Micropipettes with IDs as small as 30 nm have been produced for near-field scanning optical microscopes (*18*), and aluminosilicate pipettes with IDs of less than 10 nm have been made (*19*). The higher resistances of these smaller IDs should not be a problem; STMs have been operated with resistances thousands of times greater than our present values of 10 to 100 MΩ (*20*). The most serious limitation we have faced is that the smaller micropipette tips are extremely fragile and often break during a scan. Shorter taper pipettes may help with this problem and allow resolutions of 10 nm to be achieved.

The most promising application for the SICM is not, however, just imaging the topography of surfaces at submicrometer resolution. The SICM can image not only the topography, but also the local ion currents coming out through pores in a surface (Fig. 4). Comparison of topographic and ion current images can give a more detailed picture of the type of surface features that correlate with ion channels. In this model system, the comparison is simple: ion currents come through the holes as we would expect. Biological samples are more subtle, of course, as not every hole is an ion channel.

For images of the local ion currents, the micropipette was digitally scanned over the surface at a preselected height without movement in the z direction while the current flowing into the pipette at each point was measured (*21*). It was also possible to hold the micropipette over various locations on the imaged surface and measure local electrical properties. Thermal drift was small enough (~0.004 μm/min) that we could look, for example, at the time dependence of the ion currents above a pore, which was again simple for this model system (the current was constant), but which would be more subtle for biological samples.

The SICM offers both high-resolution topographic and ion-current images of nonconductors. Much of the necessary apparatus—micropipettes, microelectrodes, and current amplifiers—are already used routinely by electrophysiologists (*19*). Most of the positioning and feedback mechanism is the same as for the STM and is available commercially (*22*). Because the SICM operates in a saline solution or other ionic solutions, the microscope is well suited for biological applications. It complements the vibrating probe system (*23*) that can measure larger scale extracellular currents. An exciting extension of this work would be to use multiple-barrel micropipettes (*10*) with ion-specific electrodes (*19*). The total current into all of the barrels (or the current into one barrel with a nonspecific electrode) could be used for feedback, while the microscope could simultaneously measure and image the flow of different ions. We anticipate that this technique can be used in the future by electrophysiologists to combine spatially resolved ion-flow measurements and topological imaging of biological membranes.

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A Diet-Induced Developmental Polymorphism in a Caterpillar

ERICK GREENE

Caterpillars of the spring brood of *Nemoria arizonaria* develop into mimics of the oak catkins upon which they feed. Caterpillars from the summer brood emerge after the catkins have fallen and they develop instead into mimics of oak twigs. This developmental polymorphism may be triggered by the concentration of defensive secondary compounds in the larval diet: all caterpillars raised on catkins, which are low in tannin, developed into catkin morphs; those raised on leaves, which are high in tannin, developed into twig morphs; most raised on artificial diets of catkins with elevated tannin concentrations developed into twig morphs.

MANY ORGANISMS OCCUR IN TWO or more distinct forms. Developmental polymorphisms (or polyphenisms) occur when phenotypic variation is produced by differences in environmental conditions rather than by differences in genetic constitution (*1, 2*). Such developmental polymorphisms are conspicuous among arthropods with life spans that are short relative to the scale of environmental variation: examples are some color forms of caterpillars, pupae, and butterflies (*2*), winged and nonwinged morphs of water striders (*3*) and planthoppers (*4*), sexual and asexual forms of aphids (*5*), and caste sys-

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tems among social hymenopterans (*6*). Developmental polymorphisms offer the opportunity to study the complex interplay between proximate environmental switching cues, receptors that respond to these cues, and the regulation of gene expression during development. The environmental cues that trigger different developmental trajectories are in general poorly understood, but temperature, photoperiod, humidity, tactile cues, pheromones, and social conditions have been implicated for various groups (*2, 7*). I report an undescribed developmental polymorphism in which larval diet determines the morphology and behavior of a caterpillar. This developmental polymorphism is triggered by the dietary concentration of defensive compounds in the host plant.

The geometrid moth *Nemoria arizonaria* (Grote) occurs in Arizona, New Mexico, Texas, and northern Mexico (*8*). It is bivoltine, with a first cohort of adults flying in late winter or early spring and a second cohort flying in summer (*9*). I found the larvae, which have not been described, on several oak species (*Quercus arizonica, Q. emoryi, Q. undulata*, and *Q. grisea*) in southeast Arizona. Although the spring and summer broods of caterpillars look the same at hatching, they develop differently. Caterpillars of the spring brood feed on oak catkins (staminate flowers) and develop into remarkable mimics of the catkins: the integument is a rich yellow color, and densely rugose in texture with many papillae; large dorsolateral processes project from the sides of the thoracic and abdominal segments; two rows of reddish-brown, stamen-like dots occur along the dorsal midline. These morphological characteristics render the catkin morphs virtually indistinguishable from the oak catkins (Fig. 1A). Caterpillars from the summer brood hatch long after the catkins have fallen from the oak trees, and they develop instead into mimics of first year oak twigs (Fig. 1B): the integument is greenish-grey and less rugose than the catkin morph; the dorsolateral processes are not as pronounced as in the catkin morphs.

The two morphs also differ in the allometry of head and jaw morphology, and in their hiding behavior. The catkin morphs have small jaws suitable for eating the soft pollen grains from the catkins. The twig morphs have relatively large mouthparts and head capsules to accommodate the massive jaw musculature needed to eat the leathery oak leaves (*10*). The two morphs also actively seek out the substrates on which they are well hidden. The catkin morphs remain still when placed on catkins, but move onto catkins if they are placed on leaves or twigs. Conversely, the twig morphs remain still when placed on twigs, but move from catkins and leaves (*11*).

What environmental cues trigger these developmental responses? The most conspicuous and predictable differences experienced by the two broods are diet, temperature, and photoperiod. To determine whether these are used as developmental triggers, caterpillars were raised in a three-factor experiment, with two levels of temperature, photoperiod, and diet (*12*). Gravid female moths were captured at black lights near Portal, Arizona, in April and July 1988. They were kept in vials until they laid their eggs. Catkins and leaves of the host plant Arizona oak (*Q. arizonica*) were also collected near Portal and immediately frozen. The moth eggs and host plant material were transported to the University of California, Davis (*13*), where all experiments were performed. As caterpillars hatched from a brood, they were sequentially assigned to one of the eight experimental treatments. Each female moth produced more than eight eggs, so that all eight treatment groups were balanced with respect to genetic background. Caterpillars were raised in individual 5.5-ounce plastic insect rearing cups in environmental chambers. The treatments were randomized within chambers. Food was provided freely and changed daily. At 15 days of age caterpillars were categorized by a double-blind procedure (*14*).

Only diet influenced the caterpillars' developmental differentiation to a statistically significant degree. Regardless of photoperiod or temperature, all that were fed catkins developed into the catkin morph, whereas all that were fed leaves developed into the twig morph (Table 1) (*15*). The chemical composition of catkins and leaves differ in several important ways. Oak leaves are high in defensive secondary metabolic compounds (polyphenols, especially tannins), high in fiber, and low in unbound protein; catkins are low in tannin, low in fiber, and high in proteinaceous pollen (*16*). Experi-

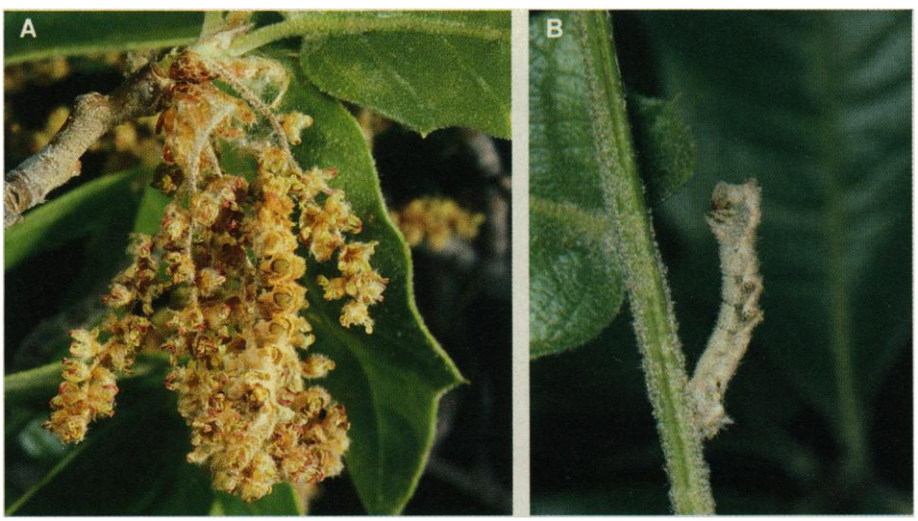

Fig. 1. Morphs of the catepillar *Nemoria arizonaria*. (**A**) A catkin morph in its normal hiding position; (**B**) a twig morph in its normal hiding position. These two caterpillars are full sibs that were raised on different diets.

Table 1. The percentage of catkin morphs produced under different conditions (zero percentage indicates that all caterpillars developed into the twig morph). Catkin and leaf diets induced the development of the catkin and twig morphs, respectively (the hypothesis that morphology was independent of diet was rejected; G [diet] = 155.3, $P < 0.0001$). Temperature and photoperiod conditions showed no statistically significant influence (G [temp] = 0.39; G [light] = 0.21).

Temperature	Photoperiod (hours)	Diet	
		Catkins	Leaves
15°C	12.5 L	100 (12)*	0 (9)
	14 L	100 (11)	0 (10)
25°C	12.5 L	100 (8)	0 (11)
	14 L	100 (27)	0 (24)

*Numbers in parentheses are sample sizes.

Table 2. The effect of four artificial diets (*17*) on larval development for caterpillars raised at 25°C on a 14-hour light cycle. The frequencies of catkin morphs produced from the artificial catkin diet differ significantly from the remaining three diets containing tannin (the diets containing tannin constitute a homogeneous subset, G tests, $P > 0.1$; all other combinations are heterogeneous, $P < 0.05$).

Artificial diet	Catkin morphs (%)
Catkins	94 (18)*
Catkins + leaves	12 (17)
Catkins + tannins	6 (16)
Leaves	6 (18)

*Numbers in parentheses are sample sizes.

ments with artificial diets (*17*) demonstrated that high dietary levels of polyphenols can induce the development of the twig morph (Table 2): most caterpillars raised on the catkin and leaf diet (intermediate tannin levels) and on the catkin and tannin diet developed into the twig morphs.

What has led to the evolution of this striking developmental polymorphism? The nutritionally superior catkin diet allows the catkin morphs to pupate more quickly than the twig morphs (Fig. 2A), attain a larger size at pupation (Fig. 2B), and survive better to pupation (*18*). The female moths produced from catkin morphs are also more fecund than those produced from the twig morphs (Fig. 2C). The fast pupation times associated with the catkin diet may also reduce the substantial risk of parasitism or predation by birds (*19*), and many confer a mating advantage, especially to early emerging male moths (*20*). Hence, although catkins are an ephemeral food resource, the marked fitness advantages associated with a catkin diet favor catkin-eating over folivory during the spring. The twig morphs are maintained in the population because the positive population genetic consequences of a second brood far outweighs their lower fecundity. A catkin morph that produced twig morph offspring will leave many more offspring (intrinsic rate of natural increase, ~9.4) than a catkin morph that waits until the following spring to produce only catkin morphs (intrinsic rate of natural increase, ~4.8). Hence, although the catkin morphs are more fecund than twig morphs, catkin morphs that did not produce twig morphs would be at an enormous selective disadvantage. The high rates of predation by visually searching predators, such as birds (*19*), have likely exerted strong selection for the high degree of crypsis.

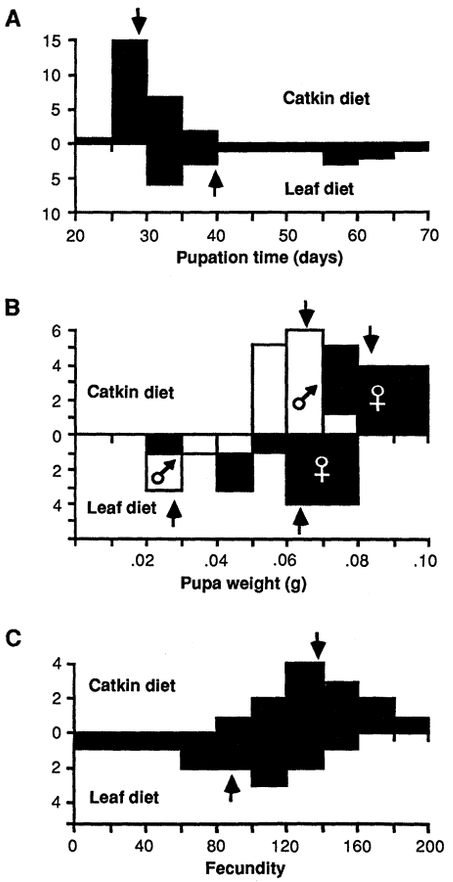

Fig. 2. Components of fitness are influenced by larval diet. These comparisons are for caterpillars raised at 25°C and 14 hours of light. The upper half of each graph shows the frequency distributions for catkin morphs; the lower half shows that for the twig morphs. The arrows indicate the medians of the distributions. (**A**) Catkin morphs pupated more quickly than the twig morphs (Mann-Whitney $U = 298$, $P < 0.0001$). There was no significant difference in pupation times between sexes within a diet treatment. (**B**) Catkin morphs pupated at larger sizes than the twig morphs (for females, Mann-Whitney $U = 101$, $P < 0.001$; for males, $U = 36$, $P < 0.001$). The sexes are shown separately since within a diet treatment females tend to attain larger pupal weights than males. (**C**) Female moths raised on catkin diets produced more offspring than those raised on twig diets (Mann-Whitney $U = 90$, $P < 0.0005$). Fecundity measures the total number of eggs produced by a female (eggs laid while alive plus eggs dissected from ovaries).

It is still not known which particular tannins induce the development of the twig morph. Tannins are a complex group of phenolic polymers, divided into two main families: condensed tannins (polymers of flavan-3-ols) and hydrolyzable tannins (polymers of gallic acid or ellagic acid). The tannin extract (*17*) used in the artificial diet is a complex mixture of polyphenolic compounds, although it is dominated by condensed tannins. It is also unclear how the dietary tannin levels effect the appropriate developmental response. A possible mechanism is that receptors respond to tannin levels, that these influence levels of circulating hormones, and that hormone levels in turn mediate gene regulation during development. Hormones, notably juvenile hormones and ecdysone, are particularly important in the mediation of development polymorphisms in other insects (*7, 21*).

Similar diet-induced developmental polymorphisms may be more widespread than we now appreciate. Many herbivorous arthropods are multivoltine, feed on several species of plants, or encounter different host plants with different biochemical properties in various parts of their geographical ranges (*2, 22*). Dietary cues may be generally important in the developmental induction of the appropriate morphological, physiological, and behavioral syndromes and may also be important in the evolution of host specificity and host races (*22*).

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10. The diameter of caterpillars' head capsules were measured to the nearest 0.1 mm using an ocular micrometer. The slopes of the regression lines relating head capsule width (HC in millimeters) to body length (BL in millimeters) were significantly different for the two morphs ($t = 16.12$, 145 df, $P < 0.001$). The regression equations (± standard errors of the coefficients) are: catkin morph, HC = 0.182 (± 0.062) + 0.0615 (± 0.002) BL; twig morph, HC = 0.071 (± 0.052) + 0.1018 (± 0.004) BL. Differences in head and jaw allometry have been related to the toughness of the diet in other foliage-chewing insects [E. Bernays, *Science* **231**, 495 (1986)].
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12. The temperatures (15°C and 25°C) and photoperiods (12.5 and 14 hours of light) are close to the mean April and July conditions at the Southwestern Research Station, Chiricahua Mountains, AZ.
13. USDA Interstate shipment permit 57-05-88.
14. Volunteers unfamilar with the experiments were presented the caterpillars in petri dishes and asked to categorize each caterpillar as either yellowish and bumpy (the catkin morph) or as greenish-grey and smooth (the twig morph). Although there is variation in skin morphology among caterpillars within a group, the volunteers had no difficulty assigning caterpillars to the two categories, and the categorizations were completely consistent among different observers.
15. In 1986 caterpillars were raised on catkins or leaves, but temperature and photoperiod were not controlled. From ten sibships of the spring brood and six sibships of the summer brood, all raised on catkins (spring brood, $n = 62$; summer brood, $n = 23$) developed into the catkin morph; all raised on leaves (spring brood, $n = 59$; summer brood, $n = 23$) developed into the twig morph. Morphology was not independent of diet (G [spring = 167.8, $P < 0.001$; G [summer] = 63.7, $P < 0.001$).
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18. For caterpillars raised at 25°C and 14 hours of light, 3 out of 28 (10.7%) raised on catkin died before pupation, whereas 10 out of 29 (34.5%) raised on leaf diet died before pupation. These proportions are thus not the same (z approximation, $z = 2.25$, $P < 0.02$).
19. Bird exclosure experiments on oak trees in the Chiricahua Mountains showed that predation by birds can reduce the biomass of foliage-dwelling arthropods by about 50% in some years [E. Greene, thesis, Princeton University (1989)]. Approximately 60% of wild-caught caterpillars from *Q. arizonica* and *Q. emoryi* were parasitized, mainly by ichneumonid and braconid wasps and tachinid flies.
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Molecular Genetic Relationships of the Extinct Dusky Seaside Sparrow

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Mitochondrial DNA from the extinct dusky seaside sparrow (*Ammodramus maritimus nigrescens*) was compared in terms of nucleotide sequence divergence to mitochondrial DNAs from extant populations of seaside sparrows. Analyses of restriction sites revealed a close phylogenetic affinity of *A. m. nigrescens* to other sparrow populations along the Atlantic coast of the United States but considerable genetic distance from Gulf coast birds. Concerns and applied management strategies for the seaside sparrow have been based on a morphological taxonomy that does not adequately reflect evolutionary relationships within the complex.

THE DUSKY SEASIDE SPARROW, A melanistic form (*1*) of *Ammodramus* sparrow discovered in 1872 (*2*), had a native range confined to Brevard County, Florida (*3*). During the 1960s, a population formerly numbering in the thousands was in severe decline, largely because of artificial flooding of marsh-grass habitat for mosquito control and conversion of land to pasturage (*4*). In 1966, the dusky was listed as "endangered" by the U.S. Fish and Wildlife Service (*5*). By 1980, only six birds (all males) could be found (*6*). Five of these were brought into captivity in a last-ditch effort to preserve genes of the subspecies. In a program of captive breeding with Scott's seaside sparrow (*A. m. peninsulae*) (*7*), first-generation female hybrids between dusky males and Scott's females were crossed to dusky males, yielding backcross progeny with an expected preponderance of dusky nuclear genes (Fig. 1). Several hybrids ranging from 50 to 87.5% dusky have been produced (*8*); they constitute the core population for a contemplated reintroduction of dusky-like birds into the wild (*7*).

The last pure dusky seaside sparrow died on 16 June 1987. Here we compare mitochondrial DNA (mtDNA) isolated from its tissues with that of other geographic and taxonomic populations in the seaside sparrow complex. The intent is to critically evaluate the evolutionary genetic history of *A. m. nigrescens*, the taxonomic entity toward which government and private management efforts have been directed.

We chose to analyze mtDNA for several reasons. First, mtDNA in vertebrates evolves very rapidly at the nucleotide sequence level (*9*), and hence provides high resolution for distinguishing recently separated populations such as those within a species (*10*). The problem of genetic distinction is especially acute for lower taxa of birds, where genetic differences are typically small (*11*). Second, because mitochondria are maternally inherited in vertebrates (*12*), mtDNA can be used to identify the matriarchal ancestry of individual animals, without the complications of allelic segregation and

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Table 1. Clonal descriptions and subspecies distributions of mtDNA genotypes observed in seaside sparrows. Letters in the descriptions, from left to right, refer to multifragment mtDNA profiles produced by digestion with Ava I, Ava II, Bam HI, Bcl I, Bgl I, Bgl II, Cla I, Eco RI, Hin cII, Hin dIII, Msp I, Nde I, Pst I, Pvu II, Spe I, Sst II, Stu I, and Xba I. Adjacent letters in the alphabet differ by a single restriction site, nonadjacent letters by at least two restriction site changes. Numbers in parentheses are references.

Clone	mtDNA description	Traditional subspecies designation	No. of birds
1	C C C C C C C C C C C C C C C C C C C	*maritima, macgillivraii* (*14*), *nigrescens*	17
2	C C C C C C C C C C C C C C C B C C C	*macgillivraii* (*14*)	1
3	C C C C C C C C B C C C C C C C C C C	*maritima*	1
4	C C C C C C C D C C C C C C C C C C C	*macgillivraii*	1
5	C B C C C C C C C C C C C C C C C C C	*maritima*	1
6	C G D C C C C D C C D C C G C C C C C	*fisheri, juncicola, peninsulae*	14
7	C G D C C C C D C C E C C G C C C C C	*fisheri*	1
8	C G D C C C C D C C F C C G C C C C C	*peninsulae*	1
9	C H D C C C C D C C D C C G C C C C C	*peninsulae*	1
10	C G D C C C C D C B D C C G C C C C C	*fisheri*	1
11	C G D C C C B D C C D C C G C C C C C	*juncicola*	1
		Total	40