at the cellular level, as well as the dynamic characteristic of intracellular lead in an isolated, intact cell system. The data indicate that lead may be mobilized by cellular stimuli that mobilize calcium, and we postulate that the mobilized lead may be available to interfere with, or participate in, the calcium-mediated cell functions elicited by these stimuli.

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Enchenopa binotata Complex: Sympatric Speciation?

Abstract. Enchenopa binotata *is a complex of six treehopper species that have diverged along host plant lines. When females were forced to oviposit on "adopted" host plants, few eggs were deposited. Fewer eggs hatched on "adopted" hosts and those that did hatch did so in response to the phenology of the "adopted" host. Mortality of nymphs on "adopted" hosts was substantially higher than on native hosts. These and other data support a sympatric model of speciation through shifts in host plants.*

Allopatric speciation was once considered the primary means by which populations differentiated into reproductively isolated species. This view has been challenged (*1*), and nonallopatric mechanisms have become more generally accepted. Bush (*2*) suggests that shifts in host plants promoted sympatric divergence of some phytophagous insects such as *Rhagoletis*. Futuyma and Mayer (*3*), after reviewing the evidence on *Rhagoletis*, accept the possibility of such a mechanism but conclude that there is little empirical evidence to support it. At issue is whether a host plant by itself can act as an effective reproductive barrier to limit gene flow after a host shift has occurred. We have recently presented data (*4–6*) which support a sympatric model of speciation by shifts in host plants.

Enchenopa binotata (Say) is a phytophagous insect that occurs from Panama throughout eastern North America. In North America it has a single generation per year and is found on six species of host plant. These hosts (*Ptelea trifoliata*, *Cercis canadensis*, *Juglans nigra*, *Viburnum prunifolium*, *Celastrus scandens*, and *Robinia pseudoacacia*) are evolutionarily diverse (*7*) and sympatric throughout the eastern United States. *Enchenopa binotata* has been considered a single polyphagous species. However, *Enchenopa* on each host differs in the coloration of nymphs, oviposition sites, nymphal feeding sites, seasonal and diurnal patterns of oviposition, and the number of eggs per egg mass. When females are given a choice of host plants on which to oviposit they select the host on which they were raised. When males and females from all hosts are confined to a single cage, there are few matings by insects of mixed host origin, and the length of mixed matings are considerably shorter. Even under conditions imposed by a cage, mating tends to occur on the host on which females were raised (*4–6*). Therefore, although we believe sympatric speciation via host plant shift does not require that females show a host preference, members of the *Enchenopa binotata* complex do demonstrate such a behavioral choice.

Allochronic life histories are important in maintaining reproductive isolation among members of this complex. Eggs hatch on each host (with the exception of *Cercis canadensis*) about the time the host is in flower. Allochronic egg hatch combined with differences in maturation produces temporal differences in mating; differences in the time of day that mating occurs further reduce the possibility of hybridization. Allochronic and diurnal differences in mating reproductively isolate adults from four of the six hosts. Members of the last pair are effectively isolated from each other by allochronic flight activity, which occurs about a week apart. On one host, almost all flight activity occurs before mating begins on that host; hence, there is very little flight by either sex once mating begins and virtually none after oviposition starts. Ovipositional attractants in egg froth tend to keep females on their hosts (*4–6*). In fact, movement by males and females throughout the summer, even among nearby conspecific hosts, is almost nonexistent (*8*).

Females only mate once; this should promote competition among males as the number of virgins decreases and increase male dispersal to new hosts. We have found that male flight activity does not increase as the number of virgins decreases. The lack of male dispersal stems from the inability of males to recognize mated females, high male mortality, and short male longevity. When mating is completed on a given host there are few or no males surviving (*4–6*).

Electrophoretically, *Enchenopa* from each host differ in the frequency and fixation of electromorphs even when collected from two adjacent tree species. There were even electrophoretic differences in the *Enchenopa* among individual conspecific trees located very close to each other. Genetic distances calculated from electrophoretic data indicate that *Enchenopa* on *J. nigra* diverged first; then divergences on *P. trifoliata*, *R. pseudoacacia*, *C. canadensis*, *V. prunifolium*, and *C. scandens* followed in the order given. Estimates of the time of divergence by means of the molecular time clock (*6*) suggest that speciation has been recent—that is, within the last 250,000 years. (In this estimate, selection is assumed to be absent.)

Wood (*4*) postulated that North American *Enchenopa* may have diverged from a tropical polyphagous ancestor. This ancestral stock encountered selection pressures to coordinate its life history with the phenology of newly exploited hosts resulting in a shift from a multivoltine life history. Colonization of North American hosts with differing phenologies and of differing nutritional quality resulted in differences in maturation that promoted allochronic life histories. Further genetic differentiation was promoted by the relative temporal permanence and spatial heterogeneity of host resources which encouraged low vagility.

We now report data on the question of whether gene flow could be reestablished by mated females ovipositing on the "wrong" host. Field-collected females from each host were forced to oviposit (*9*) on all six host species (Table 1); nevertheless, females on some "adopted" hosts refused to deposit eggs. In all cases, when females were confined to their normal host, they deposited more egg masses than did those on "adopted" hosts. The only "adopted" host that was universally accepted was *C. canadensis*. The following spring the number of nymphs per egg mass from egg masses deposited on the normal host was high (Table 1), whereas the number deposited on "adopted" hosts was very low. Egg masses on "adopted" hosts hatched at the same or close to the time that eggs deposited by females native to that host hatched (Table 2). For example, eggs from females on *Viburnum* and *C. scandens* hatched significantly earlier than those on *C. canadensis*. However, eggs from females from these two hosts deposited on *C. canadensis* hatched later than on their native host at the same time or close to the time as those eggs from females native to *C. canadensis*. The mortality of nymphs on "adopted" hosts was high during the first instar, which takes about 5 days. On "adopted" hosts only six nymphs reached the second instar and one nymph survived through the fifth instar.

Under field conditions it seems unlikely that mated females move among conspecific hosts let alone among other species of host plants. However, if such an event should occur, the probability of reestablishing gene flow seems remote. This experiment demonstrates that, if such events occurred in the past, selection on eggs and nymphs could have been intense. With such intense selection host plants by themselves could provide reproductive barriers, and it would appear that speciation of the *Enchenopa* complex was more recent than that suggested by a neutralist time clock. That eggs deposited in "adopted" hosts hatched in synchrony or close to the time of those native to that host supports the hypothesis that host phenology may have been the initial factor promoting divergence. Differential maturation of nymphs influenced by the nutritional quality of plant sap appears to be a second factor producing allochronic isolation.

Does the *Enchenopa* complex meet the objections raised by Futuyma and Mayer (*3*) that habitat or host shifts results in reproductive isolation? We can show for the *Enchenopa* complex that (i) females show a host preference, (ii) mating is allochronic, (iii) mating occurs on the host rather than in the air or nearby vegetation, (iv) there are genetic differences among the "host races," (v) there are apparent genetic differences in the ability of eggs and nymphs to survive on

Table 1. Female *Enchenopa* were collected in the field from native host plants and forced to oviposit on "adopted" hosts. Egg masses were deposited in August or September 1981 and eggs began to hatch on 4 May 1982.

Native host	"Adopted" host data					
	J. nigra	*P. trifoliata*	*R. pseudoacacia*	*C. canadensis*	*Viburnum*	*C. scandens*
J. nigra						
Females (No.)	29	20	20	23	22	20
Egg masses (No.)	52	0	0	46	0	0
Nymphs/egg mass	2.17			0.20		
P. trifoliata						
Females (No.)	48	43	46	48	44	45
Egg masses (No.)	2	313	1	19	21	15
Nymphs/egg mass	0	2.37	0	0.84	0	0.93
R. pseudoacacia						
Females (No.)	30	30	30	30	30	30
Egg masses (No.)	0	0	73	15	0	0
Nymphs/egg mass			1.60	0		
C. canadensis						
Females (No.)	27	24	23	27	25	24
Egg masses (No.)	2	0	0	73	1	0
Nymphs/egg mass	0			2.60	0	
Viburnum						
Females (No.)	25	21	21	26	38	26
Egg masses (No.)	0	0	0	24	103	0
Nymphs/egg mass				0.33	1.19	
C. scandens						
Females (No.)	50	50	50	50	50	50
Egg masses (No.)	1	0	8	63	51	165
Nymphs/egg mass	0		0	0.06	0.73	2.15

Table 2. Time (days) of hatching after *Enchenopa* eggs were deposited on native and "adopted" host plants. Survival of nymphs was determined 43 days after eggs hatched on the first host. A continuous scale beginning on 4 May 1982 when the first nymphs appeared was used to determine allochrony of egg hatch. Since individual eggs or egg masses could not be followed, the number of nymphs was used to determine egg hatch. Means are expressed with the population standard deviation. Analysis of variance ($F = 408.14$, $P = 0.0000$, d.f. = 1637) and the Student-Newman-Keuls procedure (.05 level) were used to compare means. Vertical lines indicate no significant difference.

Native host	"Adopted" host	Nymphs (No.)	Days for eggs hatched (mean ± S.D.)	Survival (%)
V. prunifolium	*V. prunifolium*	112	2.99 ± 3.78	32.04
C. scandens	*C. scandens*	277	3.71 ± 1.60	18.36
P. trifoliata	*C. scandens*	14	4.71 ± 3.75	7.14
C. scandens	*V. prunifolium*	37	5.16 ± 1.36	0.00
P. trifoliata	*P. trifoliata*	741	6.30 ± 1.28	55.60
J. nigra	*J. nigra*	113	9.47 ± 1.43	16.81
V. prunifolium	*C. canadensis*	8	9.75 ± 1.75	0.00
C. scandens	*C. canadensis*	4	10.25 ± 3.20	0.00
R. pseudoacacia	*R. pseudoacacia*	117	11.44 ± 2.38	55.60
J. nigra	*C. canadensis*	9	12.22 ± 1.30	0.00
C. canadensis	*C. canadensis*	190	12.54 ± 2.01	41.58
P. trifoliate	*C. canadensis*	16	14.50 ± 2.78	0.00

different hosts, and (vi) dispersal is low even among nearby conspecific hosts and is consistently low over several years. Although we have not proved that sympatric speciation has caused the divergence of this complex, the weight of the present evidence supports this view better than an allopatric mechanism.

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8. S. I. Guttman *et al.*, unpublished observation. Of 689 *Enchenopa* from four *Ptelea* marked and followed over an 8-week period, only 13 individuals moved between trees. Eleven of these moved between two *Ptelea* approximately 1 m apart; the other two treehoppers moved approximately 25 m.
9. Females were collected after oviposition started and mating was completed. Females were confined to branches covered with nylon cages. All host plants were grown in a garden plot to minimize the effect of environmental conditions on host phenology.
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Breathing Gas Mixtures Different from Air: An Adaptation for Survival Under the Ice of a Facultative Air-Breathing Fish

Abstract. *Gaseous respiration by central mudminnows (*Umbra limi*), particularly their use of bubbles composed of gas mixtures other than air, may have evolved as an adaptation to the oxygen-depleted, carbon dioxide–rich water of winterkill lakes. Under simulated winterkill conditions, mudminnows frequently engulfed gaseous bubbles. Use of bubbles was not related to varying methane or nitrogen content (0 to 80 percent) when all bubbles contained 20 percent oxygen. When the oxygen content of bubbles varied (0 to 20 percent), fish visited bubbles randomly but remained longer and took fewer "breaths" at bubbles with high oxygen content. High temperature (16° to 34°C) and low* pH *(6.8 to 4.5) did not stimulate increased air-breathing when dissolved oxygen was sufficient.*

Central mudminnows, *Umbra limi* (Kirtland), are continuous but facultative air breathers that use the highly vascularized swim bladder as an accessory respiratory organ (*1*). How this form of respiration evolved in the Umbridae is not known. Most species of air-breathing fishes live in warm, stagnant, hypoxic waters, although some live in well-oxygenated waters that are very acid, rich in CO_2, or subject to seasonal drought (*2*). We hypothesize that gaseous respiration by central mudminnows, particularly the ability to "breathe" from bubbles with gas mixtures markedly different from those of air, is an adaptation to the winterkill lakes that the fish commonly inhabit, in which the waters are depleted of O_2 and are rich in CO_2.

In winterkill lakes, ice prevents the fish from having direct contact with the atmosphere for 4 to 5 months of the year, but mudminnows may use bubbles trapped beneath the ice (*3*). Klinger *et al.* (*3*) found that mudminnows held in field enclosures with air bubbles in a winterkill lake, in which dissolved oxygen (DO) approached 0.0 mg per liter, survived longer than fish in enclosures without air bubbles. The average oxygen content of the naturally occurring bubbles was 3 percent (range, 0 to 11 percent) when DO was 0.5 mg per liter; bubbles also contained 1 to 75 percent methane and 23 to 98 percent nitrogen.

The severe environment (*4, 5*) of winterkill lakes was simulated in the laboratory (*6*). In a series of six experiments (Table 1) we examined how mudminnows behaved toward (i) air bubbles under both low DO–high CO_2 and high DO–low CO_2 conditions and (ii) bubbles composed of gas mixtures (nitrogen, methane, and oxygen) different from air but similar to mixtures that occur under natural conditions. To simulate ice cover, we inserted a panel of white, translucent fiber glass into each of six molded fiber glass aquariums (inside dimensions, 60 by 28 cm, and 35 cm deep). Five 4-mm holes were drilled through the fiber glass surface and countersunk; gas bubbles (2 ml) injected through holes simulated bubbles beneath the ice. We simulated cracks by leaving the holes unplugged; mudminnows could ventilate at the holes and pull oxygenated water (DO, 5 to 10 mg per liter) from above the fiber glass but could not gulp air.

Each experiment consisted of five 30-minute observation periods, and new gas bubbles were injected between these periods (*7*). Twelve fish (one large fish, 88 to 112 mm, and one small fish, 65 to 75 mm, in each of six aquariums) were placed in the aquarium before the start of experiment 1. The location and behavior of fish were recorded at 30-second intervals. Horizontal lines on the glass divided each tank into three equal volumes in the bottom, middle, and top. In addition to these three positions, fish could be at the simulated "ice" (with a part of the body touching it), at a hole, at a bubble, or at a plugged hole. We usually set DO and CO_2 concentrations the night before an experiment and measured both before the observation periods (*8*).

During experiment 1, with low DO and high CO_2 concentrations (Table 1), naïve mudminnows (those not previously exposed in the laboratory to low DO, high CO_2, or bubbles) moved up to the "ice" (62 percent of the time observed) and were often near bubbles (18 percent of the time) (Fig. 1a). After approaching a bubble a fish often raised its head and engulfed part of the bubble; additional breaths were frequently taken during the course of a visit. When a fish visited a hole it often inserted its head into the hole and pumped water from above across the gills. Although fish occasionally visited open holes, they were not recorded there (Fig. 1a), indicating that the mudminnows preferred to use bubbles than to pump oxygenated water down through holes.

The fish in experiment 1 were also tested under high DO–low CO_2 conditions (Table 1, experiment 2). When oxygen was abundant mudminnows spent a substantial percentage of time in the bottom third of the aquariums (47 percent), relatively little time at the "ice" (18 percent), and were rarely near holes or bubbles (3 percent) (Fig. 1b).

When the mudminnows were returned to low DO–high CO_2 conditions (Table 1, experiment 3), they again moved up to the "ice" (65 percent of the time observed) and were often near bubbles (25 percent of time) (Fig. 1c). The behavior of mudminnows was similar in experiments 1 and 3, indicating that the response to low DO–high CO_2 and air