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

Cantharidin content of male blister beetles *(Lytta polita)* as a function of mating and treatment with the inhibitor 6-fluoromevalonate (FMVA) or, as a control, with mevalonate (MVA). Analysis of variance rejected a multisample hypothesis of equal means. These data met the assumption of equal variances without transformation. Duncan's new multiple range (Duncan's) test and the least significant difference (LSD) test at the 0.05 level of significance were used to determine differences between pairs of treatments[12]. Means in each column followed by the same letter are not statistically different from each other using either test

Group	Treatment	Cantharidin (μg/beetle, x ± SEM)*		
		Reproductive system (RS)	Body – RS	Total
I	Unmated, untreated	135 ± 39 a	329 ± 49 a, b	464 ± 74 a
II	Mated, untreated, killed immediately	12 ± 3 c	117 ± 9 c	129 ± 9 c
III	Mated, untreated, killed 4 days later	39 ± 10 b, c	312 ± 56 a, b	350 ± 66 a, b
IV	Mated, FMVA injected killed 4 days later	12 ± 3 c	71 ± 12 c	84 ± 14 c
V	Mated, FMVA fed, killed 4 days later	28 ± 8 b, c	43 ± 10 c	71 ± 16 c
VI	Mated, MVA injected killed 4 days later	43 ± 8 b, c	383 ± 36 a	425 ± 33 a, b
VII	Mated, MVA fed, killed 4 days later	75 ± 19 b	257 ± 21 b	332 ± 36 b

* N = 7 beetles for each value. Whole body values may vary slightly from the sum of the preceding two means in each row because of rounding off.

mating, either as a single injection (0.24 mg in 10 μL saline) or as a dietary supplement (2.4 mg in 100 μl distilled water). Injection of FMVA dissolved in sterile saline (1.5% NaCl in distilled water, w/v) was effected with a micrometer-operated syringe into the abdomen of the beetle. Delivery of FMVA dissolved in distilled water onto the beetle's food was effected with the same syringe. FMVA was synthesized using published procedures[9]. As controls for possible effects resulting from administration of large amounts of metabolic substrate, beetles in two additional groups (VI and VII) were given MVA in doses matching the FMVA doses given groups IV and V, respectively. MVA was obtained by base hydrolysis of the corresponding lactone, purchased from Fluka Chemical Corp., Hauppauge, N.Y. Another group (III), as an additional control, consisted of mated beetles that were not treated with either FMVA or MVA. Beetles in groups III–VII were kept individually isolated for 4 days, during which time each continuously had access to an artificial diet consisting initially of 1 g bee pollen, 0.5 ml honey, and 1 ml distilled water mixed into a paste. At the end of the incorporation period, all beetles were individually frozen at −30 °C. Thawed beetles were dissected to remove the reproductive system because in some meloids it is a major storage site for cantharidin[4]. Two samples from each beetle, consisting of the entire reproductive system and the remainder of the body, were extracted and quantitatively analyzed for cantharidin by capillary gas chromatography[10]. The results are given in the table.

Cantharidin reserves in male *L. polita* were greatly depleted during mating. Unmated males in group I typically contained about 465 μg of cantharidin, of which 30% was present in the reproductive system. In comparison, mated males in group II averaged only 130 μg of cantharidin, indicating that approximately 70% of their stored material was transferred in copula to females. Of the cantharidin transferred (335 μg/beetle on average), approximately one third came from the reproductive system, rendering it virtually devoid of the substance, and the remaining two thirds came from body reserves.

By four days after mating, untreated beetles (group III) had restored their cantharidin reserves approximately to premating levels. Although the amounts of the substance in the reproductive systems of these beetles were less than in unmated males, cantharidin in the body minus the reproductive system, as well as in the whole insect, was not statistically different for beetles in groups I and III.

FMVA administered to mated beetles either by feeding or by injection effectively inhibited cantharidin accumulation during the 4-day incorporation period. The statistically indistinguishable cantharidin levels of beetles in groups II, IV, and V suggest that both FMVA treatments completely blocked biosynthesis of the defensive substance.

The inhibitory effect of FMVA on cantharidin biosynthesis is specifically attributable to the fluorine substituent and not simply to large doses of a substance acting as the metabolic substrate (MVA). Beetles in groups VI and VII that were given amounts of MVA equivalent to the two FMVA treatments had cantharidin levels indistinguishable from mated, untreated controls (group III). Moreover, total amounts of cantharidin in MVA injected beetles (group VI) and in unmated, untreated beetles (group I) were not statistically different.

To our knowledge, this work represents the first demonstrated use of a metabolic inhibitor to diminish the defensive reserves in an insect. Previous cases have involved either physically 'milking' the secretions of animals or, in the case of species that acquire their defenses, removing essential secondary natural products from their diets[11].

Our results show that it is possible to inhibit selectively the formation of cantharidin by administration of an analogue of a normal metabolic precursor. Importantly, this provides evidence that inhibitors can be designed to reduce intrinsic defensive substances (those that are biosynthesized from intermediary metabolites) in insects and other arthropods. Exploitation of such chemical disarmament can be expected to lead both to laboratory and to field applications. In the specific case of cantharidin, for example, because adult female meloids apparently are unable to carry out its biosynthesis[4], inhibition of cantharidin formation in males would diminish the level of protection throughout the entire population.

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