restricted to ADH release caused by osmotic stimuli[20]. Therefore, the possibility should not be excluded that central PGH_2 stimulated the ADH secretion.

It is assumed that hypertension and tachycardia induced by central PGH_2 is not due to evoked release of ADH, since antidiuresis in response to PGH_2 (5 nmoles) was roughly similar to that produced by ADH at i.v. doses of 100–200 μunits/animal, which did not change B.P. and H.R. In fact, a few milliunits/animal of ADH was required to elevate B.P. by 10–30 mm Hg in the ethanol-anaesthetized rat. In addition, this hypertension was associated with bradycardia (data not shown).

Cremades-Campos and Milton[18] reported that some stable analogues of PGH_2 increased B.T. but its isomer decreased it in conscious cats. We also observed that i.c.v. injected PGH_2 increased B.T. by 1.3–1.5 °C in the ethanol-anaesthetized rat. Unlike the antidiuretic effect, the thermogenic effect of 5–15 nmoles PGH_2 reached a maximum at 40–60 min, suggesting that both effects were independent of each other. In fact, PGH_2 at the dose of 1 nmole by which urine outflow, B.P. and H.R. were not varied significantly, produced the rise in rectal temperature.

Since PGH_2 injected i.v. at the dose of 15 nmoles/0.1 ml was without effect, it was unlikely that i.c.v. administered PGH_2 was transferred to the systemic circulation to reveal the effects observed by the central administration of PGH_2. The effects of i.c.v. PGH_2 were rather central in origin.

Further experiments are under way to elucidate the mechanisms for the antidiuretic and the thermogenic effects of i.c.v. PHG_2.

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Evidence for an aphrodisiac pheromone of female *Drosophila*

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Summary. We report here direct evidence for the involvement of a pheromone in the induction of male wing vibration, an important fixed action of the *Drosophila melanogaster* courtship pattern. This chemical stimulus is produced by mature females but not by mature males. The behavioral response is proportional to the pheromone concentration.

Chemical communication is a favoured way of communicating among insect individuals, especially for sexual communication which climaxes in the copulatory act[2,3]. One or both sexes produce pheromones which induce specific behavioral responses of the mate. According to their distance of action, pheromones can be classified as attracting and/or aphrodisiac substances. As far as their emission is concerned, pheromones are synthesized in specialized endocrine cells, very often under a neuro-endocrine control, and then are transported towards the exterior by specialized ducts. In some cases, pheromones may diffuse out towards the other individuals and in other cases, pheromones are sensed by direct contact between individuals. As far as their detection is concerned, specialized receptors are involved, which are able to transform the chemical signal into an electrical signal which is transferred via the sensory neurons towards a decoding central structure. More and more examples now provide evidence for not only 1 sex specific chemical but for several chemicals with active roles as well as regulatory roles (synergy, inhibition). For example in the Dipteron *Musca domestica*, a female specific attracting substance, cis-9-tricosene, was isolated in 1971 by Carlson et al.[4]. More recent studies have shown the male attracting power of a large number of cis-9 alkenes containing 19–25 carbons and the aphrodisiac role of other cuticular lipids including cis-14-tricosen-10-one and cis-9, 10 epoxytricosane[5,6].

Our deep understanding of the genetic technology of *Drosophila melanogaster* offers a valuable tool for the dissection of such a chemical communication system[7]. Once a male has sensed the presence of a female, he displays a set of fixed action patterns which have been described in great detail[8,9]. The nature of the female stimuli has also led to numerous and diverse studies whose results are not easy to interpret. As early as 1915, Sturtevant[10] observed that a hetero-pair of flies (consisting of a male and a female) mated more rapidly in vials which had previously held copulating pairs than in clean ones, a result which could not be confirmed by Ewing and Manning[11]. Shorey and Bartell observed that in a Y type olfactometer, males were attracted into the branch where a female odour had been blown and then tended to orient towards each other[12]. The involvement of female chemical stimuli has also been strongly suggested by studies of population genetics[13].

Wing vibration is the most conspicuous among the male's early courtship signals. We have chosen to concentrate on it and to define female sex appeal as the stimulus (or set of stimuli) which induces it in male courters[14]. We have recently reported the ontogenies of both the emission of sex appeal and its detection leading to the vibration response.

In particular we have observed that young males soon after eclosion have apparently the same amount of sex appeal as females. This female-like sex appeal of young males apparently vanishes about 1 day after eclosion, except among certain mutants, like apterous[14] and fruitless[15]and males which have been decapitated during infancy[14]. Our genetic approach to the problem has also led to the localization of the sex appeal focus in the ventroposterior region of the blastoderm[13,14]. However the physical nature of sex appeal was not clearly understood, although we had observed that blind males can start vibrating wings at 2 mm distance from a female and that females washed with hexane lose it, which suggested the involvement of a female specific odour (Jallon and Hotta, unpublished results). We report here direct evidence for the induction of male wing vibration by a female specific aphrodisiac pheromones.

To try to study the physical nature of sex appeal we allowed 100 flies of either sex to run for a given time (1 h) in a container of small volume (1 ml). The vial was then thoroughly washed with 0.5 ml hexane. A drop of the hexane mixture (20 µl) was then poured into a watchglass and the hexane evaporated rapidly. Each watchglass turned upside down on a clean glass plate forms a 'feminine' or a 'masculine sky' for a courtship chamber (3.5 cm^2; 0.7 cm^3). Clean watchglasses can be used in the same way in control experiments with or without hexane washing ('blank skies'). The male courters which can be introduced through a hole in the glass plate were at least 4 days of age and had been isolated within 12 h after eclosion. Their normal ability to court, especially to vibrate wings towards females was verified at least 1 day before the experiments reported below were performed. The behavior of courter males either alone or in homo-pairs (consisting of 2 males) under the various 'skines' were observed for 10 min and the cumulative time of the wing vibration was measured ($\overline{\Sigma T}$).

A) We have already reported the behavior of male homo-pairs - in the absence of any female constituent[14], and our observations were in agreement with Hall's published data[16]. We have once again performed this study under 'blank skies', again we often noticed a short vibration of one of the males which was repeated only in a few cases (4%) but never became long ($\overline{\Sigma T} = 2$ s, table).

B) Male testers were introduced individually under a 'feminine sky'. Only very short vibrations could be detected during 10 min observation periods ($\overline{\Sigma T} = 1$ s, table).

C) Male testers were introduced in pairs under a 'feminine sky'. After a certain latency they started to show bouts of vibration towards one another. A male towards which a characteristic courtship vibration was directed showed immediately very clear rejection responses: kicking and flickering of his wings. In the quantitative measurements of $\overline{\Sigma T}$, we considered only the courting vibrations. Males of different ages, from 4 days to 12 days after eclosion, were tested, but as they did not show any significant differences in their responses, the data have been pooled. We have studied 117 male couples in such conditions and have determined an average cumulative vibration time of 36 sec (table).

D) Males which had sojourned 10 min under a 'feminine sky' were then transferred under a 'blank sky' in the presence of a naive male courter which could be recognized by a morphological mutation such as *Curly*. The naive courters showed characteristic courtship vibrations towards the male treated under the 'feminine sky' ($\overline{\Sigma T} = 20$ s, table) while the latter showed only rejection responses while being courted.

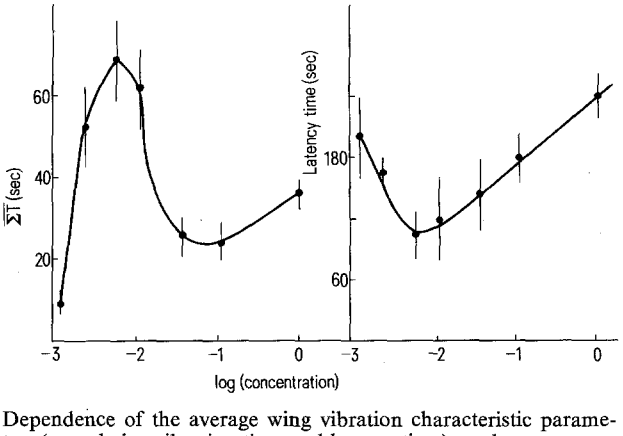
E) An experiment analogous to C was performed with homo-pairs introduced under 'masculine skies'. No significant vibration could be observed ($\overline{\Sigma T} = 2$ s, table).

This series of experiments clearly demonstrates the necessary involvement of aphrodisiac chemicals to induce the courter male's wing vibration. These aphrodisiac chemicals seem to be female-specific. The dependence of the wing vibration response upon the aphrodisiacs is shown in the figure: a clear maximum of the cumulative vibration time is obtained for about 1 one-hundredth dilution of the original extract (whose concentration has been arbitrarily chosen as unity) yielding a $\overline{\Sigma T}$ value of 65 sec to be compared with 130 sec when a real female is used[14]. A minimum value of the latency time is observed in the same conditions. At higher concentrations there is an obvious inhibition phenomenon producing both lower $\overline{\Sigma T}$ values and longer latency times. The pheromone system seems a necessary but not sufficient stimulus. Indeed, no sustained vibration could be observed with only one male courter; the presence of a 2nd fly seems to be necessary.

Preliminary gas chromatographic analysis of volatile substances from whole flies showed a special peak which is specific to females. This peak could never be observed from male materials, even when young males rich in female-like sex appeal (less than 1 day after eclosion) were studied, which suggests that the young male sex appeal[14] is linked to a different chemical. The female peak migrates very close to cis-9-tricosene, a well known component of the *Musca domestica* pheromone system[4], but we have not finished identifying it. When isolated, it induces sustained vibrations of members of homo-pairs towards each other, thus it corresponds to an aphrodisiac component of the *Drosophila melanogaster* pheromone system (Venard, Jallon, Gallois and Descoins, unpublished results). Other differences in male and female chromatograms were obvious, which had already been investigated by Hedin et al.[17].

Induction of male wing vibration by female specific aphrodisiacs. The different experimental conditions are described in the text. For each experiment, the latency time and the cumulative vibration time (ΣT) were measured during 10 min and then individual values were averaged

Group	Number of experiments	Average latency time (sec)	$\overline{\Sigma T}$ (sec)
A	43	-	2
B	54	-	1
C	117	240±40	36±8
D	26	220±15	20±3
E	37	-	2



Dependence of the average wing vibration characteristic parameters (cumulative vibration time and latency time) on the concentration of female specific aphrodisiacs. The concentration of the original extract has been arbitrarily defined as unity.

After we have determined the chemical structure of this female specific aphrodisiac, we will be able to investigate its relation with the young male sex appeal, the site and mechanism of its emission, and look for mutations that may affect either its emission or detection.

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Determination of odour affinities based on the dose-response relationships of the frog's electro-olfactogram[1]

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Summary. Electro-olfactograms (EOG's) recorded from the frog's olfactory epithelium for 11 substances were used to calculate dissociation constants which in turn serve as an index for the affinity between odorant and receptor site. These constants were calculated with and without a correction for the odour partition between water and air. For a homologous series of 7 n-alcohols these values decrease up to 1-heptanol. The dose-response relationships were based on the peaks of the EOG's since the peak/plateau-ratio was concentration-dependent for some of the substances.

Ottoson[3] compared the odorous strengths of a series of n-alcohols using the amplitudes of the peaks of the electro-olfactograms (EOG's) evoked by equimolar concentrations and using vapour concentrations of an equal stimulative effectiveness. In the present experiment dissociation constants for 11 odorous compounds, 7 of which are aliphatic n-alcohols, have been calculated using Beidler's taste equation[4]. The interaction between the assumed receptor R and the stimulus S can be written as follows:

$$S + R \leftrightharpoons SR. \quad (1)$$

The amplitude of the response r depends on the odour concentration c as follows:

$$c/r = 1/r_m (c + K_D) \quad (2)$$

in which r_m is the maximum response for the interaction between the receptor involved and a given stimulus; K_D is the dissociation constant. The latter value is given by the intercept of the concentration axis in Beidler's plot[4,5].

Tucker[6] and Poynder[7,8] found that such a representation can describe the concentration dependence of the odour-receptor interaction as reflected in the amplitude of the EOG. In this paper this concept is further developed.

In order to do so, the following implicit assumption has been made: the interaction between stimulant and receptor is at equilibrium when the peak of the EOG has been reached. The characteristic shape of the EOG consists of a peak and a plateau level. Since the latter level does not always have the same characteristics with respect to odour concentration, as will be demonstrated in this paper, this plateau level has not been used to describe the odour-receptor interaction.

Materials and methods. All frogs (n = 18) used for the present experiments were pithed. They belonged to the Dutch varieties of *Rana esculenta*[9]. Odorous chemicals of the purest grades commercially available were used as stimuli (BDH, Poole, Great Britain; Fluka, Basel, Switzerland). The odour concentrations have been calculated from the saturated vapour pressures[10].

Further preparation of the frogs and recording methods were standard. For the odour application a specially designed 'air-dilution' olfactometer was used[12]. To a relatively vast continuous air flow, serving as a carrier, controlled quantities of odorous stimuli can be added. In this way the occurrence of pressure pulses on the epithelium is prevented, since addition of odorant does not change the velocity of the flow reaching the epithelium surface. The olfactometer delivers stimuli in dilutions from 2 up to 100 times with respect to the saturated vapour. Stimuli lasted 5 sec, with interstimulus intervals of 3 min. The experiments were carried out at 22 ± 1 °C.

Results and discussion. Ratios of the amplitude of the peaks and the plateaus of the evoked EOG's were constant for acetone (figure), thus agreeing with previous observations on butyl acetate, amyl acetate, 1,8-cineole and linalool[7,8]. However, for 1-hexanol this ratio increased with the odour concentration (figure). For this substance peak and plateau overlap at low concentrations (the peak-plateau ratio approaches one). In contrast, for cumene the peak-plateau ratio decreases slightly with increasing odour concentration (figure). Other experiments in our laboratory confirmed these observations for acetone and indicated a slight increase for m-xylene (Sluyter, unpublished observations). These results show that peak and plateau levels of the EOG have not necessarily a similar dose-response relationship for the various concentrations. This suggests that at least 2 processes are involved in the generation of the EOG. The nature of the proces(ses) other