study we were unable to separate the *fan* and *fluff* phenotypes from nuclear markers in a heterokaryon test[20].

The high frequency with which *fan* and *fluff* variants are isolated, and the pleiotrophic nature of their respective phenotypes, is characteristic of a common, but poorly understood phenomenon in filamentous fungi variously called 'strain degeneration' and 'vegetative instability'. Aged cultures, and strains maintained for a long time in the laboratory with frequent subculturing, are particularly prone to such 'degeneration' which may yield visible sectors on agar medium, and which may manifest itself by changes in morphology, attenuated virulence and/or loss of secondary metabolite production. The early antibiotics literature is filled with references to strain degeneration in *Penicillium notatum* and *P. chrysogenum*[24-28].

Somatic instability in molds is attributed to mutation, heterokaryosis ('the dual phenomenon'), physiological adaptation, cytoplasmic heterogeneity, and anamolous chromosome mechanics[24,29-32]. In Actinomycetes, strain instability is associated with plasmid loss, gene amplification, and gene transpositions[33]. Genetic instability has been demonstrated in *A. nidulans* among transformants, which exhibit a wide variety of morphological phenotypes with successive subculture of individual transformants[34].

We find genetic transposition an attractive model to describe the anomalous behavior of our morphological variants in *A. parasiticus*. Whatever their origin, their high frequency from mycelial macerates would recommend against the practice of blending mycelia in an attempt to get a uniform inoculum, as has been adopted by certain workers studying versicolorin[35] and aflatoxin[36] metabolism.

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Monogenic inheritance of cyclodiene insecticide resistance in mosquitofish, *Gambusia affinis*

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Summary. Certain populations of the mosquitofish, *Gambusia affinis*, developed high levels of resistance to endrin and other cyclodiene insecticides as a result of inadvertent exposure to agricultural sprays. Genetic crossing studies show that endrin resistance is inherited as a single, autosomal, intermediate gene.
Key words. Insecticide resistance; inheritance; mosquitofish; cyclodienes; endrin; picrotoxinin.

Certain populations of the mosquitofish, *Gambusia affinis*, developed high levels of resistance to endrin and other cyclodiene insecticides as a result of inadvertent exposure to agricultural sprays. The similarities of cross-resistance patterns between insects and mosquitofish suggested that the inheritance of resistance in mosquitofish might be similar to that in insects. Numerous genetic studies of cyclodiene resistance in insects show that it is nearly always inherited as a single allele of intermediate expression (often referred to as 'semi-dominant')[1,2]. By intermediate we mean that the phenotype of the heterozygote (RS) is almost exactly the logarithmic mean of the LC_{50}'s of the homozygous resistant (RR) and susceptible (SS) strains[3]. We report here that cyclodiene resistance in *Gambusia* is a monofactorial autosomal intermediate trait, as demonstrated by crossing experiments.

Cyclodiene insecticides, including endrin, dieldrin, and toxaphene, were widely used for control of agricultural and medical pests from the early 1950's until the late 1970's, when their use

was severely curtailed as a result of environmental contamination[4]. Over 269 species of insects had become resistant to the cyclodienes by 1980 in at least some area of the world[5]. However, so far as is known, only two species of vertebrates have developed biologically significant levels of resistance to cyclodienes or any other insecticide. Endrin was used as early as 1954 for control of the pine vole, *Microtus pinetorum*. After 11 years of exposure in an orchard in Virginia, voles showed about a 12-fold resistance to endrin[6]. In contrast, the mosquitofish, *Gambusia affinis*, developed resistance to several insecticides as a result of inadvertent exposure to agricultural spraying both in Mississippi[7–9] and Texas[10].

The field-collected resistant mosquitofish are resistant to a number of cyclodienes, including endrin, heptachlor, chlordane, aldrin, and dieldrin, as well as to several other chlorinated alicyclic insecticides, including lindane, toxaphene, and strobane. Resistance levels of more than 350-fold have been recorded for endrin, heptachlor, toxaphene and strobane[8]. The resistant fish are so tolerant that endrin treated fish can excrete enough insecticide to kill susceptible fish in an untreated tank (unpublished observation).

Insects also show high levels of resistance to cyclodiene insecticides, usually at least 100-fold in homozygous resistant strains[1]. Resistance selected by exposure to one cyclodiene usually confers resistance to all other cyclodienes as well as to lindane, a phenomenon known as 'cross-resistance'[2,11]. Mosquitofish resistance to chlordane is apparently an example of cross-resistance because chlordane has never been extensively used in agricultural areas of Mississippi. Cyclodiene cross-resistance in cockroaches (and presumably other insects) extends to picrotoxinin, a plant-derived material[11]. We have found similar cross-resistance in mosquitofish. Using data on males, susceptible fish show an LC_{50} (and 95% CI) of 8.3 ppm (6.4–11.3) compared to 161 ppm (95–440) for resistant fish, demonstrating an approximately 20-fold level of resistance.

For all bioassays reported here, the toxicant was dissolved in acetone and diluted to the desired concentration in aquarium water. A minimum of 5 concentrations plus a control was used for each bioassay, except as noted below. A minimum of 32 individuals separated into at least 4 replications were tested at each concentration. Concentration-mortality data were analyzed using the POLO computer program[12].

Resistant fish were collected from irrigation ditches in an area of intense cotton and soybean production (Belzoni, Humphreys Co., Miss.). Susceptible fish were collected from a pond on the Mississippi State University campus. Assuming that the field-collected resistant fish would not necessarily be completely homozygous for the resistance alleles, we selected immature field collected fish with a high dose of endrin (100–600 ppb/48 h) prior to conducting the genetic crosses. Endrin was chosen as the toxicant for these studies because field populations were highly resistant to it, on the order of 500-fold based on a comparison of the LC_{50} values for susceptible and resistant populations. Immature fish treated at 300–800 ppb endrin were later pooled to produce a homozygous R strain.

To produce F_1 progeny, fish were sexed before sexual maturity and paired individually for reciprocal crosses[18]. Offspring were obtained and assayed from six R♀XS♂ crosses and ten S♀XR♂ crosses. The 48 h LC_{50} values (and 95% CI) for the RXS and SXR crosses were 89 ppb (62–155) and 127 ppb (96–208), respectively. Similarly, F_1 females and males gave values of 122 (108–142) and 100 (87–117). There were no statistically significant differences between the reciprocal crosses or between males and females, demonstrating resistance is autosomal, so the data were pooled. The pooled LC_{50} value for all F_1 fish was 110 ppb (92–136). The F_1 males and females were pooled for mass backcrosses to susceptible females and males, respectively.

Assuming that resistance was due to a single allele, an expected mortality curve was calculated using the data for F_1 and susceptible fish (fig. 1). Data for susceptible males and females were pooled producing an LC_{50} of 1.9 ppb (1.6–2.2) (fig. 1). The data for resistant males and females were similarly pooled, but the LC_{50} was not calculated because the endrin solubility limit was reached at about 2000 ppb. If resistance is due to a single gene, a backcross to the susceptible strain should produce 50% RS and 50% SS fish. The expected mortality for the backcross fish at any given dose should then be 50% the mortality of the susceptible strain plus 50% of the mortality of the F_1 fish.

As shown in figure 1, the F_1 fish were intermediate in resistance between the parental susceptible and resistant strains. The backcross fish showed close to a 1:1 segregation of resistant and susceptible phenotypes at discriminatory endrin concentrations of 10–60 ppb, which killed greater than 98% of the susceptible fish, but less than 17% of the F_1 (heterozygous) fish. The data from backcrosses were compared to the expected mortality at each of the doses tested using the test of significance of a binomial proportion[19]. The backcross data do not fit a possible alternative model of linearity ($X^2 = 36.8$, 5 d.f., $p < 0.001$). Only the mortality at 3 ppb was significantly ($p < 0.001$) different from the expected value. This might be explained by the segregation of a minor gene (or genes) for resistance[20], which is consistent with observations that several minor mechanisms (in-

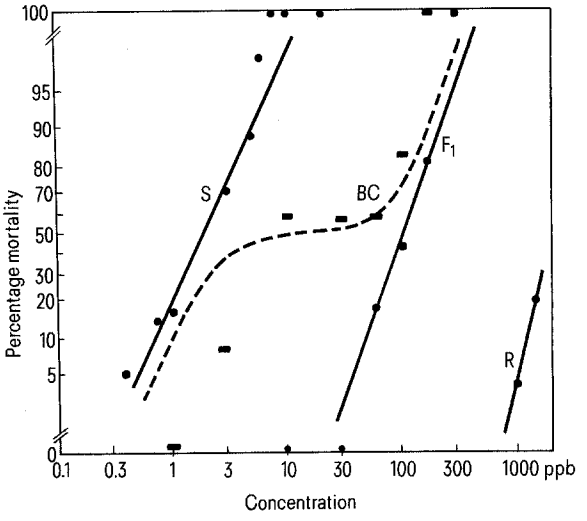

Figure 1. Genetic analysis of cyclodiene resistance in *Gambusia affinis* as assayed with endrin. R and S represent the resistant and susceptible strains. F_1 represents the pooled reciprocal F_1 crosses, and BC represents the pooled reciprocal F_1 XS crosses. The curved line shows the expected mortality for the backcross assuming monogenic inheritance, as interpreted from the S and F_1 lines.

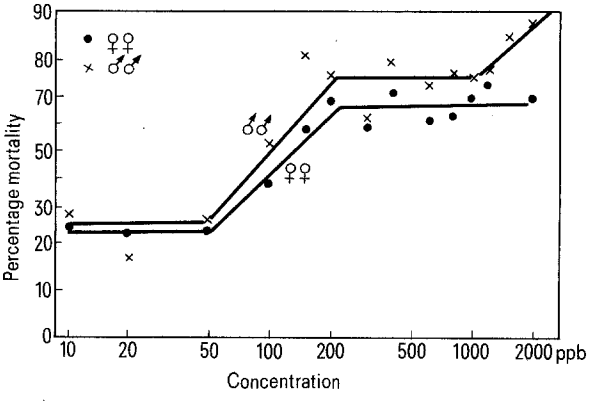

Figure 2. Segregation for cyclodiene resistance in a field population of *Gambusia affinis* as assayed with endrin. Male and female data are shown separately, with eye-fitted lines to emphasize segregation.

cluding whole body and organ uptake, metabolism and compartmentation in lipid) also contribute to resistance in these fish[13-15]. The data presented in figure 1 are very similar to dose-response curves observed in genetic crossing studies in many insect species[1].

Assays of field-collected resistant fish indicate that the wild population is segregating for resistance with a current resistance allele frequency of about 50% (fig. 2). When the resistance allele frequency is about 50%, one should have about a 1:2:1 segregation of genotypes, assuming the population is randomly mating, as if one had made an F_2 cross. As shown in figure 2, about 25% of the population died at concentrations of 10 to 50 ppb, which kill at least 98% of SS fish, but less than 10% of RS fish (fig. 1). Another 50% of the fish died when the concentration was increased to 200 ppb, which corresponds to a concentration that killed at least 85% of the RS fish (fig. 1). Concentrations of greater than 1000 ppb kill some RR fish (fig. 1), which are the last 25% of the field-collected resistant fish (fig. 2). More detailed treatment of the data imply that the R allele frequency might be closer to 55% which, assuming Hardy-Weinberg proportions ($p^2 + 2pq + q^2$), would produce 20% SS, 50% RS, and 30% RR. The data on the field-collected fish do not fit a linear model (Males: $X^2 = 28.3$, 12 d.f., $p < 0.005$; Females: $X^2 = 23.4$, 12 d.f., $p < 0.025$).

The major mechanism of resistance to cyclodienes is not well understood in any species[11]. The levels of cyclodiene resistance which mosquitofish demonstrate (30–500-fold) cannot be explained simply in terms of barriers to insecticide penetration, disposition or biotransformation[13-15]. Potentially the most significant factor in this resistance is a target site insensitivity[16,17]. This target site is believed to be the central nervous system.

The genetic and toxicological similarities of cyclodiene resistance in insects and fish suggest that the site at which these insecticides act has been highly conserved during the evolution of these groups. As has been suggested in insects[11], the primary alteration may involve a membrane receptor.

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Cantharidin biosynthesis in a blister beetle: Inhibition by 6-fluoromevalonate causes chemical disarmament[1]

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Summary. Biosynthesis of cantharidin in a blister beetle, *Lytta polita,* is effectively inhibited by 6-fluoromevalonate. Inhibition is attributed specifically to the fluorine substituent. Biochemical inhibition has not been demonstrated previously for an arthropod's defensive substance.

Key words. Coleoptera; *Lytta polita;* chemical defense; cantharidin; terpenoids; fluorine; metabolism.

The Spanish fly, *Lytta vesicatoria,* and other blister beetles (family Meloidae) are perhaps the most notorious of all poisonous insects[2]. The toxic, vesicant, and purported aphrodisiac properties of these beetles are generally ascribed to cantharidin, which is present systemically for protection from predators[3]. Cantharidin is biosynthesized by adult males and transferred in large measure to females during copulation as part of the spermatophore[4]. Biosynthesis of cantharidin involves the acetate-mevalonate-farnesol homologation process followed by an anomalous and highly complex transformation of farnesol[5]. Because 6-fluoromevalonate (FMVA) inhibits juvenile hormone biosynthesis in lepidopteran insects[6], presumably by blocking the utilization of mevalonate (MVA)[7], we hypothesized that FMVA might similarly inhibit production of cantharidin in male blister beetles. We here report on experiments designed to test this hypothesis.

We used *Lytta (Pomphopoea) polita,* a large-bodied species closely related to the Spanish fly that feeds on pollen and reproduces in early spring in the southeastern states[8]. Beetles collected at UV-light traps near Lake Placid, Florida, were sexed and individually isolated for 6–8 days prior to treatment.

We mated 42 male beetles chosen at random from a total of 49 in order to deplete their cantharidin reserves to a standardized level so that in the ensuing days they might synthesize large amounts of the substance as they rearmed themselves. The remaining seven males (group I), which served to indicate the amount of cantharidin in beetles when mating began, were killed by freezing at this time without exposure to females in the laboratory. The mated males were assigned at random to six experimental immediately after mating was complete. Beetles in two additional groups (IV and V) each were given FMVA within 2 h of