References and Notes

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4. The X was cut from stiff white plastic and had arms roughly matching the triangular paper markers.
5. The study was conducted at the Archbold Biological Station, Lake Placid, Highlands County, Florida, a relatively undisturbed, sandy, scrub oak–palmetto, highlands habitat. *Argiope aurantia*, with a vertical-band or patch stabilimentum (Fig. 1, E and F), was also abundant at the site. We made our observations from 12 to 20 August 1981.
6. We attribute such perforations to damage inflicted by larger insects that flew through the web or by others that worked themselves loose after entrapment.
7. Of the total of 60 webs (marked and unmarked), seven were destroyed and three were damaged by 6 a.m. Destruction during the night might have been caused by bats, flying squirrels, gusting wind, or large beetles or moths. Because of the invisibility at night of our markers, the comparable incidence of destruction in the marked and unmarked webs in the period before 6 a.m. was to be expected.
8. These and subsequent statistics are based on 3 by 2 chi-square contingency tests comparing marked and unmarked webs for the three categories: intact, damaged, and destroyed.
9. Scores by noon (intact, damaged, and destroyed) were as follows: 2, 1, and 27 for unmarked webs; and 14, 9, and 7 for marked webs (both types combined).
10. Calculation based on the fact that of the 27 unmarked webs that were intact at 6 a.m., 13 underwent damage or destruction by 8 a.m.
11. Observations were made from 6:30 to 8:00 a.m. on each of five unmarked webs, by four observers who were partially concealed, 7 to 10 m from the webs.
12. Because they are scarce, such mammals could not have been a major factor at our study site.
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18. We thank H. W. Levi for identifying the spiders, D. Aneshansley for statistical advice, D. Dussourd and M. Malarcher for assistance with observations, the staff of the Archbold Biological Station for hospitality during our stay in the field, and an anonymous reviewer for helpful comments. Study supported in part by NIH grant AI-02908. Paper No. 71 of the series *Defense Mechanisms of Anthropods*.

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Adaptation of Fruit Morphology to Dispersal Agents in a Neotropical Forest

Abstract. *Two-thirds of 258 fruit species from Peruvian tropical forest belong to one of two classes: large orange, yellow, brown, or green fruits with a husk; or small red, black, white, blue, purple, or mixed-color fruits without a husk. The characteristics of the two fruit classes match the size, visual ability, and jaw morphology of mammals and birds, respectively, and the animals also prefer to eat one class of fruits. Thus, most plants in this forest seem to be adapted to seed dispersal by either of two distinct broad arrays of animal taxa.*

The intricate morphological adaptations of flowers to their pollinators are considered strong evidence for coevolution, but similarly clear adaptations of fruit morphology to one or a few disperser species are scarce (*1*). Yet it has long been known that various animal groups—ants, birds, bats—feed preferentially on fruits with distinctive disperser-correlated combinations of size, color, and construction (*2*). These disperser-specific morphologies (syndromes) are thought to indicate generalized evolutionary adaptation by plants to dispersers (*2, 3*). This evidence for adaptation has two difficulties. First, it is based on compilations of nonsystematic accounts of fruit-eating by animals from many localities and habitats (*4*). If only the more obvious examples of fruit-disperser correspondence were published, the derived correlations would be biased or exaggerated. Second, even if the observed correlations are accurate, they may represent only a small fraction of the fruit species that occur in one locality. Existing evidence does not rule out the possibility that a large proportion of plants in a community are morphologically poorly matched with their dispersers (*5*). Based on an extensive systematic analysis of fruit morphology from a single tropical forest, I show that a substantial majority of plant species have fruits of one of two distinct types corresponding to birds and mammals as major dispersers.

All the plant species with fleshy fruits known to have fruited between September 1980 and December 1981 were systematically collected and described in a tropical moist forest (*6*) at the Cocha Cashu Biological Station in the Manu National Park, Peru (71°22′W, 11°52′S). These 258 species represent approximately a quarter of the known plant diversity in the study area, and over half the genera with fleshy fruits (*7*). Potential disperser taxa in the study area include ants, fish, lizards, birds, bats, monkeys, and other mammals. Because primates were especially well studied in the area, fruits eaten by monkeys might have been more thoroughly sampled. I tried to counteract this possible bias by searching for all fruits produced in the study area.

For each fruit I recorded color (*8*), size, and presence or absence of a husk. Protected fruits were defined as those in which the ripe pulp is covered by a husk—a distinct stiff layer that is not nutritious and presents a barrier to feeding or digestion. Fruits with flexible skins less than 10 percent as thick as the smallest external fruit dimension were considered unprotected (for example, oranges are protected and cherries are not).

The species of many genera varied little with respect to the characters analyzed, yet virtually no family showed similar uniformity among component genera. Thus I considered the genus as the smallest independent unit of morphological classification. In the following analysis, I counted each nonvariable genus as only one form, assigning to it the morphology of its component species. Genera with appreciable variability of color or construction among species were assigned to two or more morphological forms as appropriate.

For the nonvariable genera, the frequency of protected fruit forms shows a statistically significant variation by color (Fig. 1) (*9, 10*). Almost every color category can be objectively assigned to one of two distinct sets, according to the percentage of protected fruits (*11*). Type A fruits, with few protected genera, contain red, white, black, and mixed colors, whereas type B fruits, with mostly protected genera, are orange, brown, yellow, or green. The blue and purple color category could be included in either type, but is most similar to type A (*12*), and is included in it for further analysis.

The same statistically significant asso-

Table 1. The distribution of fruit forms (N = 172) among all possible combinations of three dichotomous characters. There is a statistically significant association among the three characters and also among each pair of characters (*22*). Numbers in parentheses are values expected assuming joint independence of characters.

Size	Set A colors		Set B colors	
	Protected	Unprotected	Protected	Unprotected
≤ 14 mm	5 (17.3)	70 (33.0)	10 (14.3)	7 (27.4)
> 14 mm	6 (15.0)	13 (28.7)	38 (12.4)	23 (23.8)

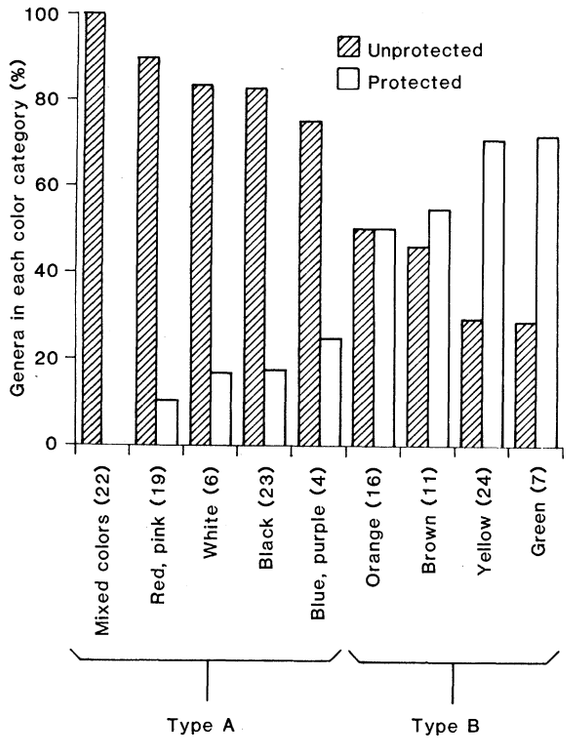

Fig. 1. Percentages of protected and unprotected fruits in various color categories. Numbers of genera for each category are in parentheses. Types A and B are homogeneous sets that differ significantly in the percentage of protected fruits (*11*).

ciation of color and presence of a husk occurs in those genera with morphological variability among species (*13*). These correlations within genera suggest small or even no effects of phylogenetic constraints, at the family and genus levels, in explaining the community-wide patterns. Thus, the significant associations among fruit characters are best explained as being sets of mutually concordant features, which may adapt them to distinct disperser groups.

In addition to color and construction differences, type A species are significantly smaller than type B fruits (Fig. 2) (*14*). The median size of all 258 fruit species is 14 mm, but 83.0 percent of type A fruits are smaller than this value, whereas 82.1 percent of type B fruits are larger. The difference is significant even if the husk of protected fruits is excluded in the measurements.

Altogether, a substantial majority of fruit genera fall into a small subset of all possible morphological combinations. There are eight possible combinations of characters among the three dichotomous character sets (protected or unprotected, type A or type B, $>$ 14 mm or $\leq$ 14 mm). The distribution in these categories of 172 fruit forms from all genera suggests highly significant associations between color, size, and presence of a husk (Table 1). The two most common combinations of fruit traits are also the most different: (i) small unprotected fruits with type A colors and (ii) large protected fruits with type B colors. Of the 258 fruit species used for this study, 175 (67.8 percent) belong to these two classes.

Although derived from a different perspective, these two fruit morphologies agree well with the bird- and mammal-syndromes reported by other investigators (*2*). The differences between these two fruit types correspond to differences between neotropical avian and mammalian dispersers in size (*15*), visual ability (*16*), and mouthpart morphology (*17*). Furthermore, birds and monkeys do not choose fruits in the proportions available but preferentially feed on distinct fruit types. In this study, there are 70 small unprotected type A–colored fruits and 38 large protected type B–colored fruits (Table 1). Birds (excluding parrots) have been seen eating 23 of these 108 fruits, all from the first category (*18*). Monkeys eat 68 of the 108 fruits, with significantly more (34 kinds) in the second category than would be expected by its availability (*19*). Monkeys seem to be more generalized feeders with respect to fruit type than are birds, which would have difficulty breaking into large protected fruits.

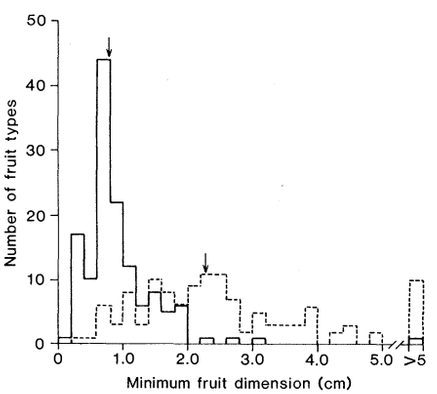

Fig. 2. Distribution of fruit size in sets A (N = 135 species) and B (N = 123 species). Minimum dimension refers only to the portion of the fruit handled by the disperser; the dehiscent husk of capsules is not included. Arrows point to the median values of the distributions.

This analysis suggests that the fruit morphology of a species frequently is adapted to the general characteristics of the animals that eat it. The strong association between size, color, and morphology even among species within genera implies that natural selection has produced the divergence in fruit form associated with bird and mammal fruit-eating (*20*). The high frequency of adapted fruit species responding to whole arrays of morphologically similar disperser taxa contrasts with the reported scarcity of fruits adapted for dispersal by only one or a few animal species (*1, 5, 21*). Several hypotheses have been suggested to explain this difference (*1, 5*), including insufficient evolutionary time, the unpredictable location and availability of good germination sites, and morphological similarity among members of a given category of dispersers. One additional factor may be that plant-disperser studies have focused on small unprotected fruits, whose morphology may make them available to most disperser species, even those from clearly different arrays. In the case of large protected fruits, it is possible that there is much higher plant-disperser specificity.

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6. Habitat classification according to L. R. Holdridge [*Science* **105**, 367 (1947)].
7. The fractions are based on plant collections since 1973 from the study site. The remaining known genera with fleshy fruits either did not fruit during the study period or are so uncommon that no specimen could be obtained. All previously uncollected fruit types were collected by me and sent to the Field Museum, Chicago, where the Manu collection is stored. All plant specimens were identified by R. Foster.

8. Fruit color was matched against the *p*H reference color scale on Riedel–De Haën (AG) wide range *p*H indicator paper, *p*H 0.5 to 5.5 and *p*H 5.5 to 9.0. Colors not on the *p*H reference scale were compared to available constant reference objects (for example, a red Victorinox Swiss Army knife). Dark shades of purple or blue were classified as black. Mixed-color fruits have two or more colors present in the ripe fruit, including the support structures.
9. All statistical methods follow S. Siegel [*Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956)], except where noted otherwise. All chi-square tests used Yates correction for continuity [R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969), p. 590].
10. Chi-square test for heterogeneity, $\chi^2(8) = 34.48$, $P < .001$. For the categories of color and morphology in Fig. 1, 132 genera had no variation among species.
11. Groups of color categories were defined by a test for homogeneity of subsets [see R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969), pp. 575–585]. I used the .05 probability level for evaluating statistically significant heterogeneity among subsets of the color categories, which were ranked by percentage of protected fruits.
12. The affinity of blue and purple with type A is supported by the high percentage of unprotected fruits of these colors, by the grading of these colors into black (*8*), and by the fact that four of five occurrences of these colors in the mixed-color category were combinations with type A colors.
13. Because of the small number of variable genera, colors are combined into type A or type B. For the resulting four categories of color and protectedness, 15 variable genera have 53 fruit forms. In the 11 genera in which only color varies, nine of ten protected forms fall into type B colors, whereas only 11 of 30 unprotected forms are type B colors [$\chi^2(1) = 6.53$, $P < .01$]. For four genera in which both color and construction vary, five of seven protected forms belong to type B colors and none of six unprotected forms are type B colors [$\chi^2(1) = 4.28$, $P < .05$]. No genera show variation only in construction.
14. Kolmogorov-Smirnov two-sample test, $D = .651$, $N = 258$, $P < .001$. Species are considered independent samples because even otherwise morphologically constant genera show appreciable variation in size among species.
15. Most fruit-eating birds in Peru weigh less than 200 g, whereas the major mammalian fruit-eaters, monkeys, weigh from 400 to 8000 g. Weights are from live measurements of birds and tamarins in the study site (J. Terborgh, personal communication) or from J. F. Eisenberg [*The Mammalian Radiations* (Univ. of Chicago Press, Chicago, 1981), p. 468].
16. Mammals usually have poor color vision, whereas birds have high sensitivity over the visible spectrum [G. L. Walls, *The Vertebrate Eye and Its Adaptive Radiation* (Hafner, New York, 1963)]. Neotropical monkeys see well in the green-yellow-orange range but have low sensitivity to and ability to discriminate among reds [R. L. De Valois and G. H. Jacobs, *Science* **162**, 533 (1968); D. M. Snodderly, in *The Behavioral Significance of Color*, E. H. Burtt, Ed. (Garland, New York, 1979), pp. 237–279].
17. Mammals have bony jaws, complex teeth, and manipulative tongues that aid in processing fruits (C. Janson, personal observation). Most fruit-eating birds have little ability to manipulate fruits with precision; the major exception, parrots, are mostly seed predators [D. H. Janzen, *Auk* **98**, 841 (1981)].
18. Chi-square one-sample test, $\chi^2(1) = 10.98$, $P < .001$. Birds were observed to eat fruits opportunistically during other studies (C. Janson, personal observation). Although not a complete description of the diets of fruit-eating birds, these observations should not be biased toward small unprotected fruits.
19. Chi-square one-sample test, $\chi^2(1) = 5.91$, $P < .02$. Fruit-eating by monkeys was recorded from systematic study of seven species in the area.
20. It is likely that fruit species not included in the two major classes show morphological adaptation to other dispersers. In particular, a number of species known to be eaten by bats are included in the class of large unprotected type B–colored fruits (Table 1).
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22. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969), pp. 601–607. The *G* statistic to test joint independence of all three characters is 118.1, allowing rejection of independence ($P < .001$ with 4 degrees of freedom, $N = 172$). The *G* statistics to test independence of color and protection, color and size, and size and protection are 49.4, 61.2, and 29.3, respectively. All allow rejection of independence ($P < .001$ with 1 degree of freedom, $N = 172$).
23. I thank R. Foster for invaluable instruction in plant identification at the study site and the Peruvian Ministry of Agriculture, Forestry Institute and ORDEMAD for support and permission to work in the Manu National Park. Several researchers contributed plant specimens and feeding observations: P. C. Wright, A. Wilson, C. Munn, S. Robinson, B. Mitchell, P. Daniels, G. Stuart, and especially R. Foster. For stimulating discussions, I am grateful to R. Foster, J. Terborgh, G. Orians, N. Wheelwright, P. Harvey, and M. Slatkin. Supported by NSF grant BNS8007381 and Sigma Xi grant in aid of research.

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Immunoreactive Dynorphin-(1–8) and Corticotropin-Releasing Factor in Subpopulation of Hypothalamic Neurons

Abstract. *Immunoreactive corticotropin-releasing factor (CRF) and dynorphin-(1–8) were visualized in rat hypothalamus by immunohistofluorescence with specific antibodies. In brains from colchicine-treated, adrenalectomized rats, neuronal perikarya with immunoreactive CRF were observed in the paraventricular nucleus of the hypothalamus. The CRF occurred together with the dynorphin-(1–8). However, the CRF immunoreactivity occurred only in a subpopulation of the dynorphin-(1–8) immunoreactive cells. These findings suggest that there may be a functional interrelationship of CRF with dynorphin-related opioid peptides and provide further evidence that neurons may contain more than one bioactive substance.*

Corticotropin-releasing factor (CRF), which consists of 41 amino acid residues, was isolated from ovine hypothalamus extracts (*1*). The peptide is a potent stimulator of adrenocorticotropic hormone (ACTH) release in vivo (*2*) and of ACTH-like and β-endorphin-like immunoreactivities in cultured pituitary cells (*1*). It has been reported that CRF originates in perikarya of the paraventricular nucleus of the hypothalamus (*3*). The paraventricular nucleus is one of the main synthesis and storage sites of vasopressin in mammalian hypothalamus (*4*). The same neurons in the paraventricular nucleus which manufacture vasopressin also contain dynorphin-(1–17) and α-Neo-endorphin, two leucine-enkephalin–related opioid peptides (*5–7*). These two opiate active substances also occur together in brain areas other than hypothalamus (*8*). We have recently demonstrated that one of the major peptide products of this α-Neo-endorphin/dynorphin neuronal system is dynorphin-(1–8) (*9*), an amino-terminal fragment of dynorphin-(1–17), which is present in much higher concentrations in brain than dynorphin-(1–17). In subsequent immunohistochemical studies, very intense dynorphin-(1–8)-like staining occurred in the same neurons that were previously demonstrated to contain α-Neo-endorphin, dynorphin-(1–17), and vasopressin immunoreactive material (*10*).

In the studies described here we investigated whether the CRF-containing neurons in the paraventricular nucleus are related to those containing the opioid peptides. To examine this question we developed a specific antiserum to CRF and compared the immunostaining produced by this antiserum with the distribution of dynorphin-(1–8) immunoreactive material. The studies were carried out on brains from colchicine-treated, adrenalectomized rats, and we found that CRF immunoreactivity is present in a subpopulation of the dynorphin-(1–8) immunoreactive neurons in the paraventricular nucleus.

The antiserum to CRF was raised in rabbits against the synthetic peptide (*11*). In a radioimmunoassay (RIA) (*12*) this antiserum bound 30 percent of a trace amount of [^{125}I-Tyr°]CRF at a dilution of 1 in 100,000. A 50 percent inhibition of this binding of [^{125}I-Tyr°]CRF to the CRF antibodies occurred by addition of a 400 p*M* concentration of authentic CRF. No inhibition of the labeled CRF occurred when 1 μ*M* sauvagine, α-Neo-endorphin, dynorphin-(1–8), dynorphin-(1–17), vasopressin, or oxytocin were added. The RIA specificity of the dynorphin-(1–8) antiserum was established in studies previously described (*9*).

Since RIA's are performed with a much higher antiserum dilution than immunohistochemistry, other populations of antibodies may be active in the latter method, therefore the antiserum specificity data obtained with the RIA are only of limited value in interpreting immunohistochemical results obtained with these same antiserums. Therefore, we also subjected the two antiserums used to immunohistochemical blocking controls, which were performed by adding an excess of various synthetic peptides