of CARNA 5 with the severe disease incited in tomato plants and the mild symptoms incited in Tabasco pepper and sweet corn plants contrasts strikingly with the absence of CARNA 5 and the resulting milder disease in tomato and severe diseases in pepper and sweet corn plants. In tobacco plants where high CARNA 5 results in reduced virus multiplication (*10*), a similar reduction in symptoms should be expected. However, with this host, the biological effects are hardly visible because the disease evoked by CMV is mild in either case.

It seems clear, therefore, that in natural hosts of CMV, which support multiplication of CARNA 5, the presence of CARNA 5 is expressed either as a new disease syndrome [shown to be lethal in at least one case (*5, 6, 8*)] or (and more often) as an attenuation of the viral infection with a resulting decrease in symptoms. We previously indicated how, during pathogenesis, the competition between CARNA 5 and the viral RNA's or the messenger capability of CARNA 5, or both, could decisively affect symptom expression (*3, 11*).

Huang and Baltimore (*12*) proposed that the competitive relationship of defective interfering (DI) particles and their helper viruses could play a major role in the evolution of viral disease. However, DI particles whose nucleic acid is directly related to the genome of their helper viruses (*12*) have not yet been described for plant viruses. On the other hand, viral satellites, equally dependent on their virus helpers for replication, are being found with increasing frequency in plant virus infections. Their genetic content has little if any relationship to the helper's genome (*13*). However, we have some evidence suggesting that they could have originated from the host (unpublished work). We therefore propose that small, satellite-like replicating RNA's such as CARNA 5 play a regulatory role in viral pathogenesis and ultimate disease expression. This report illustrates such regulatory effects by CARNA 5 in infections of CMV in three of its natural hosts, which are also important agricultural crops.

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Mortality of the Monarch Butterfly (*Danaus plexippus* L.): Avian Predation at Five Overwintering Sites in Mexico

Abstract. *Analyses of predated butterflies on the forest floor at five monarch overwintering sites in Mexico and observations of birds foraging in mixed flocks indicate that individual birds of several species have learned to penetrate the monarch's cardenolide-based chemical defense. Predation is inversely proportional to colony size and appears to be one evolutionary explanation of the dense aggregations.*

Occasional bird predation of overwintering monarch butterflies has been reported in California (*1, 2*) where small mammal predation was also inferred from wing caches and live, maimed butterflies falling from trees at night (*3*). In contrast to this minor predation in California, we report here extraordinarily heavy mortality at one previously described (*4*) and four newly discovered overwintering sites in Mexico (*5*).

Within these Mexican colonies and particularly beneath heavily laden trees (Fig. 1), accumulations of fallen butterflies increased throughout the 1977–1978 overwintering season, and by late February the forest floor was thickly carpeted in places (Figs. 2 and 3) with as many as 776 butterflies per square meter. These accumulations consisted mostly of dead butterflies. Some of the live ones were temporarily immobilized by the cold and flew off when warmed. Others were moribund, including intact individuals unable to fly, and still others, definitely predated, were missing one or more wings, abdomens, and/or heads (Figs. 4 to 11).

We collected random samples (*6*) (Fig. 3) totaling 1697 dead butterflies at site Delta on 26 March 1978, shortly after its abandonment. Undamaged butterflies constituted 11 percent of the total. The remaining 89 percent showed definite indications of predation. Of these, 35 percent lacked one or more wings, and 65 percent had all wings intact but were damaged in various other ways. A further breakdown of this last-named subgroup showed overlapping damage categories: 68 percent had damaged thoraces—from a small hole to a large eaten-out portion (Fig. 12)—and 53 percent had missing abdomens (Figs. 4, 8, 10, and 11). Moreover, of those with their abdomens still attached, 19 percent had the abdominal contents stripped, leaving only the outer cuticular shell. On three occasions, we observed a live female attached in copulo to a dead, stripped male (Fig. 13). The same categories of moribund and dead butterflies occurred at all sites.

Binocular and photographic observations at all sites revealed three species of birds preying extensively on the monarchs (*7, 8*). These were Scott's oriole (*Icterus parisorum* Bonaparte), the black-backed oriole (*Icterus abeillei* Lesson) (Figs. 14 and 15), and the black-headed grosbeak (*Pheucticus melanocephalus* Swainson) (Fig. 16). Both oriole species and the grosbeaks flew in mixed flocks of 25 to 30 birds, made repeated forays into the colonies, and attacked monarchs in the hanging clusters and, to a lesser extent, on the tree trunks. The orioles spent more time pecking the butterflies and frequently stripped the abdomens, whereas the grosbeaks often deftly snapped off and ate only the abdomens, dropping the rest of the butterfly.

In 37 percent of our direct observations (9 of 22 oriole attacks and 4 of 13 grosbeak attacks) the individual birds captured, apparently or actually damaged, and then released a butterfly without eating it. In addition, we repeatedly observed monarch wings and body parts with wings attached floating down from

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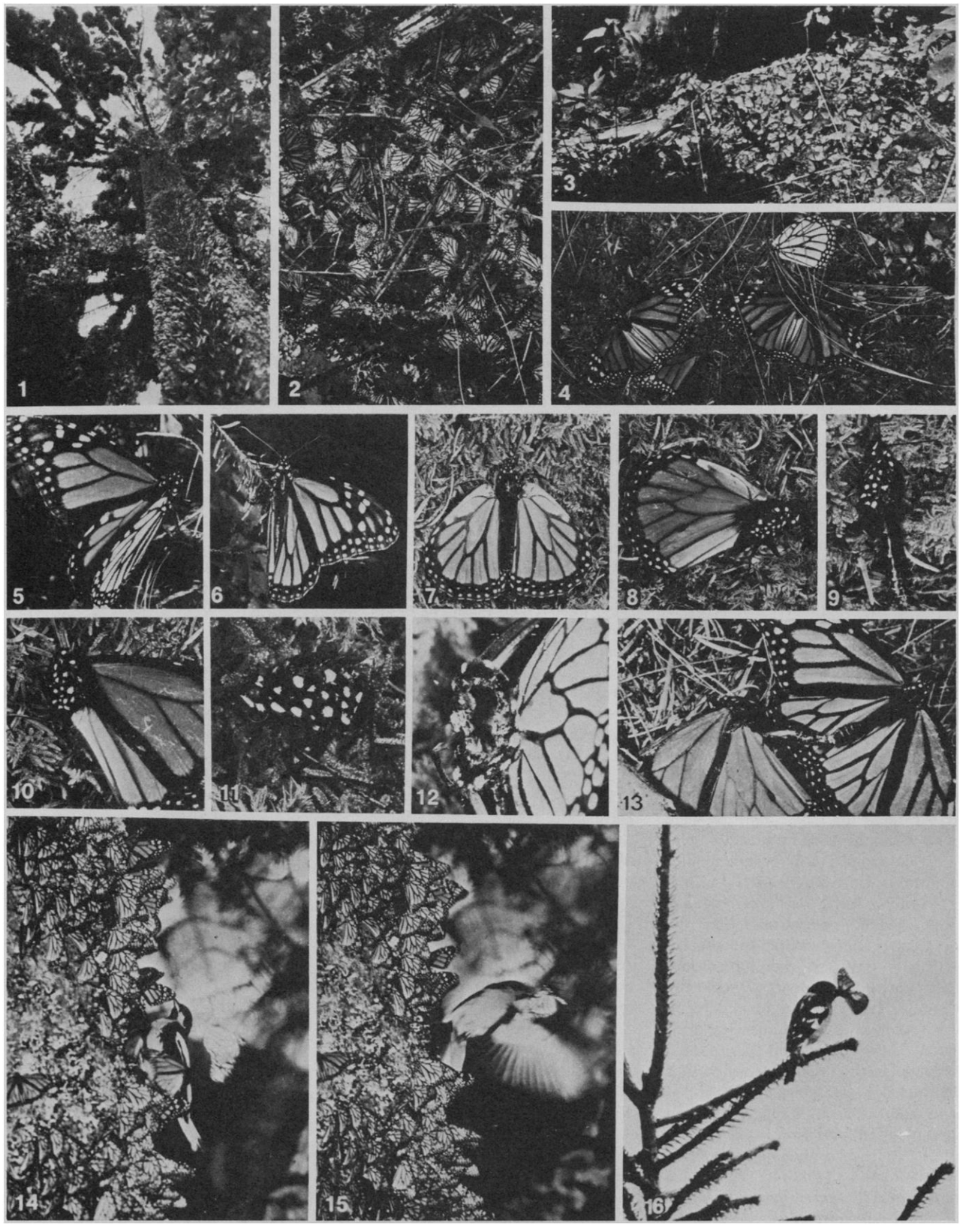

Fig. 1. Dense clusters of overwintering monarch butterflies on the trunk and boughs of a fir tree (*Abies religiosa* H.B.K.) at site Gamma, 5 February 1978. Fig. 2. Moribund and dead butterflies carpeting the forest floor beneath clusters at Delta, 28 January 1978. Fig. 3. Sample plot (0.25 m^2), site Delta, 28 January 1978. Figs. 4 to 13. Various categories of bird-predated butterflies, site $Alpha_1$, 2 February 1978. The individuals in Fig. 5 (missing right forewing), Fig. 6 (missing left fore- and hindwing), and Fig. 7 (missing both forewings) were still alive when photographed. Fig. 12. A dead butterfly from which the thoracic muscles have been eaten. Fig. 13. A normal live female still in copulo with a dead male, the abdomen of which has been stripped (site $Alpha_2$, 30 March 1978). Figs. 14 to 15. Telephoto-strobe sequence of a black-backed oriole seizing a monarch from a tree trunk cluster and flying off to consume (or reject) it, site Delta, 28 January 1978. Fig. 16. A black-headed grosbeak holding a monarch in the top of a fir tree at site $Alpha_1$, 3 February 1978. [Photo credits: L. P. Brower (Figs. 1 to 12), W. H. Calvert (Fig. 13), and G. D. Lepp (Figs. 14 to 16)]

Table 1. Summary of data for colony size and mortality due to predation and other factors at five overwintering sites of the monarch butterfly in Mexico, 1977–1978 season; S.E., standard error. Overwintering began on 1 December 1977 (*17*).

Row	Items	Sites and sample dates (1978)					
		$Alpha_1$ (3 Feb.)	$Alpha_2$ (18 Feb.)	Beta (13 Jan.)	Gamma (9 and 19 Feb.)	Delta (28 Jan.)	Mean of means
1	Number of 0.25-m^2 samples	20	15	10	27	26	
2	Dead butterflies per square meter (mean ± S.E.)	122 ± 28	112 ± 18	121 ± 27	64 ± 10	244 ± 41	133 ± 25
3	Colony area (hectares)	0.755	2.262	0.173	1.433	0.248	0.974
4	Colony mortality to date*	921,000	2,533,000	209,000	917,000	605,000	1,037,000
5	Days from beginning of overwintering	65	80	44	77	59	65
6	Dead butterflies (mean/m^2 per day)	1.89	1.40	2.75	0.82	4.14	2.20
7	Dead butterflies (mean per colony per day)*	14,000	32,000	5,000	12,000	10,000	15,000
8	Predated butterflies (mean/m^2)	81	63	96	41	190	94
9	Predated butterflies per day (mean/m^2 per day)	1.25	0.79	2.17	0.53	3.23	1.59
10	Dead butterflies that were predated (mean %)†	76	60	84	67	83	74

*Rounded to nearest 1000 butterflies. †The mean of the percent predated for each 0.25-m^2 sample at each site.

the feeding flocks within and adjacent to the colonies. Moreover, these still living or very recently dead butterfly remains corresponded to the various categories of predated butterflies analyzed in the 0.25-m^2 ground samples, and included individuals missing one to four wings, abdomens, and heads, or having gouged thoraces or stripped abdomens (or both) (Figs. 4 to 13).

Some monarch butterflies contain cardenolides (cardiac glycosides) derived from their larval food plants (milkweeds, Asclepiadaceae), which render them emetic and unpalatable to avian predators (*9*). In California and Massachusetts, samples of monarchs from all populations studied include both emetic and nonemetic individuals (*10*), and blue jays (*Cyanocitta cristata bromia* Oberholser) are capable of discriminating these by cardenolide-based taste rejection (*11*). It is therefore of great interest that, in a sample of 101 butterflies from site $Alpha_1$ in January 1977, 29 percent contained less than 10 μg of cardenolide per butterfly and, on the basis of extrapolation from another study (*10*), are therefore effectively palatable.

The observed modes of attack, damage, and rejection by the orioles and grosbeaks and the occurrence of corresponding damage categories in the ground samples, together with the mixed population of both poisonous and nonpoisonous butterflies, combine to make it plausible that these birds have broken through the monarch's chemical defense by learning to reject the cardenolide-laden butterflies by taste. Moreover, the exoskeleton of the monarch butterfly has comparatively high concentrations of cardenolides compared to the rest of the body parts (*11, 12*), and it seems likely that the abdominal stripping and thoracic muscle feeding is an additional taste-based behavior adapted to avoid the cardenolide toxicity (*13*). It thus appears that these Mexican bird predators not only discriminate the palatable from unpalatable monarchs, but also are able to distinguish palatable from unpalatable parts (*14*).

We also used 0.25-m^2 ground sampling (*6*) to compare mortality at the five sites (*15*). To obtain the total number of butterflies that succumbed to all factors as of the sampling date (Table 1, row 4), the mean number of dead butterflies per square meter (row 2) was multiplied by the respective colony areas (row 3) (*16*). To compensate for different sampling dates, the number of dead butterflies per square meter was divided by the estimated age of the colony (row 5) when it was sampled (*17*) to give the mean number of dead butterflies per square meter per day (row 6). This procedure was repeated for the predated butterflies (rows 8 and 9). The mean number of dead butterflies per colony per day (row 7) is the colony mortality to date (row 4) divided by the colony age (row 5). The percentage of dead butterflies that was predated ranged from 60 to 84 percent (row 10). Statistical analyses are shown in Tables 2 and 3.

Substantial mortality occurs at all sites, more than two-thirds of which is due to predation (Table 1, row 10). Mortality varies by a factor of 5 among the sites, and the major statistical significance derives from the predated portion; the nonpredated group does not show significant differences among the sites (Tables 2 and 3).

The areas of the sites vary 13-fold—Beta is the smallest and $Alpha_2$ is the largest (Table 1, row 3)—and show a strong inverse relation with mortality,

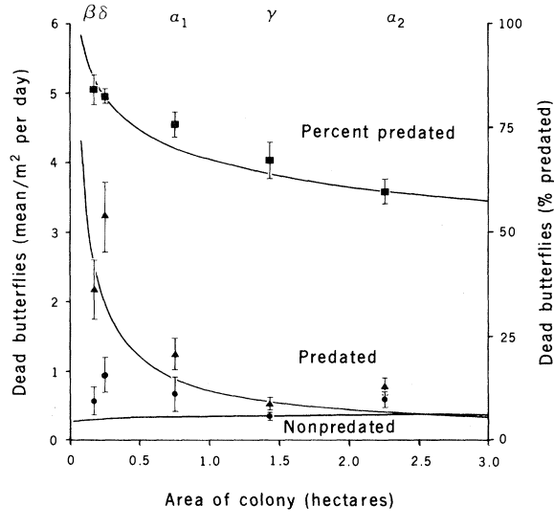

Fig. 17. Relation of colony area to mortality caused by predation and other factors at five Mexican overwintering sites of the monarch butterfly. Nonpredation mortality (lowest curve) shows no dependence on colony size, whereas the predated portion (middle and upper curves) shows a highly significant inverse relation indicating that large colonies are subject to less predation than small ones. The three curves were generated by computer in least-squares analyses (1, 96 d.f.) of the 98 0.25-m^2 samples (Table 1), and the means and standard errors are also plotted. The numbers of dead but nonpredated butterflies (lowest curve) vary randomly with respect to colony area: r for the linear function $y = ax + b$ is -0.16, $P > .10$; for the curvilinear function $y = ax^b$, $r = +.05$, with $P > .50$. In contrast, both the number (middle curve) and percentage of predated butterflies (upper curve) show highly significant inverse relation to colony area according to the $y = ax^b$ function. For the number predated, $r = -.62$, $F = 59.54$, $P < .001$, $a = 0.73$, and $b = -0.70$; for the percentage predated, $r = -0.46$, $F = 25.68$, $P < .001$, $a = 0.67$, and $b = -0.14$. The data indicate steeply increasing mortality due to predation in colonies less than 1 ha in size.

Table 2. Summary of analysis of variance of the mortality data at the five sites based on the number of individuals per square meter per day in the ground samples. The data for each ground sample were normalized by $\log_{10}$ transformations, following which three one-way analyses of variance were run.

Source	Degrees of freedom	Sum of squares	Mean squares	F ratio	P
Dead butterflies					
Between groups	4	6.70	1.68	12.66	< .0001
Within groups	93	12.31	0.13		
Total	97	19.01			
Predated butterflies					
Between groups	4	9.58	2.39	21.50	< .0001
Within groups	93	10.36	0.11		
Total	97	19.94			
Nonpredated butterflies*					
Between groups	4	1.96	0.49	1.92	.11
Within groups	87	22.12	0.25		
Total	91	24.08			

*Six zero value samples were excluded in order to make the $\log_{10}$ transformations.

Table 3. Summary of Duncan's multiple range tests comparing the five sites ($P \leq .05$).

	Sites				
	Gamma	$Alpha_2$	$Alpha_1$	Beta	Delta
Dead (mean/m² per day)	0.82	1.40	1.89	2.75	4.14
Ratio to lowest	1.00	1.71	2.30	3.35	5.05
Significance*	⊢——⊣	⊢————⊣		⊢——⊣	
Predated (mean/m² per day)	0.53	0.79	1.25	2.17	3.23
Ratio to lowest	1.00	1.49	2.36	4.09	6.09
Significance*	⊢——⊣	⊢————⊣	⊢————⊣	⊢——⊣	
Nonpredated (mean/m² per day)	0.29	0.60	0.64	0.58	0.91
Ratio to lowest	1.00	2.10	2.21	2.00	3.14
Significance*	⊢————————⊣				

*Nonoverlapping lines indicate that the means differ significantly at the $P \leq .05$ level of significance.

which is attributable to the predated portion of the dead butterflies, that is, small colonies have much more predation than do large ones (Fig. 17). Because large colonies would always experience some predation, a curvilinear relation is more meaningful biologically than a linear one, and is supported by the high r and f values (Fig. 17).

These colony area and mortality data, together with our direct observations of bird predation, provide strong evidence that some birds have penetrated the chemical defense of the monarch butterfly and that individuals in large overwintering colonies are better protected against birds than those in small ones. On the basis of our observations of extreme packing at all sites, we are confident that the number of butterflies per colony is a direct function of the colony area. Critical minimal colony size appears to be 0.5 ha, with little advantage gained by the size being larger than 1.5 ha (Fig. 17).

Our observations suggest that bird predation is concentrated on the periphery of the colonies. Because of the relation between surface area and volume, relative exposure to predation will therefore decrease in colonies of larger size. Intense competition for nonperipheral positions is expected and has probably been the cause of the dense butterfly packing (*18*). The selective basis for the upper size limit of these overwintering colonies is difficult to explain, as is the general case in all social animals (*19*). The aggregations are not food-limited during the overwintering period, because virtually no feeding occurs at this time (*4*).

These colonies occur in discrete groups, and it might be argued that the upper size limit evolved in response to local catastrophes such as volcanism, fire, or severe weather. However, because of extensive outbreeding during their annual cycle (*2*), the genetic requirements of group selection (*20*) are not met by the monarch butterfly. Thus, a causal basis for the upper size limit of these remarkable assemblages remains to be discovered.

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5. All sites discovered occur in Mexico's transvolcanic belt (*4*) in an area spanning 50 minutes of latitude and 25 minutes of longitude. We use Greek letters to designate sites on separate mountain ranges and numerical subscripts to designate sites in the same mountain range. It is very probable that many other Mexican overwintering colonies exist.
6. Ground samples were taken from 0.25-m² plots (random selection) along several transects through the colonies.
7. Bird identification follows that of R. T. Peterson and E. L. Chalif [*A Field Guide to Mexican Birds* (Houghton Mifflin, Boston, 1973)]. Chalif verified the assigned identifications of birds shown in Figs. 14 and 16. We also observed one attack by a hepatic tanager (*Piranga flava* Vieillot) on a monarch at site Gamma, and P. R. Spitzer (manuscript in preparation) saw four Steller's jays (*Cyanocitta stelleri* spp.?) feeding on them at $Alpha_2$ during February 1978. Spitzer also recorded 217 attacks by black-headed grosbeaks and 172 attacks by the same two species of orioles during 9 days of observation at $Alpha_2$ in February and March 1978. Monarch remains were also reported in the gut of a brown-crested flycatcher (*Myiarchus tyrannulus* Müller) at a Mexican overwintering site, along with other indications of bird predation (*8*).
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13. A neotropical tanager (*Pipraeidea m. melanonota* Viellot) appears also to have developed a feeding method to avoid noxious chemicals in the cuticle of ithomiinae butterflies: it squeezes the abdomen and eats the contents without ingesting the exoskeleton [K. S. Brown, Jr., and J. V. Neto, *Biotropica* **8**, 136 (1976)].
14. We consider the suggestion by M. Rothschild and D. N. Kellet [*J. Entomol. Ser. A* **46**, 103 (1972)] that certain bird species have evolved insensitivity to cardenolides at the level they occur in the Mexican colonies (maximum, 448 ug per butterfly; $N = 101$) possible but unlikely for the grosbeaks and orioles. The behavior of these birds is consistent with the less complex explanation of learned taste rejection of the toxic individuals or parts thereof (or both).
15. Disturbance of the accumulated dead butterflies throughout the overwintering season was negligible at all sites. About 20 cattle were observed eating substantial numbers of butterflies at $Alpha_1$ and $Alpha_2$, as reported previously (*4*, *8*). However, they eat only live butterflies and therefore have virtually no effect on our ground sampling procedures. Some small mammal predation occurs: seven scats containing monarch parts were found at three sites, probably from the hog-nosed skunk *Conepatus mesoleucus nelsoni* Goldman [O. J. Murie, *A Field Guide to the Animal Tracks* (Houghton Mifflin, Boston, ed. 2, 1975)], one skull of which was found near $Alpha_2$. No mammals were seen feeding on monarchs during the day, and counts of maimed butterflies falling into sheets suspended in the colonies support our data indicating that the majority of predation is due to birds. An experiment was done with the sheet method at four of five sites for a total of 200 hours, and showed a mean of 1.43 predated butterflies per square meter per day, a value close to that in Table 1, row 9. Because the overwintering occurs during the cold, dry season, decomposition of the butterflies (*4*)

proceeds slowly, and in no instance did the butterflies collected in these samples disintegrate on handling.
16. The areas of each colony were computed by azimuths and boundary distances with the use of a double meridian distance program from Hewlett-Packard's HP-25 Applications Program (1975).
17. On the basis of repeated observations at all sites, discussions with local residents, and the fact that site Beta was fully formed on 26 November 1977, we estimate 1 December as the date of colony stabilization. Shifting occurs prior to this and some consolidation occurs afterward, but the latter appears minor. Moreover, site fidelity, as in California (2), appears great: the 1977–1978 center of Alpha$_1$ differed by less than 50 m from where it was in 1976–1977.
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Prolactin Receptors in *Rana catesbeiana* During Development and Metamorphosis

Abstract. *Specific binding of ovine prolactin was found in microsomal preparations of tail, gill, and kidney of the bullfrog* Rana catesbeiana. *Binding by larval and adult liver and by kidney before larval stage XVII was low or nondetectable. Renal binding increased during metamorphic climax and in response to treatment with thyroid hormone. The emergence of renal binding of prolactin may signify a shift in the hormone's participation in the control of hydromineral homeostasis from the gill, which is resorbed, to the kidney. A renal action of prolactin during climax may facilitate metamorphosis.*

In the vertebrates, two of the basic functions of prolactin are control of growth and development and the regulation of water and electrolyte balance (*1*). Among the Amphibia, the hormone participates in the control of growth and retention of larval structures (*2*). In this regard, prolactin opposes the metamorphosis-inducing actions of thyroid hormones in larval and neotenic amphibians. In euryhaline teleosts prolactin acts at several sites to maintain hydromineral integrity; it seems to be particularly important in preventing tissue hydration in freshwater habitats (*3*). If one considers the similarities in osmotic stress experienced by freshwater teleosts and aquatic amphibians (especially larval forms), it is not surprising that there is some evidence for a role of prolactin in salt and water homeostasis in amphibians (*4*). The transformation of the bullfrog (*Rana catesbeiana*) tadpole from an aquatic to an amphibious creature must occur in a manner that is compatible with the changing demands on its osmoregulatory capabilities. In view of the involvement of prolactin in growth, development, and osmoregulation, one might expect the hormone to play an important role in the process of metamorphosis as well as in the regulation of growth of larval amphibians. Indeed, some investigators have speculated that developmental patterns may result, in part, from prolactin-controlled shifts in hydromineral homeostasis (*1*, *5*). However, the extent of this control by prolactin has not been fully ascertained (*4*, *6*). Furthermore, the amphibian organs whose activity might be influenced by prolactin have not been identified.

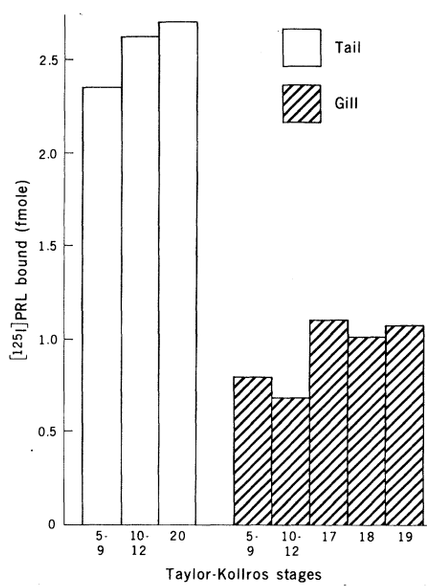

Fig. 1. Saturable binding of ^{125}I-labeled ovine prolactin (*PRL*) by tail and gill microsomal fractions. Values are femtomoles of hormone bound by 0.5 mg of membrane protein in the presence of 90 fmole of label. Arabic numbers are used instead of the more conventional Roman numerals for the different stages.

Studies of the binding of labeled prolactin to presumptive receptors in membrane fractions of mammalian (*7*) and avian (*8*) tissues indicate that the hormone shows a high degree of specific binding to established and suspected target organs. The degree of binding generally varies directly with the functional status of these organs. Hence, analysis of the occurrence of prolactin receptors in amphibian tissues should provide information on probable target organs of the hormone. However, the only information on this subject is a brief report that membranes from adult bullfrog kidney show a high degree of specific binding of ^{125}I-labeled ovine prolactin (*9*). We have studied target organ responsiveness to prolactin during amphibian development and metamorphosis. The potential sensitivity of organs in larval and adult bullfrogs to prolactin was evaluated by using the relative amount of saturable binding of ^{125}I-labeled ovine prolactin to microsomal fractions as an index of organ sensitivity. The structures examined were the tail and gill, both of which occur only in the larva, and the liver and kidney, which persist in the adult. The gill and kidney are potential sites for hydromineral-regulatory actions of prolactin, and the tail is a known target organ of the hormone.

Tadpoles of *R. catesbeiana* (11 to 20 g) were kept in tap water at 18°C with a constant food supply. After 1 week, they were staged according to Taylor and Kollros (*10*) and killed, the four structures being removed and placed in Dry Ice. The organs of two groups of 30 tadpoles each (stages V to IX and X to XII) were pooled by organ type and stage range. The organs of 15 to 20 tadpoles were pooled by type for each stage between XVII and XX and for stage XXII. The kidneys and livers of 12 adult bullfrogs were pooled by organ type. Membrane fractions of the kidney pools were prepared twice for stages XX and XXII and three times for stages V to IX and adult.

Microsomal fractions of each tissue pool homogenate were prepared by differential centrifugation according to the method of Shiu *et al.* (*11*). The initial low-speed (600*g*) centrifugation was omitted in order to minimize tissue loss. The 100,000*g* pellet was resuspended in 25 m*M* tris and 15 m*M* $CaCl_2$ buffer, *p*H 7.6. Membrane protein was estimated according to the Hartree modification of the Lowry method (*12*).

Ovine prolactin (*13*) was labeled with ^{125}I by a lactoperoxidase procedure (*14*). Labeled hormone was separated from labeled enzyme and free ^{125}I by column

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