



Behavioral Variation in Natural Populations. II. The Inheritance of a Feeding Response in Crosses Between Geographic Races of the Garter Snake, *Thamnophis elegans*

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BEHAVIORAL VARIATION IN NATURAL POPULATIONS. II. THE
INHERITANCE OF A FEEDING RESPONSE IN CROSSES BETWEEN
GEOGRAPHIC RACES OF THE GARTER SNAKE,
THAMNOPHIS ELEGANS

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The study of geographic variation occupies a central position in evolutionary biology. This is because geographic variation is ordinarily the smallest amount of evolution that can be detected in nature and because evolutionary theory, in its strongest form, applies only to small evolutionary change. Thus it is particularly surprising that there are few behavioral studies of geographic variation. These studies have demonstrated geographic variation in social displays (Boch, 1957; Sawada, 1963; Littlejohn, 1965; Ferguson, 1971), but other kinds of behavior have barely been explored (Arnold, 1980).

In contrast, decades ago morphologists implemented a vigorous research plan to determine the genetical basis of geographic variation (e.g., Sumner, 1923; Clausen and Hiesey, 1958). The first step in this plan is to rear organisms from different natural populations in a uniform environment, or, better still, in a series of environments. Geographic differences under these conditions can be attributed to genetic differences. The second step is to unravel the genetic basis of phenotypic variation within and among populations. Ordinarily this requires crossing populations for at least two generations. The contributions of dominance, additive effects of genes, and the maternal environment can be estimated from such data. Furthermore, under rather strong assumptions, the minimum number of loci responsible for geographic differences can be estimated. These loci represent both regulatory and structural genes (see Wilkens, 1970; and Templeton, 1977, for particularly elegant morphological examples).

Apparently no behavioral trait has been studied with this plan, although inbred strains have been investigated according to the second step (e.g., DeFries and Hegmann, 1970). The basic hurdle has been to identify significant, demonstrably adaptive, behavioral differences in natural populations. The following analysis of slug-eating in a garter snake jumps this hurdle and proceeds with the morphologist's paradigm for genetic analysis. Despite its novel and unseemly aspects, this analysis apparently represents the most thorough documentation of geographic variation in behavior in the vertebrate literature.

The slug-eating habit in the garter snake *Thamnophis elegans* is an adaptation that evolved in mollusc-rich environments in coastal California (Arnold, 1977, 1980). In these environments slugs constitute more than 90% of the diet. Outside the range of slugs, in inland California, *T. elegans* feeds predominantly on frogs and fish. The predilection of coastal snakes for slugs is not a learned response. Most newborn coastal snakes attack slugs on first exposure. This congenital tendency is stable, at least for the first year of life. In contrast, most newborn inland snakes refuse slugs on first exposure and will starve to death if not given an alternative food. In addition to this pronounced geographic difference, there is behavioral variation within populations. Both inland and coastal populations are polymorphic for slug-eating behavior: the geographic difference is one of degree, not a saltation. In this paper, laboratory crosses between these populations are re-

TABLE 1. *Slug feeding scores of naive, newborn Thamnophis elegans representing three parental populations and their F₁ hybrids. All three parental populations are from California. The n. coastal (Humboldt County) and s. coastal (Santa Cruz County) populations are sympatric with slugs, which are their main prey. The inland population (Lassen County) is allopatric with slugs.*

Locality	No. of litters	No. of progeny	Slug feeding score				Chi-square
			<5		≥5		
			Numbers	Percent	Numbers	Percent	
Parental populations							
Inland	68	681	567	114	83	17	} 307.10** } 321.76**
N. coast	56	463	149	314	32	68	
S. coast	15	195	30	165	15	85	
F₁ (dam × sire)							
Inland × n. coast	10	50	39	11	78	22	} 1.50 ns
N. coast × inland	15	65	44	21	68	32	
	25	115	83	32	72	28	
Inland × s. coast	8	21	17	4	81	19	} 1.28 ns
S. coast × inland	15	56	38	18	68	32	
	23	77	55	22	71	29	

ported. Since only F₁ progeny were produced, the main issues are dominance and tests for maternal effects.

Three populations, two coastal (sympatric with slugs) and one inland population (allopatric with slugs), were used in these crosses. Slug-feeding scores of newborn snakes from each of these populations are reported by Arnold (1977, 1980). Sample sizes for each population are extended here. These populations are 280 to 480 km apart.

Chemoreceptive response to slug odor also shows geographic variation (Arnold, 1980). As predicted, it coincides with differences in slug-eating response, even within populations. Distributions of chemoreception scores are continuously distributed. These distributions straddle attack thresholds such that snakes in the upper tail of each distribution show a marked tendency to attack slugs. This situation provides an excellent opportunity to study the genetics of both an underlying, continuous variable (chemoreception) and the overlying, threshold character (feeding response). Usually only the threshold character (an incidence score) is available for study and a normally distributed, underlying variable is assumed to be present (Falconer, 1960). The feeding

score distributions, reported here, are used to predict statistics that were actually observed on the underlying scale. This provides a test for the validity of standard techniques for analyzing threshold characters.

MATERIALS AND METHODS

One hundred and thirty-nine gravid females were collected in July, in five consecutive years (1975–1979), from three study populations in northern California: an inland population (Eagle Lake, Lassen County), a north coastal population (two sites, 35 km apart in coastal Humboldt County; 124°05'19"W × 41°21'56"N and 124°07'30"W × 39°57'00"N) and a south coastal population (122°13'06"W × 37°02'25"N, Santa Cruz County). These females were shipped by air back to the laboratory and 1,339 progeny were born under uniform laboratory conditions (Arnold, 1977, 1980). These progeny yielded the data on parental populations shown in Table 1.

F₁ progeny (n = 192) were produced by crossing parental populations in the laboratory. Both laboratory bred and wild-caught adults were used for the crosses. They were maintained in a temperature controlled room (28 C) on a natural (Chi-

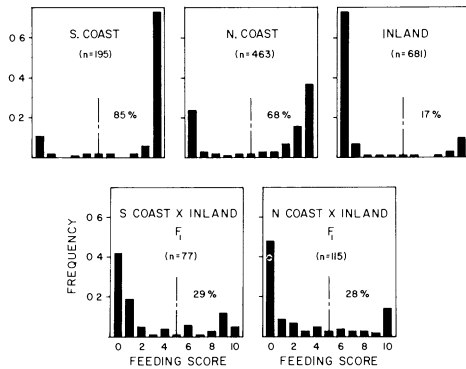


FIG. 1. Distributions of slug feeding scores in three parental populations of *T. elegans* (above) and their F_1 hybrids (below). Percentages indicate the proportions of naive, newborn snakes that were consistent slug-eaters (feeding score = 5–10).

ago) photoperiod and fed live fish or Zupreem Herp Diet (Riviana Foods) laced with fish odor two or three times a week. In the winter, prior to breeding, they were moved to a lower temperature (5–10 C) for several weeks in a room with subdued light (natural photoperiod) and high humidity. Such artificial hibernation is necessary to induce breeding. In order to ease the temperature change on transits into and out of hibernation, the snakes were moved into a third temperature controlled room (15 C, natural photoperiod) for one week in both transits. Upon return to normal maintenance temperature (28 C) after hibernation, females were paired with males in cages, and put back on a normal feeding schedule. These breeding cages were equipped with heating lamps (60–100 watts) to permit thermoregulation. Copulation occurred within a few weeks of emergence from hibernation and young were born in the summer months (June–August). The cross between the two coastal populations was not attempted, but reciprocal F_1 progeny were produced for the other two crosses.

Newborn snakes were tested for feeding response to slugs. For 10 consecutive days, beginning 14 or 16 days after birth, each snake was offered a small piece of slug (*Ariolimax californicus* or *A. columbianus*) (Arnold, 1977, 1980). The body walls of frozen slugs were cut into small

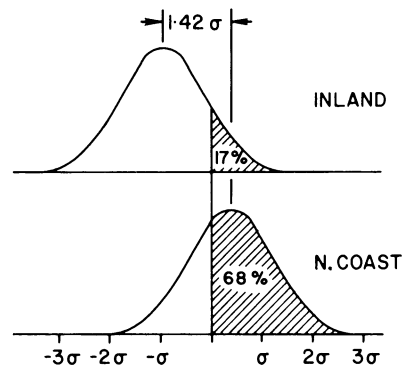


FIG. 2. Normal distributions with identical standard deviations (σ) fitted to the incidence data shown in Fig. 1. The means of the two distributions are 1.42σ apart.

pieces (2×4 mm). These pieces were so small that they did not satiate the snakes or frustrate ingestion. These slug presentations constituted the first exposure of these newborn snakes to food of any kind. On each day the ingestion or refusal by each snake was recorded, so that at the end of the presentation sequence a feeding score could be tabulated, representing the total number of feeding responses in the sequence (0–10). This constituted the feeding score of each snake. The execution of more than 15,000 feeding presentations was a considerable undertaking.

Some newborn snakes from the inland ($n = 59$) and the south coastal population ($n = 148$) were tested with a slightly different protocol (Arnold, 1977, Fig. 2). These snakes were tested at an age of 11 to 14 weeks and were fed anurans and fish prior to the 10 trial slug test. Since other experiments indicated that slug-feeding scores are stable in the face of such experience (Arnold, 1980), these snakes were included in the tallies shown in Table 1.

RESULTS

Distributions of feeding scores are strongly bimodal in parental populations and in their F_1 s (Fig. 1). Consequently it is convenient to recognize two feeding morphs in each population: a slug-refusing morph (feeding score 0–4) and a slug-eating morph (5–10).

A slug-eating morph predominates in

the two coastal populations, which are sympatric with slugs, while a slug-refusing morph predominates in the inland population, which is allopatric with slugs. The incidence of the slug-eating morph increases in the series inland, north coastal, south coastal and these differences are highly statistically significant (Table 1).

A comparison of reciprocal F_1 s indicates no maternal effect on inheritance of feeding score (Table 1). Thus F_1 produced by coastal dams show the same incidence of slug-eating as F_1 produced by inland dams.

The F_1 s show an intermediate incidence of slug-eating with a rather strong bias towards the inland population. Thus the north coastal F_1 is significantly different from both the inland (Chi-square = 8.07, $P < .01$) and the north coastal population (Chi-square = 61.32, $P < .01$). Likewise the south coastal F_1 is significantly different from both the inland population (Chi-square = 6.58, $P < .05$) and the south coastal population (Chi-square = 80.70, $P < .01$).

Despite this intermediacy, hybrid progenies resemble their inland parents more than their coastal parents. Wright (1968, 1978) suggests the following measure of departure from perfect intermediacy (due to simple or directional dominance): $\Delta F_1 / \Delta U =$ the difference between the F_1 mean and the mean of the low parental population divided by the difference between the means of two parental populations. This measure is 0.5 in the case of perfect intermediacy. Applying this measure to incidences of slug-eating, $\Delta F_1 / \Delta U = 0.22$ for the north coastal F_1 and 0.18 for the south coastal F_1 .

Incidence can also be treated as a threshold character (Falconer, 1960). Arnold (1980) showed that chemoreceptive response is a continuously distributed variable underlying feeding response to slugs. Chemoreceptive responses to slug odor were approximately normally distributed in the north coastal and inland populations with nearly identical variances and means 1.72 standard deviations (σ) apart. Normal distributions with iden-

tical variances can be fitted to the incidence data in Table 1 to provide an independent estimate of the distance between the means of the underlying distributions (Fig. 2). This gives a value of 1.42 σ for the deviation in means of the north coastal and inland populations: a value in reasonable agreement with the observed deviation of 1.72 σ . The corresponding expected deviation for the south coastal and inland means is 1.98 σ , but unfortunately there are no chemoreception data for the south coastal population which would permit a check for this expectation.

Since the threshold model is in reasonable agreement with direct observation of the presumptive underlying distributions, it will be used to provide additional measures of departure from intermediacy. Assuming identical variances on the underlying scale, $\Delta F_1 = 0.38$ for the north coastal F_1 , so that $\Delta F_1 / \Delta U = 0.26$. For the south coastal F_1 , $\Delta F_1 = 0.40$ and $\Delta F_1 / \Delta U = 0.20$. These values are close to those estimated directly from the incidence data.

DISCUSSION

An interpretation of the present results in terms of maternal effects would actually be more exciting than a genetic interpretation. Unfortunately, however, the behavioral differences do not appear to be due to a maternal influence. We can imagine that a particular flavor might be transmitted from the dam's diet, across embryonic membranes, and later influence the feeding responses of her progeny. This is not inconceivable since flavors in milk of maternal rats can later influence the diet preferences of pups (Galef, 1977). Furthermore, amino acids and nitrogenous wastes are probably transmitted between the pregnant female garter snake and her young (Clark and Sisken, 1956). Burghardt (1971), however, was unable to detect any differences in feeding responses of newborn garter snakes that could be related to the dam's diet during gestation. This suggests that although the coastal dams in the present experiment probably ate slugs in nature during their early preg-

nancy, and the inland dams did not, this maternal diet difference did not produce the geographic difference in newborn feeding behavior. If maternal failure to eat slugs during pregnancy produced slug-refusing progeny, then all F_1 progeny in this experiment should have been slug-refusers since their dams were fed only fish during pregnancy in the laboratory. Instead these F_1 hybrids differ from both inland and coastal progeny. In addition, the behavioral variation within families in all generations is easy to explain with segregating genes but it is troublesome for a theory based on maternal effects. Finally, the present results rule out some longer-term, more pervasive maternal effects, such as cytoplasmic inheritance, say by mitochondrial DNA. The reciprocal F_1 progeny show no behavioral differences.

The results strongly implicate dominance in the inheritance of slug feeding scores. Since it is not known whether this is a simple Mendelian or polygenic trait, I cannot say whether departure from intermediacy in F_1 is due to dominance at a single locus or dominance at many loci. The direction of dominance is very interesting however. One or more slug-refusing alleles are apparently dominant to slug-eating alleles.

Population data on three geographic sites enables us to narrow the field of explanations for variation within populations (Arnold, unpubl.). The intermediate incidence of slug-eating in the north coast population may reflect a balance between selection for slug predation and migration from inland sites, where the mode is slug-refusal. This is possible since the south coastal population, which is isolated from an inland migration, shows the highest incidence of slug-eating. Samples from additional sites could confirm the geographic pattern. But only additional field work could determine whether undetected environmental gradients, reflected for instance in diet, produce the pattern or whether it is best explained as a migration-selection balance.

SUMMARY

An analysis of a slug-eating response by naive, newborn garter snakes provides a particularly clear example of geographic variation in behavior with a genetic basis. Feeding responses were recorded for three parental populations and their F_1 progeny: behaviors were scored in a total of 1,531 snakes. Populations that are sympatric with slugs show the highest incidence of slug-eating snakes (68–85%), while in an allopatric population the incidence is only 17%. Thus the geographic variation in this behavior is undoubtedly maintained by selection. The behavior is congenital and stable. Crosses between populations indicate at least partial dominance for slug-refusal but the number of loci involved is unknown. Reciprocal F_1 progeny show the same incidence of slug-eating (19–32%) and give no indication of maternal effects.

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