zyme with respect to the molecular nature of the endogenous substrate or substrates remain to be determined.

Our findings extend to the transsynaptic induction of TH, the concept proposed for liver and ovary (*11, 12*) that protein kinase translocation may be a mechanism involved in transferring information to the nucleus. Moreover, since denervation reversed the decline of cytosol protein kinase and the increase in activity of the enzyme in the pellet, we suggest that protein kinase translocation is part of the mechanism whereby the sustained activation of nicotinic receptors coordinates the long-range reactions involved in the expression of the genetic code. Since adrenal denervation fails to abolish the increase in cyclic AMP elicited by cold exposure in adrenal cortex (*13*) but does abolish the increase of cyclic AMP and the induction of TH in the adrenal medulla, we can exclude a direct participation of corticosteroids in eliciting the increase of cyclic AMP in the medulla, the activation and translocation of protein kinase, and the transsynaptic induction of TH. However, we cannot rule out an indirect permissive role of corticosteroid in the regulation of TH biosynthesis.

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6. Cyclic AMP and cyclic GMP concentrations were measured by activation of a specific protein kinase after separation on alumina and Dowex columns (*13*). The baseline value of cyclic AMP was 25 ± 1.5 pmole per milligram of protein and that of cyclic GMP was 3.1 ± 0.28 pmole per milligram of protein ($N = 15$). To determine the kinase activity, four medullas were homogenized with 80 μl of $0.5M$ NaCl, 10 mM EDTA, 5 mM aminophylline, 10 mM potassium phosphate buffer (pH 6.5), modified from Corbin *et al.* (*14*). After centrifugation at 100,000g the cytosol was collected, and the pellet was homogenized again, in a mixture of 80 μl of $0.5M$ NaCl, 10 mM potassium phosphate buffer, 10 mM aminophylline, and 0.2 percent Triton × 100 (pH 6.5), and centrifuged again for 20 minutes at $2 \times 10^4 g$. The protein kinase reaction was started by adding 10 μl of [γ-^{32}P]adenosine triphosphate (500 μM), 100 mc per mmole to an incubation mixture containing (in a volume of 140 μl) 10 μl of cytosol or pellet extract, 10 μl of $0.1M$ magnesium acetate buffer (pH 6), 40 μl of $0.5M$ sodium acetate buffer (pH 6.0), 10 μl of $0.3M$ NaF, 10 μl of 65 mM aminophylline, 20 μl of calf thymus histone mixture (2 mg/ml), 30 μl of water, and 10 μl of cyclic AMP or water. After 5 minutes at 30°C, the incubation was terminated by pipetting 50 μl portions on filter paper discs and the samples were washed (*4*). In some experiments, we have characterized the kinase activity with regard to the specificity for various histone or proteins. The enzymes in the medullas in the natural state or after exposure at 4°C have different specificities for different substrate proteins (*5*); since these kinases express their maximal catalytic rates in the presence of histones, we have provisionally used calf thymus histone mixture to measure the phosphorylating activity in the cytosol and insoluble portion (pellet) of the medulla. Under these conditions, the baseline cytosol kinase activity was 163 ± 9 pmole per milligram of protein per minute in the presence of 0.7 μM cyclic AMP and 55 ± 4 pmole per milligram of protein per minute in the absence of cyclic AMP. The kinase activity in the pellet extract was 70 ± 10 pmole per milligram of protein per minute in the presence of cyclic AMP and 69 ± 7 pmole per milligram of protein per minute in the absence of cyclic AMP. Proteins were measured by the method of Lowry *et al.* (*15*). The values of RNA synthesis (*2*), TH synthesis (*3, 4*), and TH activity (*4*) in Fig. 1D have been previously reported.
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Recognition and Sexual Selection in *Drosophila*: Classification, Quantification, and Identification

Abstract. Drosophila pseudoobscura *females show a positive bias toward mating with males whose proportion in the population is low. They can perform this discrimination even when three strains of males are present. The olfactory recognition required for this discrimination entails a hierarchically ordered recognition system and a natural unit of olfactory strength.*

"This leads me to say a few words on what I have called Sexual Selection. This form of selection depends, not on a struggle for existence in relation to other organic beings or to external conditions, but on a struggle between individuals of one sex, generally the males, for the possession of the other sex. The result is not death to the unsuccessful competitor, but few or no offspring" (*1*).

Thus did Darwin state the nature of evolution resulting from competition for mates. Such competition requires two kinds of information: a recognition of different classes of potential mates and a bias in selecting a mate from one class rather than another. Petit, Spiess, Ehrman, and others have shown that, in drosophilids and in some other insect species, one type of mate selection bias depends on the proportion of the two classes (that is, genetic strains) in a population, with a positive bias for the strain in least abundance (*2*). This "rare male advantage" is mediated by odor in *Drosophila pseudoobscura* and possibly in other species. A recognition system mediated by odor raises several questions with regard to the type of neural networks required to organize and quantify olfactory information and about the nature of olfactory cues.

Drosophila mating behavior has been extensively studied and appears to involve a complex of auditory, chemical, tactile, and visual cues that govern the interaction between males and females, leading to persistent courtship by the males and to final acceptance (or repeated rejection) of a male by a female (*2, 3*). During this interaction in *D. pseudoobscura*, males are indiscriminately active while females are discriminatingly passive. Each female must classify potential mates on at least three levels. Level 1 recognition determines whether the male is of the right species. Because this is a right or wrong binary choice under heavy selection pressure, it is undoubtedly genetically programmed. It also appears to involve more than one sensory modality; several studies have been conducted concerning the features required for species recognition (*4*). Level 2 recognition has also been the subject of several studies; it involves assessment of the "vigor" of the courting male (*2*). Some genetic strains are more readily accepted as mates than others. For example, there are mutant strains of males that are virtually incapable of competing with wild-type males for mates, even with females of their own genetic strain. The recognition mechanism here may involve assessment of the male's execution of the mating ritual, as judged against an internal (genetically programmed) or external (learned) standard. Level 3 recognition controls the rare male advantage. Three separate kinds of information are required for it. (i) The population must be classified into the various strains present. (ii) The classified strains must be quantitated to measure their proportion in the population. (iii) Each male must be identified as a member of the minority or majority strain.

We have previously shown that the rare male behavior in certain strains of *D. pseudoobscura* can be controlled by olfactory cues (*5*). However, this work was conducted with only two strains present in the mating chambers, and it is not evident a priori that a quantitative recognition based on olfactory cues can generalize to choices between more than two strains, although a multistrain population is probably a more natural situation. For testing this generalizability we worked with two wild-type strains, Arrowhead (AR) and Chiricahua (CH), which differ in a third chromosome inversion, and an orange-eyed mutant strain, *or*, of the standard third chromosome arrangement.

Mating trials were conducted in Elens-Wattiaux observation chambers with 24 pairs of flies per chamber (*6*). Females were equally divided between AR and CH strains, while males were used in a variety of ratios. Virgin flies of both sexes (raised at 23°C and 60 percent relative humidity) were collected within 3 hours of eclosion, separated by sex under mild etherization, and used in mating trials 4 days later. The CH and AR flies were marked by wing clipping during collection to enable visual scoring of matings. In these trials males mate repeatedly while females mate only once. Data were used only from chambers in which all females mated (*7*).

On the basis of vigor alone, CH and AR males are equally acceptable as mates. However, an *or* male is only 50 percent as likely to mate as either wild-type inversion strain under the same conditions. The expected mating frequency is corrected for this disadvantage [see (*8*)].

Results of the mating trials are given in Table 1. Data are tabulated as the natural logarithm of the ratio of the observed mating frequency to the expected frequency. This function is symmetric about the expected frequency; a positive sign for an entry indicates an advantage for that particular strain of male.

Matings where males are present in a 5:5:2 ratio show classical rare male behavior, with the single rare strain enjoying a significant advantage. However, the interpretation of the other four cases investigated is more complex. In the 1:1:1 case there is a slight advantage for the *or* strain, while in the three 4:1:1 cases there are two rare strains, but no more than one strain enjoys an advantage. Advantages in all trials are of two types: (i) when the number of AR and CH are unequal, the minority strain is advantaged; and (ii) when the number of AR and CH males are equal, the *or* males enjoy an advantage if they are the minority strain relative to the sum of the two nonmutant strains.

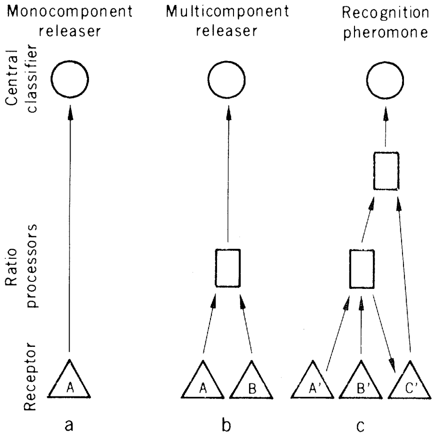

Fig. 1. Representation of minimal neural schemes for classifying olfactory signals and making the classification available (from the central classifier level) for behavioral effectors. A and B represent specific receptors for single compounds; A', B', and C' may not be specific receptors, but may represent odor patterns received by several individual receptors.

Level 3 recognition of AR and CH males can be controlled by olfactory cues (*9*). In order to accomplish such recognition, the female must classify particular odor patterns as "CH" or "AR," quantitate the patterns to arrive at the relative proportion of these two strains, and identify a potential mate as "AR" or "CH." Since she has had no contact with males other than the very brief contact with males of her own strain after eclosion, she must accomplish these tasks without relevant prior experience. Furthermore, it has been shown that she can recognize several genetic strains of males (and even two populations of the same genetic strains raised at different temperatures) in the absence of prior experience. The recognition system provides a set of patterns that is usable for classifying potential mates, as well as a "unit of olfactory strength" that can be calibrated to equal one male. The nature of the advantages in the three strain crosses places one further requirement on the system—the recognition must be hierarchically organized. The proportion of AR to CH is determined first (in priority if not temporally); if it is close to unity, then the *or* : (AR + CH) proportion is behaviorally important. Thus, the characteristics of the recognition system are as follows.

Table 1. *Drosophila pseudoobscura* males (*24*) in the ratios indicated were presented to females (12 AR:12 CH), and the males accepted as mates are tallied by strain. Values are $\log_e$ [(observed matings)/(expected matings)] where the expected matings have been corrected for differences in male vigor. A positive log odds value indicates a mating advantage for that particular strain; a negative value indicates a disadvantage. (Each ratio was tested in a total of 144 matings.) Boldface entries are statistically significant when tested by χ_1^2 against the other two strains of males in the trials. Symbols in parentheses indicate levels of significance: $* = P < .05$; § = $P < .01$; ‡ = $P < 10^{-5}$; † = $P < 10^{-7}$.

Ratio of males	Log of odds for male mating success		
AR : CH : *or*	AR	CH	*or*
1 : 1 : 1	−.054	−.130	**.310**(*)
4 : 1 : 1	−**.378**(†)	**.701**(†)	.410
1 : 4 : 1	**.644**(‡)	−**.285**(‡)	.173
1 : 1 : 4	.060	.134	−.113
5 : 5 : 2	−.108	.160	**.855**(‡)
5 : 2 : 5	−.076	**.362**(§)	−.237
2 : 5 : 5	**.407**(§)	−**.261**(§)	.059

1) *Specificity*. A particular strain can be identified in the presence of other strains.

2) *Quantifiability*. The proportion of each identifiable strain in the population can be determined in such a way as to provide behaviorally important information.

3) *Apparent ability to deal with novel information or combinations of information*. The choice of strains used is arbitrary and, in earlier studies (*2*), a wide range of different strains has been used. No prior experience with any particular strain of males is required for the females to exhibit specific and quantitative discrimination.

4) *Hierarchical ordering of information*. Information about the separate strains is not treated as though it is of equal behavioral importance.

To interpret these results, we draw on the theoretical tools of pattern recognition, a field that largely owes its existence to the digital computer (*10*). Initial work in computer-based pattern recognition can be categorized as discriminant (or decision theoretic). The central focus of this approach is classification (*11*). Patterns to be classified are input as mathematically expressible "features"; for neural networks these could be the amplitude and frequency of neuronal firings. The input features are processed by a variety of quasigeometrical and statistical methods to categorize them into similarity clusters. These clusters may be derived either with or without reference to a set of labeled (preclassified) patterns (*12*).

Although such clustering techniques are useful in many applications, they are inefficient if used in such difficult but important problems as scene analysis. Furthermore, studies on such disparate topics as mammalian vision and human speech indicate that such an atomistic approach to classification provides an inadequate description of the recognition involved in such tasks. Recent work has opened up structural (or syntactic) approaches to these problems (*13*). Structural recognition begins with features too, but these are first classified into pattern primitives that are the basis of the recognition system. This limited set of basic patterns is then input into a hierarchically organized recognition system whose output is a significant message. However, unlike the inputs, which form a very limited set, the output messages can be essentially unlimited because the repetition of basic units also conveys information. The English language, for example, combines a set of about 45 phonemes into words, words into phrases and sentences, and sentences into larger structures of meaning. Thus, a relatively limited sensory basis can provide very complex signals for cuing behavior.

In understanding olfactory stimuli, specifically pheromones, as inputs, we need to consider their mode of action. The pheromones that have been the object of most recent investigations are of the type classified by Bossert and Wilson as releasers—reception triggers a characteristic, immediate, and overt behavioral response in the affected organism (*14*). Characterization and synthesis of such releasers, together with behavioral and electrophysiological studies of pheromones and related compounds, have shown a remarkable specificity in reception and action, often coupled with a large number of receptor sites for the active compound (*15*). The recognition system appears to be discriminant (rather than structural), with classification occuring at or near the receptor level as diagrammed in Fig. 1a. Recent work on multicomponent pheromones does not contradict this basic picture, although the requirement that the compounds be present in the proper ratio does require more feature processing capability than the monocomponent system that is shown in Fig. 1b.

While releaser pheromone systems certainly show specificity and tracking behavior requires at least some quantifiability, they lack the other two properties exhibited by the *D. pseudoobscura* rare male system—ability to deal with novel information and hierarchical structure. These two characteristics are specifically present in structural recognition systems. Other structural recognition systems, such as mammalian vision, are already well known in comparative psychology. The pattern primitives in the pheromone system could be individual substances or particular concentration patterns of substances. Chemical investigations in our laboratory so far indicate the latter, and such multicomponent systems can certainly carry more information with fewer substances than monocomponent ones (*16*). These pattern primitives are then hierarchically processed according to rules laid down by the limitations of the nervous system. This processing suppresses some information [such as the proportion of *or* males when there is a type (i) advantage] on the basis of other signals present, as schematized in Fig. 1c. The females' ability to respond to a broad range of olfactory stimuli, to process these stimuli in a hierarchical fashion, and to quantitate the population by these stimuli points toward a more complex processing of olfactory cues than had previously been suspected.

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7. This procedure is standard in our laboratory to guarantee the vitality of the stocks. No chamber in this study was rejected; in 42 chambers (1008 females) no female failed to mate within 3 hours of introduction into the chamber.
8. Correction for this disadvantage is made by the formula

$$m_{\exp,i} = \frac{Nf_i}{n_{AR}f_{AR} + n_{CH}f_{CH} + n_{or}f_{or}}$$

where $m_{\exp,i}$ is the expected matings involving the ith strain of males; N = total number of matings expected; n_i is the number of males of the ith strain present, and f_i = mating frequency of males of the ith strain, relative to AR males when i and AR are present in equal numbers ($f_{AR} = f_{CH} = 1.0; f_{or} = 0.5$). For example, for 144 matings and AR:CH:*or* = 1:1:1 (48 each type), the expected ratio is 57.6:57.6:28.8 (observed ratio was 55:51:38).
9. From current data we cannot demonstrate that *or* recognition is mediated by odor. However, if it is not, the recognition system—classification, quantitation, and identification—must be significantly more complex because it must integrate data from different sensory modalities.
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Dufour's Gland: Source of Sex Pheromone in a Hymenopterous Parasitoid

Abstract. *Females of* Apanteles melanoscelus *and* Apanteles liparidis *produce a sex pheromone in Dufour's gland of their reproductive system. Males of both species exhibit premating behavior when in contact with filter paper smears of the gland of their respective females.*

Sex pheromones are known to occur in a number of hymenopterous parasitoids (*1*). However, the pheromone source has apparently not been found, although the general body surface (*2*), male pygidial glands (*3*), and thorax (*4*) have been implicated in different species. Dufour's gland, a prominent structure of the female reproductive system in Hymenoptera, is known to produce pheromones, but to my knowledge none of these has previously been identified as a sex pheromone (*5*).

I have found that Dufour's gland does produce sex pheromones in two hymenopterous parasitoids. The insect primarily investigated, *Apanteles melanoscelus*, is a small braconid wasp, which attacks larvae of the gypsy moth, *Lymantria dispar*, throughout the latter's range in North America and Europe. Both *A. melanoscelus* and *A. liparidis*, the other species used, were maintained in the laboratory on gypsy moth caterpillars.

Courtship in *A. melanoscelus* is as fol-