out all areas of the superior colliculus, although it tends to appear somewhat denser anteriorly and medially (Fig. 1C). This situation remains throughout the life of the animal (*5*).

These results show that during development a neural pathway may distribute broadly and then retract to its normal adult pattern. In the developing rat, the retinal projections from both eyes initially overlap in the superficial layers of the superior colliculus, with most of the uncrossed axons eventually retracting or dying back at least as far as the optic chiasm, if not to the retina. It remains to be seen whether the transitory uncrossed axons are branches of contralaterally directed axons, as appears to be the case in older animals (*9*).

Other studies of the developing visual system have also found an initial overlap of the inputs from the two eyes (*2, 3, 10*). In marked contrast to the present observations, however, these studies show that as development proceeds, the two inputs segregate into adjacent laminae or stripes of roughly equal size rather than one's causing the almost complete elimination of the other. This is true for the retinotectal pathway of the monkey in which the inputs from each eye spread evenly throughout the superior colliculus on fetal day 78 and then segregate into alternating stripes between days 124 and 144 (*3*). It is not clear why the retinotectal pathway of rats and monkeys should develop so differently from an initially similar pattern. The mechanisms whereby segregation or retraction of afferents occurs are not known either. Since our experiments show that removal of one eye at birth prevents the elimination of uncrossed axons, it appears that the crossed optic pathway plays a major part in this process, perhaps by competitively inhibiting the maintenance of connections of uncrossed axons.

These observations also provide a cautionary note regarding the interpretation of the results of early lesions based solely upon examination of animals at much later times. In our own earlier studies on the effects of neonatal eye removal in rats, we hypothesized that the enlarged uncrossed pathway was most likely the result of sprouting rather than, as seems to be the case, retention of an earlier developmental state (*4, 11*). It is necessary to consider the likelihood that other anomalous projections observed after early lesions also may be due to failure of retraction of a transitory pathway rather than to sprouting.

It is possible to speculate about the relation of the events observed here to the normal pattern of development of the optic chiasm. The initial distribution of axons at the optic chiasm to crossed and uncrossed sides may not be precisely controlled, although axons originating from the temporal retina may be most likely to project ipsilaterally and nasal axons may be least likely. Such a distribution could simply be a product of the mechanics of growth through the region of the chiasm. Subsequently, the retraction process, resulting from competitive interaction between uncrossed and crossed axons, may eliminate inappropriately distributed axons and lead to a precise definition of the decussation point at the chiasm. Thus the pattern of distribution of axons at the optic chiasm may not, in fact, be due to specific interactions between the axons as they grow through the chiasm; it may instead be the result of a two-part process in which there is an approximate sorting depending on mechanical factors at the chiasm and a secondary adjustment depending on competitive interactions between terminal branches of axons. The study points to a relation between early eye removal and this developmental pattern. It would be interesting to reinvestigate the anomalies found in albino animals with the current model in mind.

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8. We have also observed the presence of transient uncrossed retinotectal projections in normal newborn rats which received intraocular injections of tritiated proline [P. W. Land and R. D. Lund, *Neurosci. Abstr.* **4**, 576 (1978)]. However, the excessive tissue background produced by isotope injections in young animals made it difficult, even with grain counting, to achieve the resolution we obtained with HRP. For example, we were unable to detect any expanded projections beyond postnatal day 4 in autoradiograms from normal animals, whereas the HRP method revealed numerous uncrossed fibers as late as day 7.
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11. The failure of retraction of a transitory pathway certainly seems to account for the abnormal projections we observed after eye removal at birth. However, a limited degree of axonal sprouting has been reported in rats enucleated as adults [D. C. Goodman *et al.*, *Brain Behav. Evol.* **8**, 27 (1973)]; we have confirmed this result by using axonal transport of HRP in animals with one eye removed at 25 days of age (P. W. Land and R. D. Lund, unpublished observation).
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Tomatine and Parasitic Wasps: Potential Incompatibility of Plant Antibiosis with Biological Control

Abstract. *α-Tomatine, an alkaloid in tomato plants, is toxic to an endoparasite of a major lepidopterous pest of tomatoes. The parasite acquires the alkaloid from its host after the host ingests the alkaloid. This form of interaction creates a potential dilemma to controlling herbivorous pests through chemical antibiosis in plants.*

Plant resistance to insects can result from the presence in various plant tissues of naturally occurring chemicals that are antagonistic or antibiotic to insect pests (*1*). Some host-plant resistance (HPR) programs are searches for selectively breeding for a high content of chemicals toxic to insects (*2*). However, little is known about the possible detrimental effects of these plant antibiotics on biological control agents (such as parasites and predators) of insect pests.

We have found that a parasite of a major agricultural pest could be poisoned by an antibiotic, α-tomatine, from tomato plants (*3*). The parasite was an ichneumonid, *Hyposoter exiguae* (Viereck), in one of its larval hosts, *Heliothis zea* (Boddie) (*4*). α-Tomatine forms a one-to-one insoluble and biologically inactive complex with many β-sterols and may induce toxic effects by impairing sterol utilization (*5*). Also, it is sapogenic and causes cytolysis (*6*). Because certain β-sterols are essential nutrients for insects (*7*), α-tomatine may be useful as an antagonist in the control of tomato pests. However, we found that the parasite *H. exiguae* can be toxified by α-tomatine, acquiring it from its less sensitive host, *H. zea*.

Our results are based on the use of artificial diets for the host containing 0.3 and 0.5 percent (wet weight) α-tomatine (*8*). Host larvae were fed on diets in five groups of 20 larvae per diet type (*9*). To

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Table 1. The effects (means ± 1 S.E.) of different diets of the host, *Heliothis zea*, incorporated with α-tomatine and β-cholesterol on certain parameters of the parasite *Hyposoter exiguae*. Means followed by different letters within a column are significantly different ($P < .05$). Abbreviation: NS, not significant.

Diet	Successful parasitization* (%)	Larval period (days)	Pupal period (days)	Pupal eclosion* (%)	Adult weight (mg)	Adult longevity (days)
Control	67.6 ± 6.1 (NS)	7.3 ± 0.2 b	6.7 ± 0.1 c	86.3 ± 3.3 a	2.5 ± 0.1 a	21.2 ± 0.9 a
0.3 percent tomatine	63.6 ± 7.3 (NS)	8.8 ± 0.5 c	6.3 ± 0.3 bc	57.7 ± 4.4 c	1.9 ± 0.03 b	12.7 ± 2.1 b
0.3 percent tomatine + β-cholesterol	69.4 ± 4.9 (NS)	6.6 ± 0.2 a	6.3 ± 0.2 bc	93.8 ± 5.4 a	2.2 ± 0.1 ab	16.0 ± 1.6 b
0.5 percent tomatine	61.8 ± 7.6 (NS)	11.3 ± 0.2 e	5.7 ± 0.2 a	60.8 ± 2.2 bc	1.6 ± 0.1 c	5.8 ± 1.3 c
0.5 percent tomatine + β-cholesterol	62.1 ± 3.4 (NS)	10.0 ± 0.2 d	5.7 ± 0.2 a	71.6 ± 4.3 b	2.1 ± 0.1 b	14.9 ± 2.5 b

*Retranslated from arc-sine transformed angles of the percentages.

determine whether α-tomatine-induced toxicity by impairing sterol utilization, we attempted to nullify toxicity in separate tests by feeding insects on artificial diets containing α-tomatine admixed with equimolar amounts of β-cholesterol as a sparing agent. Next, we traced the development of individual progeny of *H. exiguae* reared from hosts fed the various diets.

None of the diets had a significant effect on the percentage of successful parasitization ($F_{4,16} = 1.83$, $P > .05$) (Table 1); the overall degree of parasitization was ≃ 65 percent. The larval period of *H. exiguae* reared from hosts fed a control diet lasted ≃ 7 days. However, duration of the parasites' larval period was significantly prolonged ($F_{4,20} = 12.41$, $P < .01$) when α-tomatine was present in the hosts' diet (Table 1). The larval period of *H. exiguae* was extended to 8.5 days when hosts were fed on a diet containing 0.3 percent α-tomatine. Parasites reared from hosts fed on a diet containing 0.5 percent α-tomatine had larval periods of more than 11 days. The addition of β-cholesterol to the 0.3 percent diet fed to the hosts resulted in shortening the larval period of the parasites as compared to that of the control groups. However, the addition of β-cholesterol to the 0.5 percent diet did not result in a total reversal to normal parasites' larval period. Although the larval period was shortened significantly by about 1 day, it was still much longer in duration than that of parasites reared from hosts fed the other diets.

The toxicity of α-tomatine to *H. exiguae* was also manifested by a significant reduction ($F_{4,16} = 8.12$, $P < .05$) in the percentage of pupal eclosion (Table 1). Whereas the controls exhibited nearly 90 percent success in pupal eclosion, parasites reared from hosts fed the 0.3 or 0.5 percent diet exhibited only 60 percent success. Incorporation of equimolar amounts of β-cholesterol into the 0.3 percent diets resulted in normal eclosion of the parasites; but in the 0.5 percent diet containing β-cholesterol, the increase in pupal eclosion was not significant. Significant variations ($F_{4,20} = 2.97$, $P < .05$) in the duration of the pupal period were associated with different diets (Table 1). However, it appeared that a shorter pupal period could be correlated with higher levels of α-tomatine in the diet. This occurred in those fed the 0.5 percent diet (with or without the addition of β-cholesterol), where the surviving parasites (that is, lower percentage of pupal eclosion) developed more rapidly. The addition of β-cholesterol to the diets had no effect on the duration of the parasites' pupal period.

The weights of adult parasites (wasps) reared from hosts fed on diets containing α-tomatine with or without β-cholesterol were significantly lower ($F_{4,20} = 3.72$, $P < .05$) than those of adult parasites reared from hosts fed on the control diet (Table 1). Higher concentrations of α-tomatine in the diet resulted in smaller adult parasites. Adult parasites reared from hosts fed the 0.3 percent diet were significantly smaller than the adult parasites from the 0.3 percent and control groups. Addition of equimolar amounts of β-cholesterol to the diets fully alleviated the manifestation of lower weights in *H. exiguae* when the 0.3 percent diet was fed, but only partially alleviated it when the 0.5 percent diet was given.

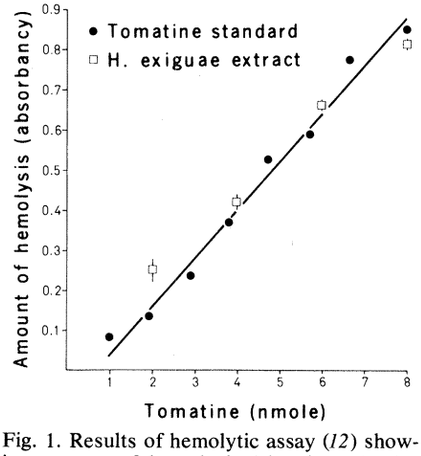

Fig. 1. Results of hemolytic assay (*12*) showing amount of hemolysis (absorbancy) of a standard solution of α-tomatine in comparison with the parasite extract (*10*). The amounts of hemolysis of the parasite extract are based on means of three tests (vertical bars are ± 1 S.E.). The α-tomatine content of the parasite extract was quantified by gas-liquid chromatography (*11*).

The presence of α-tomatine in larval tissue of the parasites would indicate that the parasites were either absorbing or ingesting the toxin from their hosts or that it was not totally metabolically altered by the hosts or parasites (*H. exiguae* does not excrete or defecate until the adult stage). To determine whether α-tomatine was present in the parasites, semipurified extracts (*10*) of third instar *H. exiguae* were analyzed by gas-liquid chromatography (GLC) (*11*), hemolysis (*12*), and ligand (*13*) assays. The GLC assay showed that the extract of the 20 *H. exiguae* larvae contained a total of 1.2 μmole of tomatidine; therefore, the extract prior to hydrolysis probably contained a glycoside of tomatidine (*14*). The hemolysis assay revealed consistent quantitative results with that of the GLC, showing 1.1 μmole of tomatine from the same nonhydrolyzed extract. To determine whether the extract of the parasites contained different glycosylated forms of tomatidine we compared its hemolytic activity with a standard α-tomatine. The hemolytic activity of the extract of *H. exiguae* was exactly that of the standard α-tomatine solution (Fig. 1) (note the close fit of activity of the *H. exiguae* extract to the standard curve of α-tomatine). Also, the ligand assay revealed that the addition of extramolar β-cholesterol to the parasite extract negated its hemolytic effect. This consistency of specific activity as determined by the hemolytic assay, abolishment of activity by ligand formation, and GLC analysis demonstrates that *H. exiguae* contained α-tomatine. Thus, the presence of α-

tomatine in larval tissues of the parasites indicates that antibiosis against the parasites is a result of absorption of the α-tomatine from the hosts.

Our studies demonstrate the potential incompatibility of biological control with HPR programs based on chemical antibiosis. The partial alleviation of α-tomatine antibiosis by the addition of β-cholesterol to the diet suggests that the presence of sterols or steryl esters in tomato plants may mask tomatine toxicosis to insects. However, the prolonged larval period, reduced pupal eclosion, smaller size, and shortened adult longevity of a parasite (*H. exiguae*) resulting from toxicosis by a plant antibiotic (α-tomatine) presents an enigma to integrated pest management in breeding high concentrations of a particular plant toxin to inhibit insect pests. This incompatibility may be exacerbated even further if the pest population evolves tolerance to the antibiotic, while the parasite population remains sensitive; the analogous problem is commonly encountered today with chemical insecticides (*15*). Alternatively, there may be compatibility if biological control agents that contact fewer plant antibiotics via the "physiological filter" of the host are able to evolve tolerance to these antibiotics more readily than their hosts, which must evolve tolerance to a multitude of plant chemicals while facing mortality induced by the parasite. Furthermore, according to present theory insects should be at a disadvantage while feeding on plants containing antibiotics (*16*). However, our results suggest that this putative disadvantage may be outweighed by the ability of these herbivorous insects to serendipitously utilize these antibiotics as prophylactics against their parasites.

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10. The excised parasites were ground in boiling methanol. The homogenate was filtered, diluted to 25 percent methanol in H_2O, acidified to *p*H 2, and extracted once with petroleum ether and once with ethyl ether. The aqueous solution was readjusted to *p*H 8 and extracted three times with ethyl ether; the residue was dried under nitrogen gas and redissolved in 2 ml of 50 percent methanol. The sample was stored at 0°C until used for hemolytic (*12*) and GLC (*11*) assays.
11. A 100-μl portion of partially purified parasite extract (*10*) was hydrolyzed with 2*N* HCl at 100°C for 2 hours to liberate tomatidine. The solution was then adjusted to *p*H 8 and extracted three times with ethyl ether. The ether fractions were pooled and dried under vacuum. Prior to GLC analysis, tomatidine was derivatized by Tri-Sil 'Z' (Pierce Chemical Co., Rockford, Ill.). β-Cholesterol was added as an internal standard. The GLC analyses were performed on a Varian 3700 GC equipped with a coupled flame ionization and thermionic specific detector (for N); we used a stainless steel column (0.5 m by 2.2 mm) containing 5 percent OV-101 on Chromsorb W; the temperature program was 200° to 300°C at 10° per minute; the carrier gas was helium, at 30 ml/min.
12. For the hemolysis assay, the indicator system consisted of 3 ml of washed sheep red blood cells (5 percent) in normal saline plus tris buffer (*p*H 8). Microliter portions of standard α-tomatine solution were compared with parasite extract (*10*) for hemolytic activity. The portions were added to the indicator system, incubated at room temperature for 30 minutes, and centrifuged at 3000 rev/min for 10 minutes. Released hemoglobin was measured colorimetrically at 542 nm.
13. β-Cholesterol (10 μmole) was added to hemolysis system. The β-cholesterol was stored as a 50 percent methanol solution; it precipitates α-tomatine and abolishes hemolytic activity.
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Sexual Difference in Pattern of Hormone Accumulation in the Brain of a Songbird

Abstract. *After adult zebra finches (*Poephila guttata*) received injections of tritiated testosterone, fewer hormone-concentrating cells were found in females than in males in two brain regions involved in song: hyperstriatum ventrale pars caudale and magnocellular nucleus of the anterior neostriatum. In some other regions, no sexual difference was detected. It is, therefore, possible that sex differences in the sensitivity of specific neural populations to hormones underlie the striking anatomical dimorphism observed in neural regions controlling song.*

Sex steroids act on the brain to bring about a number of behavioral and neuroendocrine effects. One step in the mechanism of steroid action includes limited-capacity binding of steroids by certain neurons, binding that can be detected by autoradiography (*1*). Numerous autoradiographic studies have allowed generalizations about the topography of cells that concentrate steroids in the vertebrate brain. One is that the distribution of steroid target neurons has been fairly stable throughout vertebrate evolution, since labeled cells are found in homologous brain regions in fish, amphibians, reptiles, birds, and mammals (*2*) [although there are exceptions to the general pattern (*3, 4*)]. A second generalization, that the distribution of labeled cells is similar in males and females of any given species (*5*), implies that the distribution of hormone-concentrating cells is not changed by whatever processes determine the sexual differentiation of the brain. We now report a definite sex difference in the numbers of cells accumulating hormone in specific brain regions of a songbird, the zebra finch (*Poephila guttata*), after injection of tritiated testosterone.

Male zebra finches sing a short courtship song learned in early life from the father (*6*). Castrated adult zebra finches sing much less frequently than intact birds, and testosterone propionate injections reverse the effects of castration (*7*). Autoradiographic evidence in the adult male (*4*) indicates that after tritiated testosterone is injected, labeled neurons are found in several brain regions implicated in the control of song or other vocalizations. These include the caudal nucleus of the hyperstriatum ventrale (HVc), magnocellular nucleus of the anterior neostriatum (MAN), nucleus intercollicularis of the midbrain (ICo), and the tracheosyringeal motoneurons (nXIIts), which are the hypoglossal motoneurons innervating the vocal organ, or syrinx. Behavioral and anatomical evidence in the canary indicates that these brain regions are interconnected and that they

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