibility for genetic control because it would allow "recombination" of a released cytoplasm with indigenous Indian genome (Curtis 1976).

As noted in Figure 4, ISB 49 differed from De 19 in being incompatible with aposymbiotic females. The results reported suggest a gradation of properties in males as follows: (a) De 2 and Pa; (b) ISB 20; (c) ISB 49; (d) De 19; and (e) aposymbiotic. Whether this gradation reflects an underlying gradation in density of rickettsiae, or of some other particle, remains to be determined.
The effects of tetracycline treatment of larvae were not uniform; in addition to the TT5 and TT7 (aposymbiotic) derivatives of the treatment, other types were found (Figure 4) some of which superficially resembled ISB 20. Unlike the latter stock, these tetracycline-derived segregating stocks all ultimately reverted to the parental Pa type. By contrast, from ISB 20, in addition to the Pa-type revertants [ISB 20(13), ISB 20(14) and ISB 20(9)f in Figures 2 and 3], there were ISB 49-type sublines [ISB 10(1), (2), (5), (8), and (15) in Figure 2 and ISB 20(7)g in Figure 3] and lines where segregation of male types continued for further generations [ISB 20(7)a-f and h-m, and ISB 20(9)a-e and g-o in Figure 3].

Adult treatments with tetracycline at different concentrations gave lines which initially simulated ISB 49 and ISB 20. However, once again the tetracycline lines reverted to Pa type after a few generations, unlike ISB 49 and ISB 20. The eventual reversion to parental type of lines derived from tetracycline treatment of larvae or adults suggests that in many cases rickettsiae are incompletely cleared by tetracycline (as also found by Portaro and Barr 1975) and that normal concentrations can be restored after several generations.

The effect of tetracycline on adults could be of importance in a genetic control program because the chickens kept for blood feeding of mosquitoes in a mass-rearing colony (Singh et al. 1975) have been dosed with antibiotics to protect them from intestinal infections. Studies are required of the effects on incompatibility of feeding mosquitoes on chickens dosed with various concentrations of tetracycline.

This paper and other recent publications have shown that the earlier “all-or-none” picture of cytoplasmic incompatibility is complicated by the following phenomena:

(a) Polymorphism of cytoplasmic types in wild populations, e.g., De 19 and De 2 in Indian populations (Subbarao et al. 1974 and 1977) and a similar phenomenon in Bangkok (Krishnamurthy and Laven 1976).

(b) Segregation between the sons of a single female in their compatibility properties, e.g., ISB 20 (Figure 1).

(c) Segregation of sublines from the daughters of one female, the males of any one subline showing invariable properties, e.g., French (1970) and ISB 20(7)g and ISB 20(9)f in Figure 3.

(d) Partial compatibility of certain wild-type males leading to partial egg hatch of individual rafts, e.g., Hanford females × Scauri males (Barr 1970).

(e) Partial compatibility of aged Pa males with De females (Singh, Curtis and Krishnamurthy 1976).

(f) Partial compatibility of aposymbiotic females × De 19 males (Figure 4).

(g) Partial compatibility of newly tetracycline-treated males (Yen and Barr 1974 and 1975, and Figure 4).

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LITERATURE CITED


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