depressions between each cuticular fold appear free of crystalline or other substances. Papillae from dried samples were semierect or collapsed in the lower parts, suggesting that the folds are functional for living cells as supporting devices. The apex of a dried papilla appears bulbous. The cross-sectional view of a single cell (Fig. 2) reveals that the papilla is not an empty cellular extension, but contains a concentration of cellular material within the apex in addition to that present in the flattened basal portion.

Additional experiments were conducted to determine if pigments other than flavonoids were contributing to UV absorbance. Prolonged extraction of the intact ligule in a soxhlet apparatus with dichloromethane removed the carotinoid pigments and some fatty materials. The UV appearance and the structural features exhibited with scanning electron microscopy were not significantly altered by this treatment. Similar treatment with methanol removed the flavonoids and other phenolic compounds. After the methanol procedure, all UV-absorbing parts of the petals were converted to UV reflectance; we were unable to convert UV-reflecting parts to UV absorbance.

The combined results for these plants are consistent with the interpretation that intense UV absorption is associated with concentrations of flavonoids in the tips of specialized epidermal papilla cells. The upper surfaces of these cells are largely convex or conical and are held in an erect position by substantial cuticular folding. A maximum of flavonoid material is present at the cell-cuticle interface above the general epidermal surface level; this makes for efficient UV absorption as the angle of incident light changes during the daylight hours. Similar cells lacking the flavonoid concentration in the tip and other forms of epidermal cells lacking flavonoids reflect UV. Flavonoid-containing cells other than those with papillae generally reflect UV but may function for intermediate UV shading. These properties are particularly evident in floral parts of the Compositae and are present in other plant families as well.

More than 70 years ago the light-receptive properties of papillate epidermal cells of leaves were described and investigated (*12*). The planoconvex surface of the papilla tip functions as a condensing lens and the light rays are concentrated on the interior tangential walls of the cell. In addition, the lens curvature enables these cells to focus light on the interior when the incident light strikes the cell obliquely as well as vertically. The shape, thickness, and surface configurations of the cuticle apparently are associated with the optical properties of these cells (*13*) but critical experimental evidence on this issue is lacking. Papillate epidermis has been recorded on the floral organs of various taxa (*10, 14*) with little discussion of their functional relation to light other than the suggestions that they are associated with shading and texture (*15*).

It is now reasonable to propose that these petal cells function with respect to optical properties in a manner equivalent to those on leaves and that the dense accumulations of flavonoid pigments in the papillate extension facilitates efficient UV absorption during all daylight hours.

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8. Flavonoids generally are soluble in most aqueous and alcoholic solutions and the customary microtechniques and histochemical procedures remove them from the cells. Special techniques minimizing the use of these solvents were devised. Fresh petals in glycerine were frozen at −20°C on a microtome block and sections 20 to 30 μm thick were cut, mounted on a cold slide, and examined under a Zeiss UV fluorescence microscope [H. P. Guerin and P. G. Delaveau, *C. R. Hebd. Seances Acad. Sci. Ser. D Sci. Nat.* **267**, 726 (1968)]. After spraying with a mist of 1 percent aqueous $AlCl_3$ (*9*, pp. 51–56), epidermal layers with flavonoid concentrations were distinguished by a rapidly dispersing yellow fluorescence, which was documented photographically (Fig. 1a).
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11. On exposure to ammonia vapor, bright, uniformly yellow ray-flowers of *Lasthenia chrysostoma* become visibly patterned. The bases of the ligules and the entire disk become a vivid red, while the tips remain yellow. An identical patterning is produced under UV light without the ammonia, the bases and disk showing absorbance and the tips reflectance (Fig. 1b). These floral parts contain aurones and chalcones as well as other flavonoid compounds [B. A. Bohm, N. A. M. Saleh, R. Ornduff, *Am. J. Bot.* **61**, 551 (1974); M. Shimokoriyama and T. A. Geissman, *J. Org. Chem.* **25**, 1956 (1960)]. Whole dried ligules were mounted in a minimum amount of dilute Aerosol OT in 20 percent methanol and were treated with freshly prepared methanol saturated with sodium methoxide; the cover slip was sealed with paraffin. In this alkaline reagent aurones turn a vivid red, in a manner similar to, but slower than, the ammonia reaction [M. Shimokoriyama, in *The Chemistry of Flavonoid Compounds*, T. Geissman, Ed. (Macmillan, New York, 1962), pp. 301–308; Mabry *et al.* (*9*, pp. 227–229)].
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Behavioral Taxonomy in Canids by Discriminant Function Analyses

Abstract. *The technique of linear discriminant function analysis was applied to behavioral data that were collected on infant canids. The method was first tested with a known cross between a wolf and a dog and proved to be valid, indicating that quantitative analyses of behavioral phenotypes can be used in assessing taxonomic relationships. In addition, the controversial New England canid was determined by behavioral analysis to be more closely related to coyotes than to wolves.*

Behavioral characters have been used to formulate or substantiate taxonomic schemes based on other criteria (for example, anatomical), mainly in the class Aves (*1–4*). Indeed, classical ethological approaches have had classification as one of their main goals following the early work of Heinroth (*1*), Whitman (*2*), Lorenz (*3*), and others (*4*). Yet rigorous quantitative methods have not been applied to behavior taxonomies. We report here the use of discriminant function analyses to construct a behavioral taxonomy; to the best of our knowledge this is the first such use of this method. In addition, we attempt to determine where an enigmatic, controversial canid, the New England canid or "Eastern coyote" (*Canis latrans* var.), falls in relation to other selected members of the genus *Canis* (family Canidae). Because of the great variation in behavior among domestic dogs (*5*), we placed greater emphasis on comparing the New England canids to wild congeners. The results of this analysis may be used to examine the congruence of taxonomies based on behavioral and anatomical characters. Linear discriminant function analyses based on

Table 1. Discriminant functions differentiating species of canids on the basis of four behavioral characters. The top numbers are weights for the characters and define the axis that discriminates to the greatest degree between the species. The higher the number, the better the discrimination. The numbers in parentheses indicate the observed proportion of the behavior in relation to the total number of interactions observed in the stated time period for each species pair. Ranges of proportions are reported for the ten pairs of New England canids. For example, 0.47 and 0.25 are the proportions of observed agonistic behavior (character 1) for the two pairs of coyotes, and 0 and 0 are the corresponding proportions of agonistic behavior for the two pairs of wolves.

Combination	Agonistic behavior		Play behavior	
	Character 1 (days 21–28)	Character 2 (days 29–35)	Character 3 (days 21–28)	Character 4 (days 29–35)
Coyote/wolf	46.61 (0.47,0.25/ 0,0)	0 (0.36,0.27/ 0.11,0.09)	312.99 (0.01,0/ 0.21,0.16)	0 (0.14,0.09/ 0.34,0.30)
Dog/wolf	0 (0,0/0,0)	0 (0,0/ 0.11,0.09)	71.76 (0.33,0.38/ 0.21,0.16)	160.20 (0.42,0.42/ 0.34,0.30)
Coyote/New England canid	9.60 (0.47,0.25/ 0.09–0.32)	35.09 (0.36,0.27/ 0.07–0.23)	110.31 (0.01,0/ 0.03–0.10)	65.88 (0.14,0.09/ 0.11–0.20)
Wolf/New England canid	63.75 (0,0/ 0.09–0.32)	56.92 (0.11,0.09/ 0.07–0.23)	116.56 (0.21,0.16/ 0.03–0.10)	297.20 (0.34,0.30/ 0.11–0.20)

skull and dental characters have placed the New England canid close to the coyote, and suggested an introgression of genes from both the wolf and the dog (*6, 7*).

The animals observed included two pairs each of wolves, coyotes, beagles, wolf-dog (malamute) hybrids (*8*), and a litter of five New England canids from the Saint John–Allagash wilderness of western Aroostook County, Maine. All animals were hand-reared, treated similarly, and observed from 21 to 35 days of age (*9*). Clear-cut behavioral differences have been consistently demonstrated during early development in hand-reared animals allowed 15 minutes of interaction per day (and otherwise housed alone), so this method was used for the paired animals (*10*). The intact litter of New England canids was observed for 105 hours (ten possible paired interactions). A total of 4227 interactions was analyzed for the 18 pairs of animals and data were converted to proportions in order to make direct comparisons between the groups. The frequency of occurrence of approximately 50 actions was recorded either by hand or on audio- or videotape (*10, 11*). Since the most significant behavioral differences concern the frequency of occurrence of play and agonistic (for example, fighting and other aggressive, offensive acts eliciting submission) interactions, we decided to use these two behaviors for analysis. Data were organized into 1-week intervals, resulting in four analyzable characters.

To test the validity of using discriminant function analyses on behavioral characters, we first analyzed the data for the wolves, dogs, and wolf-dog hybrids. The two characters separating the wolves from the dogs (Table 1) were play between 21 to 28 and 29 to 35 days of age, the dogs being the more playful of the two. The average positions on a wolf-dog discriminant function axis for wolves, dogs, and wolf-dog hybrids were 64.54, 92.27, and 79.20, respectively. There was no overlap between the groups. The intermediate position of the wolf-dog hybrids in this analysis of behavior is in agreement with an analysis of Lawrence and Bossert (*6*), who demonstrated the intermediacy of coyote-dog hybrids with biometrical characters. This suggests that behavioral characters, just as anatomical characters, may be used to assess taxonomic relationships, and that the characters analyzed have a polygenic basis, a point stressed by Lorenz (*3*) in his earlier writings.

We next compared the New England canids to coyotes and wolves. Both morphological and behavioral data (*6, 7*) have suggested that the New England canid is more closely related to the coyote than to either the wolf or the domestic dog. In order to determine the behavioral relationship and compare it to the data of Lawrence and Bossert, appropriate discriminant analyses were performed. Table 1 shows that both the coyotes and the New England canids were more aggressive than the wolves and also that the wolves were more playful. A summary of the results of pairwise analyses for the coyotes, wolves, and New England canids (Table 2) shows that the coyotes and the New England canids share approximately the same relationship to the wolf [D^2 values (distances in discriminant function units) of 72.01 and 75.48, respectively, when compared to the wolves] and that the New England canids fall very close to the coyotes (D^2 = 14.97). On a linear discriminant function axis between coyotes and wolves, the mean positions of coyotes, wolves, and New England canids were 14.14, −57.88, and −8.90, respectively (*12*). Again, there was no overlap between the groups.

Table 2. Distances (D^2), in discriminant function units, based on pairwise analyses of four behavioral characters in coyotes, wolves, and New England canids. Numbers in parentheses are distances computed from skull and dental characters [from Lawrence and Bossert (*6*)].

	D^2	
	Wolves	Coyotes
Coyotes	72.01 (64.10)	
New England canids	75.48 (29.84)	14.97 (26.83)

Our results demonstrate that linear discriminant analysis may be applied to behavioral data, and that quantitative analyses of behavioral characters are useful in deriving taxonomic relationships. These results also suggest that play and agonistic behavior have a substantial genetic component. On the basis of behavioral criteria, the New England canid is seen to be more closely related to the coyote than to the wolf. Our results agree with those of Lawrence and Bossert (*6*), who showed a similar relationship based on anatomical measurements.

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Sex Recognition in the Crayfish *Procambarus clarkii*

Abstract. *Male crayfish,* Procambarus clarkii, *show different behaviors toward males (aggression) and females (submission, courtship). Behavioral and neurophysiological tests with water in which the crayfish had been held demonstrated the existence of sex pheromones. The inner rami of the antennules are the site of reception, and the chemicals are probably carbohydrates.*

In the crayfish *Procambarus clarkii*, copulation is preceded by courtship behavior (*1*). Courtship consists initially of aggressive behavior by both male and female but is succeeded by a series of nonaggressive interactions. Male-male interactions are persistently aggressive. A similar system has been described in the banded shrimp *Stenopus hispidus* (*2*) and in snapping shrimp (*3*). The difference in male-male and male-female interactions in the crayfish indicates that sex recognition occurs. We now report the existence and nature of sex pheromones in *P. clarkii*.

After being shipped from Louisiana, specimens (40 to 61 mm, cephalothorax length) were kept in separate, aerated aquaria.

To show that a male can discriminate sex without physical contact, the following experiment was performed. For each trial, a male was held in a 10-gallon (38-liter) aquarium for 2 to 5 days. An opaque plastic container (15 cm in diameter, 19 cm tall, and perforated with 1-mm holes), was placed in the aquarium near the source of aeration. A stimulus animal was placed in the perforated container, and the behavior of the test animal in the aquarium was observed. Control tests using opaque but nonperforated containers were negative.

All tests were run between 1100 and 1500 hours, when this species is normally less active. After a stimulus animal was placed in the plastic container, the response latency was recorded, and the amounts of time spent by the test animal in the following activities were recorded for 60 minutes: (i) searching, either 1 cm or less from the test container or elsewhere; (ii) resting, either 1 cm or less from the container or elsewhere; (iii) chelae raised posture; (iv) feeding movements; and (v) curled telson and uropod. There were 15 stimulus replications, 10 male and 5 female (Table 1).

In neither searching time nor resting time did the test animals respond differentially to male and to female stimulus animals. However, males showed submissive behaviors (feeding movements, chelae down, curled telson) when females were in the test container (90.6 percent of observation period) while the raised chelae posture (an index of agonistic behavior) was prevalent when the stimulus animal was a male (84.0 percent of period; $\chi^2 = 77.9$, $P < .0001$).

A second series of experiments was made with the same test animals after they had been blinded (*4*). The behaviors were timed as in the first series (Table 1). Normal and blinded animals did not differ in the amount of time they spent searching. The blinded test males searched longer when females rather than males were in the perforated container ($t = 3.44$, $P < .005$). Again, males spent most of the observation period (82 percent) in aggressive postures when the stimulus animal was a male, but showed submissive behaviors (89 percent of observation period) when females were in the test container ($\chi^2 = 78.0$, $P < .0001$).

The first and second experiments showed that neither vision nor physical contact was necessary for sex recognition. In the remaining tests, chemical means of communication were studied. Aged, aerated water from a container in which a male or a female crayfish had been held for 24 to 48 hours (called male or female water) was used to test the responses of isolated males. Glass tubes were connected to two funnels, one used for the male or female water and the other for control water (aged, aerated, tap water). The flow of water from the funnels into the tank of an isolated male was at a rate of about one drop every 3 seconds. Durations of behavior patterns were recorded as above for 30-minute periods. We made 12 replications of this experiment (Table 1). There was a significant difference in the time spent within a 2-cm radius of the test source (male or female water) and the control source ($t = 28.6$, $P < .001$). Males showed aggressive behavior toward male water but were submissive when female water was introduced ($\chi^2 = 51.8$, $P < .0001$).

Ablation experiments were done to establish sites of chemosensory organs. Eight males whose antennae were removed responded appropriately to male or female water. When the antennules of these individuals were removed, they no longer responded to test water. In addition, ten males with intact antennae but antennules removed showed no response to test water (each male tested on three separate days). Furthermore, when just the inner flagella of the antennules were removed, test animals were no longer responsive.

The following tests were performed (by the procedure of the third experiment) to establish some characteristics of the chemical factors involved.

1) Water from five males and five females was filtered separately through Sargent filter paper No. 500, and the filtrates were tested on ten males. All males responded differentially toward the filtrates.

2) The water from the males (six samples) and females (four samples) was boiled, cooled, and tested. Three male and two female samples were boiled for 5 minutes, the others for 30 minutes. The results were positive and appropriately different for the two sexes.

3) The water from males (four samples)

Table 1. Average time (in minutes) spent by a male crayfish in different behaviors. Numbers in parentheses represent number of trials with male and with female stimuli. The timing of the observation period began with the initial response. In the third experiment, times in parentheses are response times to aged, aerated, tap water.

Stimulus	Latency	Searching		Resting		Chelae up	Feeding	Curled telson
		Near source	Elsewhere	Near source	Elsewhere			
Normal males, stimulus animals in container, 60-minute observation periods								
Male (10)	9.8	49.9	3.8	2.9	3.4	50.4	0.5	2.8
Female (5)	10.2	43.0	3.2	5.2	8.6	1.8	52.2	54.4
Blinded males, stimulus animals in container, 60-minute observation periods								
Male (10)	6.6	45.6	6.8	3.4	4.2	49.3	0	0.7
Female (5)	11.8	50.6	4.0	3.6	1.8	0.6	53.4	52.2
Normal males, test water added, 30-minute observation periods								
Male (7)	4.0	24.9 (1.1)	3.3	0.4 (0.4)	0.6	27.6	0	1.4
Female (5)	7.8	22.6 (1.0)	2.8	2.4 (0.4)	1.0	0.8	27.6	26.8