cis and *syn* all-*trans* 3-hydroxyretinal oximes were observed in the chromatogram obtained by analysis of about 400 larval brains (fig, B). Peaks of *anti* 11-*cis* and *anti* all-*trans* 3-hydroxyretinal oxime were not clear in this chromatogram possibly because of the minor amount. In addition to 3-hydroxyretinal, *syn* and *anti* all-*trans* retinal oximes were detected in the larval brain (fig. E). Contents of 3-hydroxyretinal and retinal were estimated as follows; one compound eye contains about 5 pmol of 3-hydroxyretinal and one larval brain contains about 0.01 pmol of 3-hydroxyretinal and about 0.03 pmol of retinal, respectively.

3-Hydroxyretinal is reported to be the chromophore of the insect rhodopsin[16–19]. The present HPLC analysis demonstrates that the *Bombyx* compound eye contains 3-hydroxyretinal. Geometrical isomers of 3-hydroxyretinal are also found in the larval brain, while only all-*trans* retinal was detected in this organ. Though we have not examined the reversible interchange in the amount between these isomers, which might reflect the photoconversion of a chromophore in the photoreceptor pigment in the brain, the presence of both 11-*cis* and all-*trans* isomers in the brain suggests that 3-hydroxyretinal in the larval brain is also the chromophore of a functional pigment involved in the photoperiodic response. A plausible assumption is that the pigment actually involved in photoperiodic light perception forms a complex with an apoprotein. It is unlikely that free 3-hydroxyretinal and retinal, the absorption spectra of which fall mainly in the UV-light region, function in photoreception. The photoreceptor pigment is probably a retinoid protein complex, with its main absorption in the blue-green region[7]. Further identification of the photopigment active in the photoperiodic response of the silkworm is now under way in our laboratory.

Acknowledgment. This work was supported by a Grant-in-Aid (59 540 459) from the Japanese Ministry of Education, Science, and Culture.

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0014-4754/88/010074-03$1.50 + 0.20/0

Age-related perseveration of the precopulatory behaviour in male *Drosophila melanogaster*

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Summary. In response to an interruption of their courtship, males of *D. melanogaster* exhibit a lasting sexual arousal (up to 30–60 min), expressed behaviourally by characteristic wing displays. A study of this effect centered on two 'memory mutants' of different ages suggests that it can be related to an ageing-dependent perseveration, rather than to modifications in memory processing.
Key words. Drosophila melanogaster; sexual behaviour; ageing; memory mutants.

The courtship of male *Drosophila melanogaster*[3] is normally performed in close contact with the female. However, in small observation chambers it can commonly be observed that, when a female moves off, the male does not always follow immediately. Remaining behind, he subsequently performs a few courtship movements in her absence before moving off. Brief wing displays can sometimes be seen as the male moves around the chamber and before he makes contact with the female again.

Such observations strongly suggest that, following stimulation, a male's sexual arousal does not immediately decline. It might be possible to regard this phenomenon as expressing a sexual 'central excitatory state', analogous to that described for food in *Phormia*[4] and in *Drosophila*[5] itself. We have now begun to study this persistent courtship in order to discover its extent and control.

To make reasonable measurements, we require a situation in which a male can be given limited contact with a female, who is then removed whilst observations continue on the male. We used an observation chamber made in two adjacent circular compartments, each 25 mm in diameter and 5 mm deep, separated by an opaque moveable partition. Our basic procedure was to introduce a young, virgin female (24–36 h from eclosion) into one compartment and a mature, but inexperienced male into the other. The partition was kept closed whilst the male was allowed 3 min to settle down before his locomotor activity was measured each minute, for a further 5 min. This was done by dividing the floor of the compartment into quadrants and counting the number of quadrants entered with all six legs.

Any wing movements related to courtship (i.e. scissoring or vibration) were also recorded. At the end of this period, the

partition was temporarily removed and the flies allowed to meet, then closed again when both partners were in the same compartment. Once courtship was initiated, the male was allowed 4 ± 1 contacts with wing display to the female, before she was again separated from him (either by opening the partition again and letting only one of the insects move through; or by allowing the female to exit through the top of the chamber, which had a moveable coverglass). Records were kept of latency and duration of this interrupted courtship. Following this encounter, the male's behaviour was recorded as before. In contrast to the pre-courtship period, wing display was now often conspicuous; records of this (and locomotor activity) were kept minute by minute until 3 consecutive minutes had elapsed with no wing display. The time from the separation of the female until this criterion was reached we call the 'persistence time' for sexual arousal.

We shall report elsewhere on the general features of this persistence, the effect of varying contact with the female, and how it interacts with some aspects of behavioural activation. Here we concentrate upon one set of observations; since persistence time not uncommonly exceeds 30 min, one eventuality is that persistence could reflect some kind of learning and memory of the male's contact with the female. We have therefore examined this persistence in two of the so-called 'memory mutants', in some of which sexual behaviour and experience have been shown to be affected. Thus the normally increased latency to court of males who have been kept with unreceptive females for a few days is strongly depressed in the mutant *amnesiac*[6].

In our tests, we first used the *dnc*^{2} allele of the *dunce* mutation[7]; second and mainly, a thermosensitive mutation of the *Ddc* gene (allele *ts*^{2}, first studied in a behavioural context by the group of Tempel[8]), the dopadecarboxylase enzyme (DDCase) being implicated in the metabolism of two neuromodulators, dopamine and serotonin.

Our *dnc*^{2} stock was on a Canton-S background and accordingly was compared with wild-type males of the reference strain Canton-S, stock 231 (*C-S*) kept in the same conditions: reared at 21 ± 1 °C and tested at 3–4 days of age.

The *ts*^{2} mutant of *Ddc* was reared in conditions as similar as possible to those described by Tempel et al.[8], that seem optimal to control the expression of the mutation in the adult fly. Our experimental groups (E and E_a) were maintained from egg to eclosion at 20 ± 0.1 °C, then kept at this permissive temperature during the first 3 days of their adult life; this allows the cuticle to harden and melanin to develop normally (since the decarboxylase blocked by the mutation under restrictive thermal conditions is involved in the melanin pathway). Males of the experimental groups were then placed into an incubator at 29 ± 0.1 °C for 3 days; this treatment results in an almost complete lack of serotonin in the central nervous system. These males were then removed from the incubator, placed into the observation room at 21 °C and tested the next day (group E) or when 19 ± 3 days old (group E_a). There were three control groups: first C_1, a group of *ts*^{2} males reared and held throughout at 20 ± 0.1 °C, transferred into the observation room when 6 days old, then tested there on day 7. Similarly, C_a was reared in the same way, but compared with the E_a group at the age of 19 ± 3 days. Finally, the control group C_2 were also reared at the permissive temperature of 20 ± 0.1 °C until 6 days as adults, then transferred to the observation room, but these animals were not tested until they were 10 days old. Our reasoning here was that keeping flies at higher temperatures (like the E group) has been found to accelerate ageing in some *Drosophila* species[9]. So that, if we found differences between the E and C_1 flies, they could be due, either to the mutation per se, or to differences in the physiological age of the animals. If we make the rough assumption that ageing takes place twice as rapidly at 29 °C as at 20 °C, then our 10-day-old group C_2 should control for this eventuality. For each experimental vs. control group, a total of 24 males were observed, each with one virgin female from the 'Dahomey' wild-type stock. Since there is a fairly strong diurnal rhythmicity of sexual activity, care was taken to measure equal numbers (n = 8) of males during three main periods of the flies' day; early morning (08.00–09.39 h), late morning (09.40–12.59 h) and early afternoon (13.00–17.00 h); these times refer to the start of each observation.

Results. A) Data from the *dunce* mutant. Comparison of mutant males with the wild-type *C-S* does not reveal any behavioural peculiarities of the *dunce* flies in this situation. As shown by the table, there are no significant differences between the two strains in any of the pertinent variables. Although the early morning peaks in initial activity and sexual reactivity to the departure of the female, found in the wild flies, are not seen in the mutant, no significant statistical interaction could be found between time factor and genotype for the persistence of sexual arousal.

Hence the interpretation of persistence in terms of memory processing is not supported by the results on *dunce*.

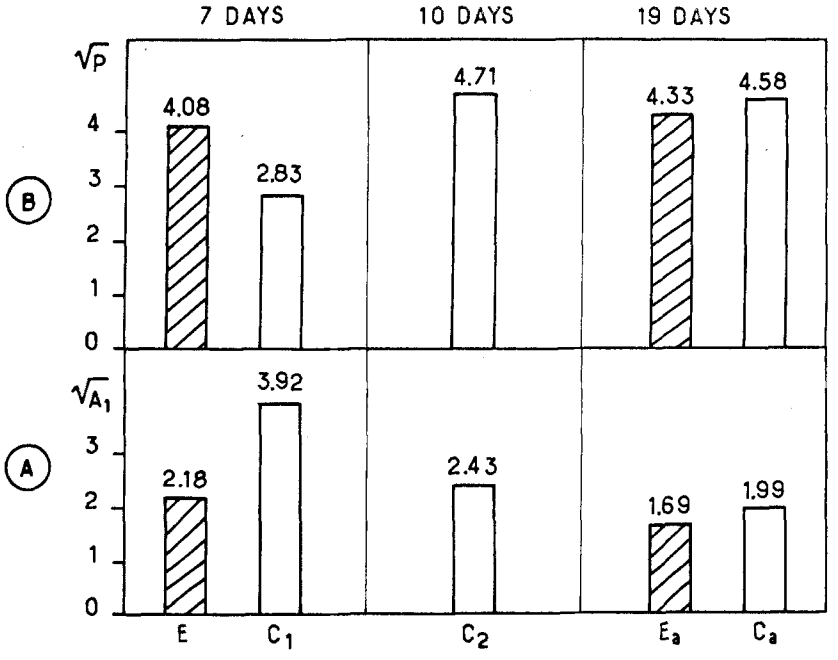
B) Data from the *ts*^{2} mutation of *Ddc*. As this study was focussed on sexual behaviour, we could not use a strain bearing a chromosomal deficiency of the structural gene coding for DDCase for such deficiencies only allow survival until pupation, at best[10]. Therefore we directed our attention toward one of the thermosensitive mutants blocking the expression of the gene *Ddc*, following a thermal shock delivered during adult life. The variables that were analysed are the same as for *dunce* (table).

1) At 7 days of age, there are no significant differences between groups E and C_1, for the time variables latency (Student's $|t| = 1.30$) and duration ($|t| = 0.66$) of the courtship bouts. On the other hand, the level of initial activity (A1 in 5 min) is significantly reduced ($p < 0.05$) by the thermal treatment (fig., A). Last, but not least, this treatment significantly lengthens ($p < 0.02$) the average persistence level (fig., B). This result is in agreement with those obtained on *dunce*; no memory mechanism seems to be involved, for the maximal persistence recorded (in a male of the E group) exceeded one hour! Alternatively, this increased persistence of the sexual arousal could suggest that the strong reduction in DDCase enzyme synthesis, especially in the CNS, induces a decrease in the inhibitory capacities which might be expect-

Performance of the mutant *dunce*^{2} in the test of sexual perseveration

	Lat.	Dur.	A1	P
dnc^2	2.18 ± 0.13	1.67 ± 0.07	3.26 ± 0.58	2.36 ± 0.33
C-S	1.97 ± 0.12	1.64 ± 0.07	3.36 ± 0.62	1.95 ± 0.26
(Student's (|t|)	(1.19: NS)	(0.31: NS)	(0.14: NS)	(0.98: NS)

3–4-day old males of the mutant strain *dnc*^{2} (n = 24) are compared with wild-type males (n = 24) of the reference strain *Canton-S (231)* here denoted as *C-S*. Values given in the first two lines are mean ± SE. The third line presents the values of Student's |t|. Lat. = log latency (s) for courtship initiation. Dur. = log duration (s) of the allowed 4 ± 1 bouts of courtship. A 1 = initial activity, measured as the square root of the number of quadrants entered by the male in 5 min before encountering the female. P = sexual persistence, measured as the square root of the male's sexual perseveration (min) after female's departure.



Sexual perseveration in *D. melanogaster* thermosensitive mutant ts^2 for the dopadecarboxylase gene (*Ddc*). Variations (A) in male's initial activity A 1; (B) in persistence of the courtship wing display. Modulations by imaginal age and thermal treatment. E, E_a experimental groups (72 h at 29 °C, between days 4 and 6 of adult life). C_1, C_2, C_a, control groups without thermal shock.

ed to damp down the remnant expression of the male's sexual arousal, after departure of the female. Such a decrease in central inhibitory effectiveness can more probably be ascribed to the serotonin deficit consequent to the expression of the ts^2 mutation than to the deficit in dopamine. However, one other interpretation (at least) remains possible; behavioural modifications consecutive to the thermal treatment of experimental males could be a result of their physiological ageing, faster than in animals of the control group C 1.

2) Therefore a second control group (C 2) was studied when 10 days old. If ageing per se is effective, an increase in sexual persistence could be expected, as in our 7-day-old experimental flies. Contrary to our expectation, this result actually emerges very clearly from our data (fig., B); the average sexual persistence is significantly increased in the C 2 flies, as compared with the C 1 group; $m = 4.71 \pm 0.39$, against 2.83 ± 0.39 ($p < 0.002$). The mean level of C 2 even exceeds that found in group E (4.08 ± 0.33), although this difference is not significant. On the other hand, no statistically significant differences between the groups could be found, either in the level of initial activity (even though the mean value of this variable is lowered in the C 2 group, i.e. in the older flies), or in the time variables (latency vs duration of the permitted courtship bouts).

3) To investigate further the role of ageing, we were able to compare two still older groups (E_a vs C_a, see above). These groups, treated exactly as were E and C 1, were tested for sexual persistence at 19 ± 3 days of age. By this time, about 13 days had elapsed since E_a had been subjected to the heat treatment. The fact that there were no significant differences between E_a and C_a in latency or duration of courtship mean activity (fig., A) or mean persistence (fig., B) suggests that there are no long-lasting effects of heat treatment per se. Compared with younger flies, the 19-day-old males do not show increased mean persistence (fig., B) but nevertheless they do have the highest individual values; 37.5% of them exceeded 30 min, compared with 29.2% of 10-day-old flies and 10.4% of 7-day-olds ($\chi^2 = 9.67$, 2 df: $p < 0.01$).

C) Age-related effects in a wild strain. Finally, to investigate whether these rapid ageing-like effects could result from some specific characteristics of the *Ddc-ts*^{2} genotype, we also compared two samples of wild-type males (*C-S*, n = 24 per sample), 3–4 vs 13 ± 1 days old (rearing temperature 21 ± 1 °C). The age-related differences found in this case compare quite well with those discovered in *Ddc-ts*^{2}: 1) No significant differences in latency vs duration of the interrupted courtship. 2) A decrease in initial activity (A 1), even more pronounced than in the mutants. 3) Once more, a significant increase ($p < 0.05$) of the sexual persistence with age; hence this effect seems more probably to be related to ageing itself than to a more specific intervention linked to the level of synthesis of the enzyme DDCase; although the two interpretations are by no means contradictory.

Discussion and conclusions. These results, taken as a whole, suggest that ageing may be an important factor which increases the regular persistence of sexual arousal following loss of contact with a female. In some ways, one is reminded of one effect of human ageing described by Luria [11] as a special loss of the ability to inhibit the perseveration of some behaviour patterns. The differences that we observed in males carrying the *Ddc* ts^2 mutation between those flies in which the temperature sensitive gene was operating and those in which it was inactivated appear to be an indirect outcome of an accelerated ageing at high temperature, even in comparatively young adults. They are unlikely to be due to the specific action of the gene on the level of synthesis of serotonin in central neurons. However, it is worth stressing that the sexual perseveration observed does seem to be more pronounced in the ts^2 mutants than in the wild-type males. For instance, 10-day-old mutants of the group C 2, although untreated by heat, show an average level of sexual persistence markedly higher than in 13-day-old wild males; 4.71 ± 0.39 vs 2.92 ± 0.35 ($|t| = 3.42$; $p < 0.001$). This divergence may be ascribed to a more rapid ageing process in the mutant flies, even if very incompletely expressed in flies reared at a permissive temperature. It is very likely that one aspect of ageing, at a neurochemical level, may consist of less

effective synthesis of some neuromodulators. On the other hand, neither our results on *dunce*[2], nor those bearing on the *ts*[2] mutant of the gene *Ddc* support an interpretation of the 'sexual perseveration' effect in terms of mnesic deterioration. This is not the first time that the generality of impact of some 'memory mutations' has to be qualified[12-14], especially as far as the *dunce* mutation is concerned.

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2 Acknowledgments. This research was supported by an exchange grant of the Royal Society. We are grateful to Dr Linda Partridge for advice and loan of apparatus.
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0014-4754/88/010076-04$1.50 + 0.20/0

A source of cutaneous maternal semiochemicals in the mink?

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Summary. Unique hypertrophic apocrine sweat glands are described in the neck, perineal and inguinal skin of mink kits. These glands enlarge after birth, only to regress rapidly and become vestigal by weaning. No similar phenomenon has been recognized before in mammals. Behavioral studies indicate a possible role for the glandular secretion in maternal recognition of the young.
Key words. Mink; apocrine glands; pheromones; maternal behavior; semiochemical.

Maternal care in mammals depends upon the exchange of a variety of sensory stimuli between the mother and her young and is further influenced by the endocrinological status and prior experience of the dam[1-4]. Olfactory cues provided by semiochemicals are likely the most important of the sensory stimuli mediating the mother-young interaction[2-5]. The olfactory cues which mediate the attraction of the young to their mother have been identified in some species; for example, a 'maternal pheromone' has been described in the feces of the female rat[6-9]. Little is known, however, of the source or nature of olfactory cues which emanate from the young to influence maternal behavior and/or offspring recognition. This paper describes hitherto unrecognized hypertrophic cutaneous adnexal glands in the young mink and reports behavioral studies supporting a putative function in chemical communication.

The cervical apocrine glands were first identified upon histological examination of skin from mink kits suffering a fatal staphylococcal dermatitis known as 'pimply kit' disease[10]. The unusual prominence of these glands prompted a histologic and morphometric study of their development during the neonatal period. Four mink kits, 2 of each sex, were sacrificed during the first week of life and at weekly intervals up to 6 weeks (the age of weaning). Mink were skinned and the subcutaneous surface was examined to determine the extent and location of the glands. Skin samples from dorsal neck, inguinal and perianal areas from fetal mink, kits and adults were fixed in 10% buffered formalin. Tissues were processed routinely for histological examination and stained with hematoxylin and eosin (H&E). The area occupied by the cervical glands was measured by tracing the perimeter of all sections of glands lying beneath a standard length of epidermis using a Zeiss Videoplan. Means of total glandular area were calculated for each of 3 sites (cranial, middle or caudal cervical) and for age of mink (newborn, 1, 2, 3, 4, and 5 weeks). The mean areas were log transformed to stabilize error variance and analyzed as a split plot design with the whole units in a completely random arrangement[11].

The cervical region of the newborn mink is very prominent (fig. 1a). The skin is palpably thicker than that of other body regions and is covered by fine, yellow, crusts, presumably dried glandular secretion. The cervical gland is situated on the dorsolateral aspect of the neck and extends from the occiput to the thorax. It is light yellow-brown and the subcutaneous surface is finely nodular (fig. 1b). The gland is present in the fetus. The cervical gland diminishes as the young mink grow and is inapparent macroscopically at 6 weeks.

Histologically, the gland is composed of coiled tubular apocrine glands which show morphological evidence of active secretion (fig. 1c, d). By comparison, at 5 weeks of age, the glands are virtually inactive (fig. 1e). All glands undergo devolution by apoptosis, or programmed cell death (fig. 1f). Morphometric assessment shows that the tubular area of the cervical glands increases markedly over the first 2 weeks of life before decreasing (fig. 2). Glandular area varies significantly with age of mink and site of sampling ($p < 0.0001$). The cervical glands do not exist in the adult, although regular apocrine sweat glands are present throughout the haired skin.

Small foci of hypertrophic apocrine sweat glands, similar to those in the cervical region, occur sporadically in the inguinal and perianal skin of neonatal mink. The latter are not equivalent to the perianal glands associated with scent production in sexually mature animals. These scent glands are rudimentary in neonates.

Behavioral experiments were designed to test the hypothesis that secretion from the glands may function in maternal recognition of the young. Experiments were performed at a commercial mink farm. Secretion from the cervical glands of live 5–15-day-old mink was collected onto unscented absorbent cotton pads by gentle skin massage for 5 min (Neck). Control samples were collected by the same procedure, per-