

bility of a pluripotential spread of the virus in subacute spongiform virus encephalopathies. However, the available results in scrapie—namely, spread of the infection after intracerebral inoculation to visceral tissues (4, 5) and the reverse, spread of the infection from the periphery to the brain (5, 6)—can most easily be explained by viremia rather than by propagation of the virus along neural pathways, either centrifugally and centripetally from and to the brain. The demonstration of viremia in experimental Creutzfeldt-Jakob disease may also have implications for the disease afflicting man.

Although it is not known how the infection spreads in human spongiform virus encephalopathies, the virus of Creutzfeldt-Jakob disease has been found in the liver, kidney, lung, lymph nodes, and cerebral spinal fluid, and the virus of kuru has been found in lymph nodes, kidney, and spleen of humans (16). It is conceivable that the hematogenous spread of the infection is also implicated in man. The presence of virus in the blood in experimental Creutzfeldt-Jakob disease suggests that this may be true and that there may well be a danger of transmitting this disease via blood transfusions from humans harboring the agent during the incubation period, when the clinical disease is not readily apparent. Gajdusek mentioned that two humans harboring Creutzfeldt-Jakob disease were professional blood donors until shortly before the onset of their symptoms (3).

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 14. The animals with clinical signs were: No. 1 of the 1st week group, No. 1 of the 2nd week group, No. 3 of the 3rd week group, No. 2 of the 12th week group, No. 3 of the 15th week group, Nos. 1 and 2 of the 20th week group, No. 2 of the 24th week group, and Nos. 2 and 3 of the 25th week group (Table 1).
 15. Autopsies of these guinea pigs revealed: hemopericardium (No. 2 of the 2nd week group),

stomach torsion and dilatation of stomach (No. 1 of the 13th week group), liver abscesses (No. 1 of the 25th week group), and polycystic kidneys (No. 1 of the 26th week group).

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Search Image for Leaf Shape in a Butterfly

Abstract. *The butterfly Battus philenor forms search images for leaf shape when searching for its two larval host plants in southeast Texas. This behavior increases the rate of discovery of host plants and permits females to track changes in relative host plant suitability for larval growth. Apostatic selection resulting from search image formation is a likely explanation for divergence in leaf shape by the two host plants.*

The significance of a plant's leaf shape has been attributed to abiotic environmental factors (1). In contrast, Gilbert (2) noted that the species in some tropical plant families that support populations of coevolved herbivorous insects differ greatly in leaf shape. He suggested that apostatic selection (3, 4) exerted by those herbivores may have produced the observed leaf shape diversity. Central to Gilbert's argument is the assumption that searching insects are able to discriminate leaf shapes and that individuals searching for one leaf type are less likely to respond to another. One mechanism that can lead to such differential response is search image formation. I report here that (i) ovipositing females of the pipevine swallowtail butterfly, *Battus philenor*, search selectively for either broad- or narrow-leaved larval host plants; (ii) females can switch preference from one leaf shape to another on the basis of experience and hence form true search images; (iii) a search image for one leaf shape results in host plants with that leaf shape being discovered in greater proportion than their abundance in the habitat; and (iv) butterflies with strong search images discover larval food plants at higher rates than butterflies with weak search images. In addition, I suggest that *B. philenor* is the primary selective agent responsible for divergence in leaf shape by its two larval host plants in southeast Texas.

I conducted this study between 22 March and 22 May 1977 in the open longleaf pine uplands of the Big Thicket region of southeast Texas. Adults of *B. philenor* are common in the pine upland habitat at that time of year; females can be found searching among the herbaceous vegetation for the two larval food plants *Aristolochia reticulata* and *A. ser-*

pentaria (Aristolochiaceae) (5). The two host plants, perennial herbs reaching a maximum height of 40 to 50 cm, are closely related within the genus *Aristolochia* (6), yet differ in leaf shape. The more common species *A. reticulata* has the broad, ovate leaves characteristic of the genus (6), whereas all *A. serpentaria* plants in areas of sympatry with *A. reticulata* have long, narrow, parallel-sided leaves resembling grass blades.

Ovipositing *B. philenor* can be followed easily in the field, permitting observation of host plant search behavior under natural conditions (5). My preliminary observations suggested that females use leaf shape as a visual cue for locating host plants. As a female flies slowly above the herbaceous vegetation, she periodically approaches and lands on a plant and "tastes" it, presumably with tarsal chemoreceptors similar to those present in other insects (7). If the plant is not an *Aristolochia*, she immediately resumes search flight. If the plant is an *Aristolochia*, she either lays a small cluster of eggs or resumes search flight without ovipositing. Since the two *Aristolochia* species constitute less than 5 percent of the plants that females approach and "taste," it seems unlikely that the butterfly recognizes a plant as an *Aristolochia* by employing long-distance olfactory cues such as are used by some other insects (8).

To test the hypothesis that leaf shape is an important cue used in initiating approach to a plant, I observed ovipositing females in an approximately 80-acre (32 ha) area of open longleaf pine upland in the Kirby State Forest, 15 miles (24 km) north of Kountze in Hardin County, Texas. All herbaceous plants and shrubs growing in the area were classified as having either long, narrow leaves or

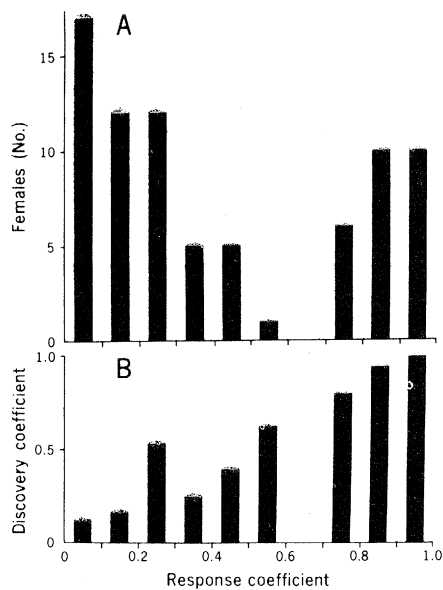


Fig. 1. Host plant discovery patterns with respect to leaf shape. (A) Frequency distribution of response coefficients (RC) for 78 female butterflies. (B) Females with search image for broad leaves (high RC) discovered primarily *A. reticulata* (broad leaves); those with search image for narrow leaves discovered primarily *A. serpentaria* (narrow leaves). Bars are means for RC intervals of 0.1. The Spearman rank correlation coefficient from original data was $r_s = .772$, $N = 71$, $P < .001$.

broad leaves (9). Individual females were followed for 30 minutes or until they flew out of the area. During the first 10 minutes, I counted the number of narrow- and broad-leaved plants of all types that a female approached and "tasted" (the mean number per female per 10 minutes was 65.6). From these counts I determined a response coefficient (RC), defined as the fraction of all broad-leaved plant species that a female landed on. A high RC indicates a preferential response to broad leaves, and a low RC indicates a preferential response to narrow leaves. For the entire 30-minute period I recorded the time of discovery of each *Aristolochia* plant and each oviposition.

If females do not respond differentially to leaf shape, they should approach broad-leaved plants in the proportion in which they occur in the habitat. The distribution of RC values would consequently be unimodal with a mean equal to the percentage cover formed by the broad-leaved species. The distribution I observed, however, was bimodal (Fig. 1A), indicating that individuals are highly selective in their responses to leaf shape. The two modes correspond to two classes of females: those searching for narrow-leaved host plants (RC between 0.00 and 0.60) and those searching for broad-leaved host plants (RC between 0.60 and 1.00). A bimodal distribu-

tion could have arisen if the habitat were divided into large patches of predominantly narrow-leaved or predominantly broad-leaved plants and if females remained within one patch for a large portion of the observation time. Any such heterogeneity was not apparent, however; if it existed it was only on a scale much smaller than the distance a female moved during the 10-minute counts (10).

Further observations suggest that the leaf shape preferences are learned behaviors and do not reflect a genetically fixed polymorphism of response to leaf shape. Females that lay eggs on only *Aristolochia* plants with leaves of the shape being searched for do not change search modes during the 30-minute observation periods. For six females I compared the RC during the first 10 minutes of observation with that during the final 10 minutes. Five of the six females showed no significant change in the fraction of approached plants that were broad-leaved. The one statistically significant change ($P = .03$) was from a weak preference for narrow leaves (RC = .39) to a stronger preference for narrow leaves (RC = .13). An overall test of significance demonstrated any changes to be nonsignificant, however ($\chi^2_{12} = 13.75$, $P > .10$) (11). Thus, females whose search image is continually reinforced show constancy of response to leaf shape. In contrast, two females that discovered and laid eggs on *Aristolochia* plants with leaf shape different from that being searched for behaved differently. One female, originally in the narrow leaf mode (RC = .32), "accidentally" discovered and oviposited on an *A. reticulata* (broad leaf) after 16 minutes of observation. Her RC during the last 10 minutes of observation was .69, a value characteristic of the broad leaf search mode. A second female shifted from searching for broad leaves (RC = .74) to searching for narrow leaves (RC = .28) after "accidentally" discovering and laying eggs on an *A. serpentaria*. Both of these shifts were highly significant ($G = 11.74$ and 13.04 , respectively, G -test, $P < .005$ in both cases). Females thus appear capable of modifying leaf shape preference on the basis of relatively little experience (12, 13), although it is unlikely that they always do so.

If leaf shape preference is to cause apostatic selection and divergence in leaf shape, a female searching for one leaf shape must discover host plants with that leaf shape in greater proportion, and host plants with different leaf shape in lesser proportion, than they occur in the habitat (3). This is true of *B. philenor*.

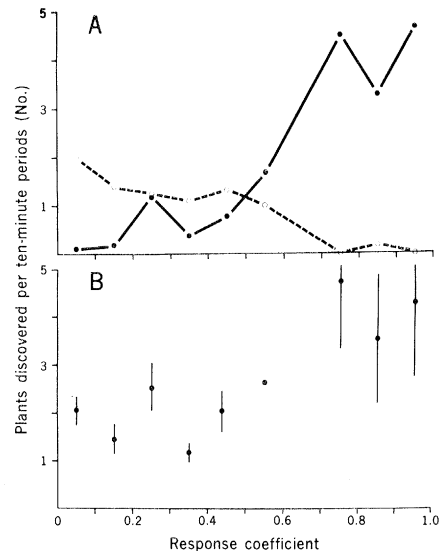


Fig. 2. Host plant discovery rates as a function of RC. (A) Discovery rates of each host plant species considered separately. The values are means for RC intervals of 0.1. Solid line is the rate at which *A. reticulata* (broad leaves) is discovered; the broken line is for *A. serpentaria* (narrow leaves). The Spearman $r_s = .780$ and $.678$, respectively, $N = 55$, and $P < .001$ for both species. (B) Overall discovery rate of both host plants considered together. The values are means for RC intervals of 0.1. The error bars represent 1 standard error. The Spearman $r_s = .415$, $N = 55$, $P < .01$.

The fraction of *Aristolochia* plants landed on that are *A. reticulata* (discovery coefficient) is highly correlated with the RC (Fig. 1B; Spearman $r_s = .772$, $N = 71$, $P < .001$). Females responding preferentially to narrow leaves during search discover mostly the narrow-leaved *A. serpentaria*; those responding preferentially to broad leaves discover mostly the broad-leaved *A. reticulata*. Furthermore, females with strong preference for a particular leaf shape (RC near 0 or 1) discover a greater proportion of host plants with that leaf shape than females with weak preferences do.

The ability of vertebrates to form search images is well documented (4, 13-16). By contrast, true search images have not been described for invertebrates although insects, at least, are capable of rudimentary shape discrimination (17). The behavior of *B. philenor* satisfies the three commonly accepted criteria for forming a search image. (i) Females perform an internal filtering of the visual stimuli that initiate the motor response of approaching and "tasting" a plant (13, 14). The bimodal distribution of RC values indicates that such a filtering process occurs. Since a female does not recognize a plant as an *Aristolochia* until she touches it, the filtering must be of some property of the object sought, in this case leaf shape. (ii) The differential

response to leaf shape is a learned behavior (18), as is indicated by the ability of females to shift between different search modes. (iii) The objects of the search image are discovered in greater proportion than their abundance in the habitat (13-15, 18) (Fig. 1B).

It is precisely this last property that can account for the divergence in leaf shape by the two species of *Aristolochia* in southeast Texas. During any brood, *Battus* may lay eggs on up to 50 percent of the plants of one of the host species. Larvae normally consume 40 to 45 percent of the yearly crop (19) and are thus a potentially strong selective agent acting on *Aristolochia*. The following scenario is a likely route that evolution may have followed. *Aristolochia reticulata* is 20 to 40 times more abundant than *A. serpentaria* in the open pine uplands. In addition, *A. serpentaria* begins producing leaves 1 to 2 weeks later in the spring than does *A. reticulata*, so that the relative abundance of the latter is even greater at the time when *B. philenor* breaks winter diapause and begins ovipositing. At this time of year all *A. reticulata* plants are suitable for oviposition and females become entrained to a broad-leaf search image. Through continual reinforcement the search images become stronger, and the probability of discovering a narrow-leaved plant correspondingly diminishes. An *A. serpentaria* plant that then produces long, narrow leaves will have a lower probability of being discovered than one that produces broad, ovate leaves. To the extent that the amount of defoliation is inversely correlated with plant reproductive success (20), natural selection will favor the replacement of broad leaves with narrow leaves.

The adaptive significance of a search image has been postulated to be an increased prey discovery rate (15, 18). This is true for each host plant of *B. philenor* considered separately (Fig. 2A). The number of plants discovered per 10-minute search is highly correlated with the RC (Spearman $r_s = .678$ and $.780$ for *A. serpentaria* and *A. reticulata*, respectively, $N = 55$, and $P < .005$ in both cases). The total number of plants of both species discovered per 10-minute search is also correlated with the RC (Fig. 2B, $r_s = .415$, $N = 55$, $P < .01$). The highest rate of discovery thus corresponds to a "perfect" search image for *A. reticulata* (that is, RC = 1.00). If natural selection favored individuals that maximized the total discovery rate of host plants, all females would adopt a search image for broad leaves. Many females search in the narrow-leaf mode,

however, indicating that selection does not maximize total discovery rate.

Search image formation in *B. philenor* probably enables females at any particular time to discover most rapidly plants of the species conferring greater juvenile survival. In southeast Texas there is a seasonal shift in the relative egg and larval survival on the two host plants. Survival is greater in the early spring on *A. reticulata* because these plants contain more edible tissue than *A. serpentaria* plants. Larvae, which must feed on several plants to complete development, are consequently larger when leaving their first plant if it is an *A. reticulata* and are more likely to discover another plant. By late spring most leaves of *A. reticulata* become tough and inedible. Because *A. serpentaria* leaves remain edible throughout the season, however, this plant confers high larval survival in the late spring. Associated with the change in value of the two host plants is a marked shift in the percentage of eggs laid on each species (19), although on any given day females having both types of search image can be found.

Because females do not lay eggs on plants having only tough leaves, the rate of oviposition on *A. reticulata* plants decreases over the spring as the abundance of plants with young leaves declines. If the rate of egg-laying on *A. reticulata* falls below that on *A. serpentaria*, then differential reinforcement could cause the search image to shift from being predominantly for broad leaves to being predominantly for narrow leaves (21). Such a mechanism probably ensures that females search most efficiently for the host species yielding greater juvenile survival.

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- Of the 31 species most commonly approached and "tasted," those categorized as narrow-leaved were: *Panicum* (three sp.), *Andropogon* sp. (Gramineae); *Yucca* sp. (Liliaceae); *Commelina erecta*, *Tradescantia hirsutiflora* (Commelinaceae); *Liatris acidota*, *L. elegans*, *Vernonia texana*, *Solidago nitida* (Compositae); and *Schrankia histicina* (Leguminosae). Those categorized as broad-leaved were: *Cnidocolus texana*, *Croton argyranthemus*, *Tragia gracilens*, *T. urticifolia* (Euphorbiaceae); *Gelsemium sempervivum* (Loganiaceae); *Sassafras albidum* (Lauraceae); *Rhus toxicodendron*, *R. copalina* (Anacardiaceae); *Rubus aboriginum* (Rosaceae); *Galactia volubilis*, *Rhynchosia latifolia* (Leguminosae); *Quercus laurifolia* (Fagaceae); *Cornus floridana* (Cornaceae); *Asimina parviflora* (Annonaceae); *Vitis rotundifolia* (Vitaceae); *Vaccinium staminium* (Ericaceae); *Aster* sp., *Berlandiera betonicifolia*, *Hymenopappus artemisifolia* (Compositae). Mean ratios of length to width were determined from five leaves of each species. The mean ratio for the 12 narrow-leaved species was 34.47, with a range of 4.99 to 124.30. The mean ratio of length to width for 18 broad-leaved species was 2.11, with a range of 0.92 to 3.35. There was no overlap between the two sets of species ($P < .001$, Mann-Whitney U-test). The ratio of length to width for the narrow-leaved *A. serpentaria* was 15.02 ± 2.79 ; for the broad-leaved *A. reticulata* it was 1.63 ± 0.06 .
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