

## REVIEW

# THE FLEXIBLE FLY: EXPERIENCE-DEPENDENT DEVELOPMENT OF COMPLEX BEHAVIORS IN *DROSOPHILA MELANOGASTER*

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### Summary

We demonstrate that *Drosophila melanogaster* provides a rich model system for studying behavioral development. Two additions to the many well-known advantages of this species are exploited here. First, as in mammals and higher vertebrates, early experience affects behavioral development of *Drosophila*. Second, the affected behaviors are complex and yet readily studied in the laboratory. Thus, *Drosophila* can be used to study the developmental mechanisms by which organisms can optimize their behavioral repertoires to enhance their chances for survival. Evidence that early experience affects development of preferences for specific visual patterns, male courtship and female responsiveness to courting males is reviewed; in each case, experience modifies responses to behavioral targets. Our results demonstrate that developmental plasticity allows adjustment of intrinsically determined responses to visual targets so that they can take into account the actual characteristics of the developing animal's environment. Furthermore, plasticity makes it possible to introduce 'cultural' and 'social' elements into courtship and mate choice in insects. This previously unrecognized role for developmental plasticity in insects has broad theoretical and practical implications.

### ***Drosophila*: a model system for studying developmental behavioral plasticity**

Early experience can affect the development of the brain and behavior of vertebrates. Exposure to an impoverished environment typically diminishes performance of the adult, whereas exposure to an enriched environment can have beneficial consequences. Growing up in total darkness retards the development of visually guided behavior of cats (e.g. Timney *et al.* 1978). In contrast, being housed in an enriched environment rather than in standard laboratory cages leads to improved performance of rats in learning tests (Greenough, 1976). Similarly, deprivation adversely affects human children's performance on intelligence tests (Dennis, 1973), whereas enrichment can have long-

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term beneficial effects (Tamis-LeMonda and Bornstein, 1987). The consequences of early experience can be very specific. Exposing cats or rats to a visual environment consisting of lines of only one orientation produces long-lasting changes in the cues to which the animals will attend (Hirsch, 1985; Tees *et al.* 1980). Exposure to appropriate stimuli early in life also has specific and dramatic effects on the behavior of adult birds. For example, some species of songbirds must hear species-specific songs during early development if they are to sing normally when they become sexually mature (Marler, 1987, 1991), and exposure to specific imprinting stimuli during a bird's early life can affect its choice of mates (Immelmann, 1972).

At which anatomical sites and by what mechanisms does early experience produce long-term changes in adult behavior? Brain structures that are affected by early experience have been identified in birds, and biochemical, physiological and morphological changes in those structures have been associated with song-learning and imprinting (Bradley and Horn, 1987; Nottebohm, 1991). The behavioral effects of early experience or deprivation have also been related to neural changes in mammals (Hirsch, 1985; Greenough, 1988; Fregnac and Shulz, 1994). Nonetheless, we need to examine a broader range of organisms to find additional model systems for studying both the functional significance of experience-dependent changes in the nervous system and the underlying cellular and molecular mechanisms. Preferably, these should be systems in which it is possible to elucidate the effects of experience at many different organizational levels in the nervous system.

Developmental plasticity was long thought to be an exclusive characteristic of mammalian brains, but for more than a decade evidence has been mounting that the nervous systems of lower vertebrates and invertebrates can exhibit similar plastic phenomena (e.g. Murphey, 1986; Lnenicka, 1991; Meinertzhagen and Hanson, 1993; Schmidt, 1994). Several years ago, we realized that the common laboratory fruitfly (more correctly, pomace fly), *Drosophila melanogaster*, provides numerous advantages for an analysis of both neuronal and behavioral developmental plasticity. First, adult flies perform dozens of simple and complex behaviors in laboratory settings. Second, flies have a short generation time (10 days at 25°C) and are easy to culture, making it possible to manipulate their early exposure readily. Third, large numbers of flies can be maintained under the same environmental conditions at different stages of the life cycle, making it possible to look for critical periods during which early experience can have effects on the nervous system and on behavior. Fourth, geneticists have isolated hundreds of single-gene mutations that disrupt the development and function of the *Drosophila* nervous system (Hall, 1982; Lindsley and Zimm, 1992), which can be used as molecular scalpels to disrupt, and thus study, the effects of experience on neuronal and behavioral development.

The first step in utilizing the potential offered by *Drosophila* was to determine whether adult behavior of this species shared with that of vertebrates the property of being influenced by early experience. We focused on the preference for specific visual patterns that walking flies of either sex exhibit, the courtship that male flies perform in response to sexually attractive virgin females, and sexually receptive females' opening of their vaginal plates to permit intromission in response to a courting male's attempt to copulate.

The advantages of these behaviors are that much is already known about the behaviors themselves, the stimuli to which the flies respond when performing the behaviors and the manner in which the central nervous system functions during the execution of these behaviors. Furthermore, although responses to visual targets can be measured within 24 h of eclosion, and are thus likely to be determined intrinsically, it is possible that visual experience adjusts and stabilizes this behavior, as it appears to do for localization of targets in some vertebrates (e.g. Knudsen, 1987). The other two behaviors mature gradually during the first few days of the adult stage; flies do not become fully competent to perform courtship or indicate their receptivity to copulation until several days after they eclose from their pupal cases (Manning, 1967; Ford *et al.* 1989). It is possible that experience before and during the appearance of these behaviors could play a role in their development, much as it does for singing and mate choice in birds.

Will any conclusions from laboratory studies be applicable to flies in a natural setting? In their natural habitat, flies utilize visual cues to orient themselves within their environment (Heisenberg and Wolf, 1984); at food sources, where flies of this species engage in sexual behavior, males perform courtship and females respond to courting males as they do in laboratory settings (see Spieth and Ringo, 1983; Tompkins, 1984). Thus, we are confident that what we are studying in the laboratory are manifestations of a neural plasticity that has been selected because it is advantageous in the flies' natural habitat.

### **Early experience affects responses to visual patterns**

#### *The behavior*

Hecht and Wald (1934) first studied the 'reflex response' to a large array of vertical stripes by allowing a fly to go back and forth along a very narrow glass corridor paralleling the pattern; movement of the pattern in the direction in which the fly was walking caused the fly to stop, proceed backwards, and then turn and head in the opposite direction. By varying the spatial frequency and intensity of the pattern, they were able to measure the visual acuity of *Drosophila*, which they then related to the morphology of the compound eye. Their thoughtful approach to studying the visual responses of *Drosophila* set a foundation for much of the work that has followed.

Flies' responses to visual targets can be studied in a large, cylindrical arena that has black vertical stripes mounted on its white interior wall (Fig. 1). A fly that has been rendered flightless and is released on the floor of such an arena usually walks rapidly towards the patterns (Horn and Wehner, 1975; Wehner, 1972; Wehner *et al.* 1969). Once the fly reaches the wall, it is likely to climb up it, often proceeding along the pattern or its outer edge (Osorio *et al.* 1990).

Vertical stripes are useful as stimulus patterns because they elicit two different responses. First, the flies appear to use vertical patterns to help them maintain a constant heading (Wehner and Wehner-von Segesser, 1973; see also Collett, 1988). Thus, flies may use an interocular difference in the movement of the pattern across the receptor surfaces as a signal that they have made a turn, i.e. that there has been a change in course (Wehner and Wehner-von Segesser, 1973). In addition, flies are attracted to stripes,

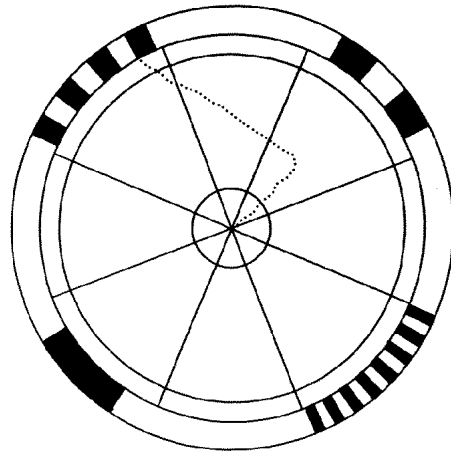


Fig. 1. Cylindrical arena (61cm tall and 51cm in diameter) for testing visual choices of walking flies. The four sets of patterns consist of black vertical stripes of different widths (one single wide stripe, two stripes, four stripes and eight narrow stripes; the two, four or eight stripes together are as wide as the single stripe). The wall is blank between patterns. Since the white stripe separating any two stripes in a pattern is as wide as the black stripe on either side, the overall luminance of the four patterns is the same. The patterns are positioned  $90^\circ$  apart so that flies will not be likely to head for the blank spaces between them. The dotted line shows a path taken by a fly. The sector in which the fly crossed the outermost concentric ring, which is 90% of the way from the arena center to the wall, was recorded. The fly shown started to head towards the two-stripe pattern, then turned and walked to the four-stripe pattern. Adapted from Fig. 1 of Hirsch *et al.* (1990).

which can serve as targets. The flies' final destination, as indicated by their pathway, suggests that they are attracted by the darkest region in their visual field (i.e. they are exhibiting scototaxis; Osorio *et al.* 1990).

If two patterns are present on the cylinder wall, the flies appear to make a choice. When the two patterns are less than  $60^\circ$  apart, they tend to head for the space between them (Horn and Wehner, 1975); when they are more than  $60^\circ$  apart, they tend to approach one pattern or the other. By varying the characteristics of two or more patterns that are at least  $60^\circ$  apart, it is possible to determine quantitatively the effects of stimulus variables, such as stimulus width and number, on flies' preferences (Wehner, 1972; Hirsch *et al.* 1990).

#### *The effects of visual deprivation*

Early experience affects the responses of flies (Hirsch *et al.* 1990; Mimura, 1986, 1987) and of other insects (e.g. crickets, Campan *et al.* 1987; Meille *et al.* 1994) to visual patterns as well as the responses of crickets to sound stimuli (Shuvalov, 1990). Experience may thus play an important role in the development and/or maintenance of the neuronal mechanisms that govern responses to visual (and auditory) targets.

To study the effects of early visual experience on flies' preferences for black vertical stripes, two groups of *D. melanogaster* were raised from the egg stage, a control group housed in normal cycling illumination (12h of light followed by 12h of darkness, 'light-

reared') and an experimental group kept in total darkness ('dark-reared') (Hirsch *et al.* 1990). Flies from the two groups were placed one at a time at the center of a cylindrical arena (Fig. 1), which had four sets of black vertical stripes mounted on its white inside wall. Each fly was observed for up to 2min to determine whether it approached the cylinder wall and, if so, which set of vertical bars or intervening blank wall segment was approached. Most subjects were from the flightless mutant strain *raised* (Mahaffey *et al.* 1985) chosen for the ease of testing. For comparison, flies from the Canton-S normal laboratory strain which 1h prior to testing had had their wings clipped under CO<sub>2</sub> anesthesia were also studied.

Irrespective of the rearing conditions, most flies of either strain walked to the one-, two- or four-stripe patterns, avoiding both the eight-stripe pattern and the blank wall between patterns. Thus, development of the visually guided approach to patterns on the cylinder wall does not require that flies be given prolonged exposure to light during early life. The flies' responses to targets in the arena could be tested within 24h of eclosion. At that point in development, the preferences of the light-reared flies (which had received 12h of light exposure as adults) and of the dark-reared flies (which had received between 1 and 2h of light exposure as adults and none as larvae or pupae) were indistinguishable (Fig. 2; Hirsch *et al.* 1990). However, the flies' responses changed as the duration of the dark-rearing and the age at the time of testing increased. The distribution of responses to

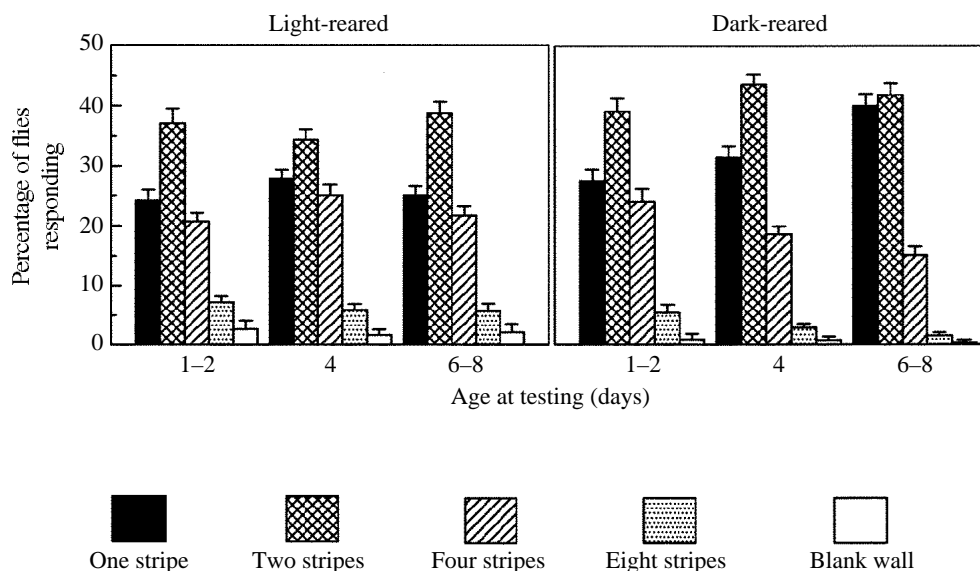


Fig. 2. Averaged responses of light- and dark-reared *raised* flies tested at different ages of adult life to the four patterns presented in the test arena shown in Fig. 1. Responses to the blank wall are also shown. Note that responses of the light-reared flies (left) are quite stable, as are responses of the dark-reared flies (right) to the two-stripe pattern. Responses of dark-reared flies to the one-stripe pattern increase with age and/or duration of the deprivation, whereas responses to the four- and eight-stripe patterns and to the blank wall decrease. Error bars indicate s.e.m.,  $N=1020$ , ages 1-2;  $N=1697$ , age 4;  $N=1229$ , ages 6-8.

the four patterns for light- and dark-reared *raised* flies at 1, 2, 4, 6 and 7–8 days of adult age (Hirsch *et al.* 1990) revealed that differences between light- and dark-reared flies gradually developed over the course of the first 8 days of adult life. These differences reflected changes in the dark-reared flies and not in the light-reared ones. As Fig. 2 shows, responses to the four sets of patterns and the intervening blank wall remained relatively constant over the first 8 days of adult life for the light-reared *raised* flies. Similarly, responses to the two-stripe pattern remained relatively constant throughout this period for the dark-reared flies. In contrast, responses to the one-stripe pattern increased with age for the deprived flies, while responses to the four- and eight-stripe patterns and to the blank wall decreased.

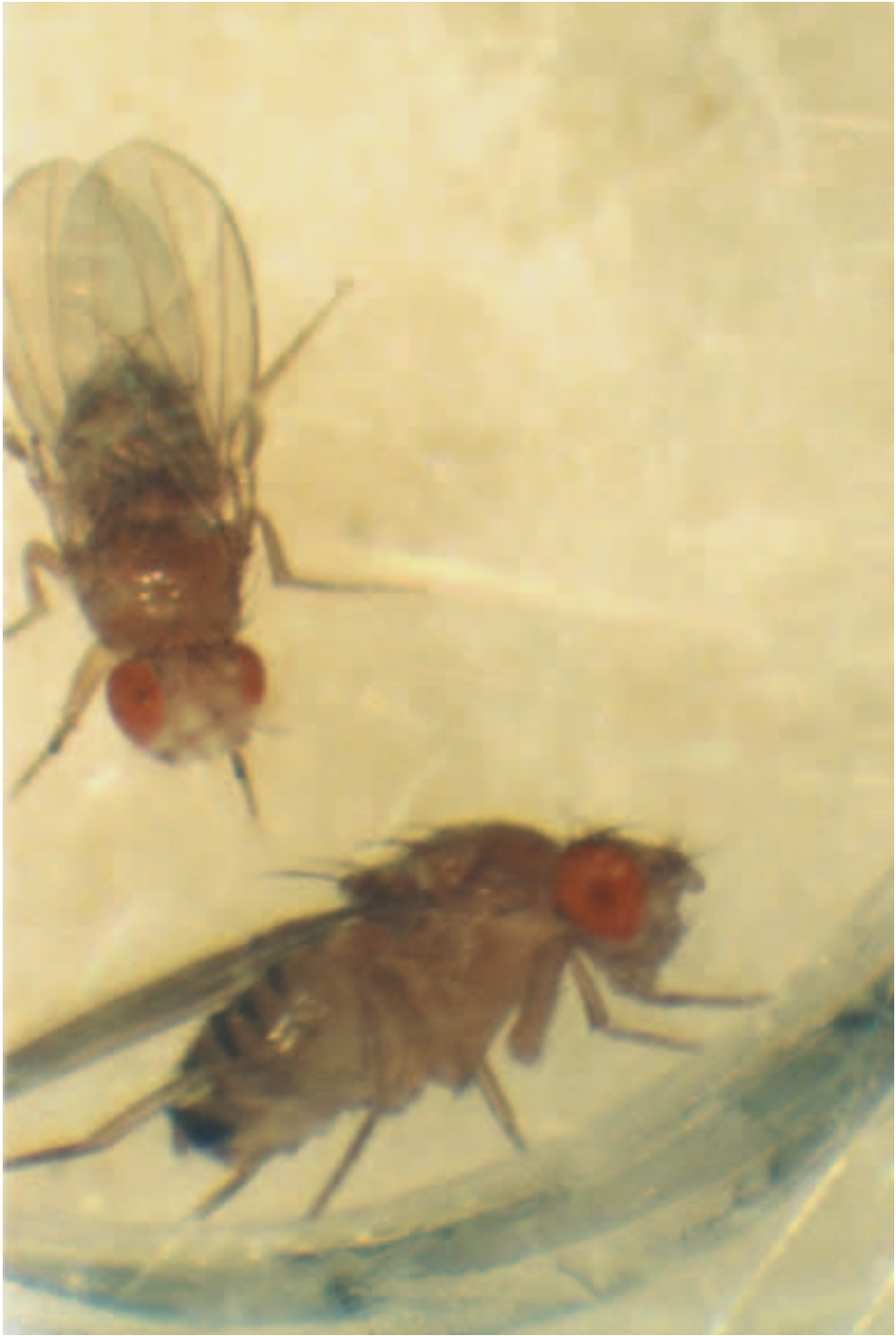
The timing as well as the duration of the deprivation is important (Hirsch *et al.* 1990). First, darkness alone for several days before testing was not sufficient to produce a behavioral change; flies reared in normal illumination until day 4 of adult life and then kept in total darkness for the next 6 days did not differ in their preferences from 10-day-old light-reared controls. Second, dark-rearing during larval and pupal stages did not have as pronounced an effect on visual preferences in the adult stage as it did during the first 4 days of adult life. These results suggest that there is a ‘critical period’ during the first few days of *Drosophila* adult life for the effect of visual deprivation on pattern preference.

*Experience may adjust response characteristics of developing sensory systems*

The environment poses challenges and provides opportunities to all species. Each individual’s behavior in a given situation represents its response to those challenges and opportunities and can be thought of as the product of a unique combination of information – some gathered by past generations, the rest during the individual’s life span. This information is used by the programs that construct and modify the organism’s nervous system (e.g. Hirsch, 1985; Hirsch and Tieman, 1987; Lnenicka *et al.* 1991; Schmidt, 1994). By examining the ‘design characteristics’ of sensory systems, we may gain some insights into the functional significance of developmental plasticity.

One approach to the design of a sensory system is to optimize its function by using past and current inputs to predict those in the future. For example, sensory neurons have a limited response range (e.g. the voltage range over which receptor potentials can change), and this response range must in some way be matched to the range over which stimuli actually vary in the animal’s environment. Information theory provides one approach to optimizing this matching; it is based on knowing the relative frequency with which different stimuli occur in the organism’s sensory world (Laughlin, 1981). Such information about the organism’s world can be transmitted either genetically or by allowing the developing organism to ‘sample’ the actual inputs to which it is exposed during early life, then using this sample to match the response range of cells in its sensory pathways to the characteristics of its early environment (Laughlin, 1984). The neurophysiological effects of exposing cats to a limited range of stimulus orientations during early life are consistent with such a hypothesis (Hirsch, 1985).

In this regard, we note that, in light-reared *Drosophila*, responses to the four sets of visual patterns remain relatively constant over the first 8 days of life. In contrast, when dark-rearing of *Drosophila* continues for more than 1 or 2 days of adult life, deprived flies



increase their number of responses to the one-stripe pattern and decrease their number of responses to the four- and eight-stripe patterns as well as to the intervening blank wall. The number of responses of dark-reared flies to the two-stripe pattern remains relatively constant. Dark-rearing thus shifts at least some of what seem to be intrinsically set preferences for vertical targets. Ultimately, these preferences must stabilize, since dark-rearing flies from 6 to 10 days does not produce any shifts in preferences (Hirsch *et al.* 1990). Whether experience plays a role in this stabilization, as it may in vertebrates (Hirsch and Tieman, 1987), remains to be determined. The neural basis of such stabilization may be related to changes in the density of feedback synapses, which occur in the fly visual system during the first 4 days of adult life (Meinertzhagen, 1989; Kral and Meinertzhagen, 1989; reviewed in Meinertzhagen and Hanson, 1993).

### Early experience affects courtship

#### *The behavior*

The courtship of *Drosophila melanogaster* males was first described by Alfred Sturtevant (1915), one of the founders of modern genetics. Sturtevant noted that males from the species *D. ampelophila*, as *D. melanogaster* was then called, extend and vibrate one wing in response to females, lick the female's genitalia and attempt to copulate by curling their abdomens under their thoraces. If the flies are observed in shallow acrylic courtship chambers (volume approximately 2cm<sup>3</sup>) with a dissecting microscope, it is apparent that male courtship is more complex than Sturtevant realized. When an unanesthetized male and virgin female are transferred into an observation chamber with an aspirator, the male initiates courtship within a few seconds; he approaches the female, orients his body so that he is facing the female's abdomen while standing beside her, and briefly vibrates his own abdomen. He then taps her abdomen with one of his foretarsi (Fig. 3) and vibrates the wing that is closest to the female in order to produce a courtship song. If the female runs away, the male maintains contact with her, pivoting to maintain his orientation relative to her abdomen ('orient back' behavior) or following her from behind. As Sturtevant noted, a male that has performed several minutes of courtship will usually lick the female's genitalia and attempt to copulate; in addition, the male often performs 'orient front' behavior, in which he moves in semicircles in front of the female. Analysis of gynandromorphs (male-female mosaics) has revealed that a small group of neurons on one side of the dorsal posterior brain is required to be male for orientation, following, tapping and wing extension. For wing vibration, there is an additional requirement for male neurons in the thoracic ganglion (Hall, 1977, 1979; von Schilcher and Hall, 1979). If a female is stimulated by a male's courtship to be receptive to copulation, she will respond to one of the male's copulation attempts by opening her

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Fig. 3. A *Drosophila melanogaster* male (top) courting a conspecific female. The male is performing two courtship behaviors: he is pivoting to face the female's abdomen as she runs along the side of the observation chamber ('orientation') and he is extending his left foreleg to tap her abdomen ('tapping'). Photograph taken by Laurie Tompkins with a Yashica FX-D SLR camera, focused through an aus Jena Citoval stereomicroscope, using Fujicolor 400 film. Magnification approximately 70×.



vaginal plates to permit intromission. Analysis of gynandromorphs has revealed that a fly must have a small group of female neurons on both sides of the dorsal anterior brain to mate with a courting male (Tompkins and Hall, 1983).

Courtship behavior has a complex structure. First, it is not a continuous process, but occurs in bouts. Irrespective of whether he is ultimately successful in stimulating the female to be receptive to copulation during the observation period (typically 10–15min), a *D. melanogaster* male usually performs several bouts of courtship, each lasting from a few seconds to more than a minute, and attempts copulation at least two or three times. To quantify the vigor with which a male performs courtship, it is useful to define a *courtship index* (the percentage of time during the observation period, or before mating if it occurs, that a male performs courtship).

Second, courtship bouts are not invariant. Different females elicit courtship that varies with respect to the timing and duration of the component behaviors and the sequence according to which one component follows another. Markow and Hanson (1981) developed a multivariate courtship profile to describe and quantify the transitions between different courtship behaviors. By studying courtship performed by mutant sensory-deficient males in response to wild-type females, Markow (1987) was able to demonstrate that visual input and, to a lesser extent, chemical stimuli associated with females are very important to males and affect the transitions between different stages of courtship and the probability of copulating (reviewed by Tompkins, 1984). For their part, female flies are stimulated by males' courtship to open the vaginal plates so that an attempted copulation by the male can result in intromission. Analysis of mutant sensory-deficient females' responses to normal males and normal females' responses to wingless males has revealed that auditory stimuli produced when males vibrate their wings play a major role in stimulating females' receptivity to copulation, whereas chemical stimuli associated with courting males have relatively minor effects (von-Schilcher, 1976; Tompkins *et al.* 1982; Markow, 1987).

To describe courtship further, its outcome can be quantified in two ways. First, one can define a population's *copulation frequency* (the percentage of pairs that initiate copulation during an observation period). Second, one can define a male–female pair's *copulation latency* (the interval between the time that the male starts courting and the time that the flies begin to mate, if they copulate during the observation period). Copulation latencies and frequencies are affected by the age of the flies (3- to 6-day-old flies have the highest copulation frequencies and the lowest copulation latencies); by the temperature and humidity (for flies that were raised at 22 or 25°C: testing temperatures of 22–25°C and moderate humidity are optimal); by the time of day (the proportion of pairs copulating rapidly peaks during a 1h 'window' 6h after sunrise); and by the social and experiential factors described below.

#### *Visual deprivation and mate choice*

To study the effects of visual deprivation during adult life on courtship behavior, two males, one light-reared (raised throughout life in normal cycling illumination) and one dark-reared (raised in the same cycling illumination until eclosion, then maintained in total darkness during the first 4 days of adult life), were placed into a courtship chamber

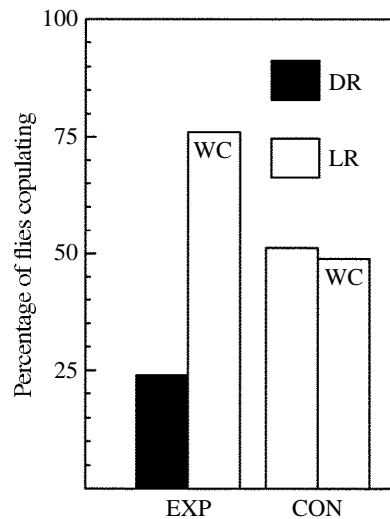


Fig. 4. Mean copulation frequencies of dark- and light-reared males competing for light-reared females. EXP, copulation frequencies of dark-reared (DR), intact males were significantly different from those of light-reared (LR), wing-clipped (WC) males. CON, wing-clipping cannot account for this difference, since results for intact and wing-clipped light-reared males were not significantly different.  $N=88$  per group.

along with a single light-reared female. To identify the male, if any, with which the female copulated, each triad was observed for a maximum of 15min, during which time the female elicited courtship from both males. To distinguish the light-reared males from the experimental males, they were tagged by ‘wing-clipping’ (amputating the distal tip of one wing during the first day of the adult stage), which did not have a significant effect on either copulation frequency or latency (H. V. B. Hirsch, M. Barth, L. Sun, H. Sambaziotis, M. Huber, D. Possidente, H. Ghiradella and L. Tompkins, in preparation). If the female mated during the observation period, her copulation latency was calculated. Males that had been deprived of light during the first 4 days of the adult stage were significantly less likely to copulate than were the control males (Fig. 4). Thus, dark-rearing of males for the first few days of adult life has a dramatic effect on their courtship success in a competitive situation (H. V. B. Hirsch, M. Barth, L. Sun, H. Sambaziotis, M. Huber, D. Possidente, H. Ghiradella and L. Tompkins, in preparation).

To see whether the effects of deprivation could also be demonstrated in a non-competitive situation, individual males were observed with individual females (Barth, 1994). The exposure conditions were varied during the first 4 days of adult life for both males and females. In the control group, males and females were light-reared; in the three experimental groups, one or both members of the pair were dark-reared. In those experimental groups in which the two members of a pair had received *different* visual exposure as adults, copulation frequencies were lower and copulation latencies were longer than for the control group (H. V. B. Hirsch, M. Barth, L. Sun, H. Sambaziotis, M. Huber, D. Possidente, H. Ghiradella and L. Tompkins, in preparation). The deficit

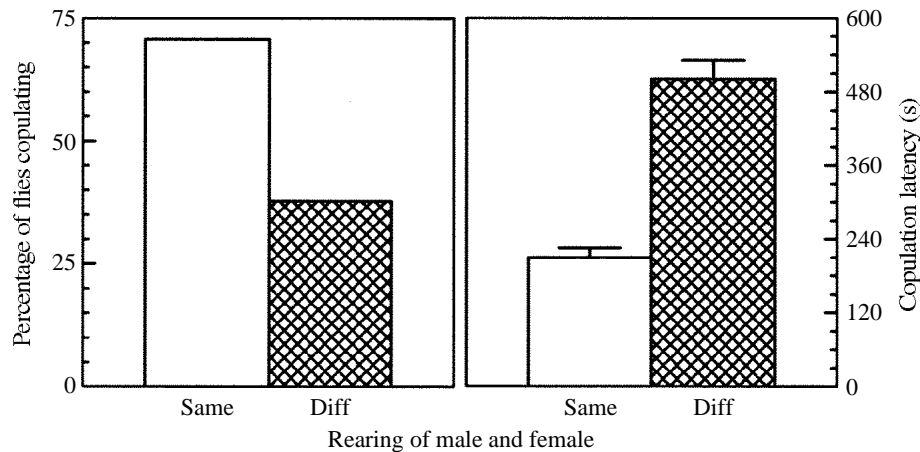


Fig. 5. Mean copulation frequencies and latencies for pairs of flies in which the male and female had the same (Same) exposure history (both light-reared or both dark-reared) and for pairs in which the male and female had different (Diff) exposure histories (one light-reared and the other dark-reared). Pairs having the same exposure history were more likely to copulate and did so more rapidly. Error bars indicate S.E.M.,  $N=116$ , Same;  $N=122$ , Diff.

resulting from dark-rearing was approximately the same whether it was the female or the male that had been deprived. Surprisingly, when *both* the males and the females were dark-reared, their copulation latencies and frequencies were indistinguishable from those of the control group in which both flies were light-reared (H. V. B. Hirsch, M. Barth, L. Sun, H. Sambaziotis, M. Huber, D. Possidente, H. Ghiradella and L. Tompkins, in preparation) (Fig. 5). Thus, visual deprivation affects both the courtship responses of males and the likelihood that females will open their vaginal plates to permit intromission. Moreover, the effects are complementary in the two sexes and, for each sex, the consequences of dark-rearing depend on their partner's exposure history.

#### *Homosexual courtship and adult males' copulation success*

The courtship targets of *Drosophila melanogaster* males are not always virgin females. When males first eclose from their pupal cases, they are as attractive to older, sexually mature males as are virgin females (Tompkins *et al.* 1980; McRobert and Tompkins, 1983). After 4h, males gradually lose their 'sex appeal'; by the time they are 2–3 days old, they elicit very little courtship (Curcillo and Tompkins, 1987). During the time that males lose their sex appeal, they also become sexually mature, in the sense that they can perform the aforementioned courtship behaviors and copulate with females (Ford *et al.* 1989).

Since performance of courtship entails an expenditure of time and energy and renders the courting male vulnerable to predation, the evolutionary significance of homosexual courtship was not obvious. Accordingly, to determine whether immature males' experience of being courted by older males affected their behavior as adults, two groups of males were raised. In one group, isolated males were housed in individual vials from

Table 1. Mean copulation latencies and frequencies for young males exposed to homosexual courtship from either intact forked males or from wingless (and thus non-singing) forked males, or raised in isolation without exposure to any other males

Experience	Copulation latency (min)	Copulation frequency (%)
Intact males	5.89±0.6	82
Wingless males	9.72±1.6	80
None	12.26±1.9	76

Copulation latencies are shortest for males courted by intact males and longest for males raised in isolation; copulation latencies for males exposed to wingless males are intermediate.

There are no significant differences in mean copulation latencies among the three groups. Taken from McRobert and Tompkins (1988).

Values for copulation latency are mean ± s.e.m.,  $N=50$  per group.

the time they were larvae until they were 5 days old; in the second group, newly eclosed males were confined with 3–5 mature *forked* males (*forked* is an X-linked mutation that does not affect males' sexual behavior, but does alter the length and morphology of their bristles, making it possible to distinguish *forked* and wild-type males). After 3 days, the *forked* males were removed, and the wild-type males in the second group were stored individually in vials for 2 days. When the isolated males and the males that had been confined with mature males were 5 days old, they were tested individually with virgin females. Males that had been courted (by older males) when they were immature had shorter copulation latencies than males that did not elicit courtship, although their copulation frequencies were not significantly different (Table 1), suggesting that homosexual courtship benefitted the immature males (McRobert and Tompkins, 1988).

The species-specific courtship song, produced by vibration of courting males' wings, is a prominent element of courtship in this species. To test the hypothesis that early exposure to the courtship song of mature males affects males' courtship (McRobert and Tompkins, 1988), the experiments described above were repeated, substituting mature *forked* males whose wings had been surgically removed for intact *forked* males; wingless males perform courtship that is qualitatively and quantitatively normal except for the absence of courtship song (S. P. McRobert, F. B. Schnee and L. Tompkins, in preparation). Males courted by wingless males subsequently mated more quickly than isolated males, but not as quickly as males courted by intact males (Table 1). We interpret these results to mean that early exposure to the visual and/or auditory stimuli associated with courtship song is important, but it is not the only stimulus component that affects the adult behavior of males exposed to homosexual courtship during early life.

#### *Developmental plasticity of courtship in a mutant strain lacking circadian rhythmicity*

In the course of measuring circadian variations in copulation latency of wild-type flies, evidence was obtained suggesting that a well-known null mutation of the *period* gene, identified through its effects on circadian rhythms (Konopka and Benzer, 1971), may also modulate the effects of dark-rearing on copulation latency. Investigation of this mutant,

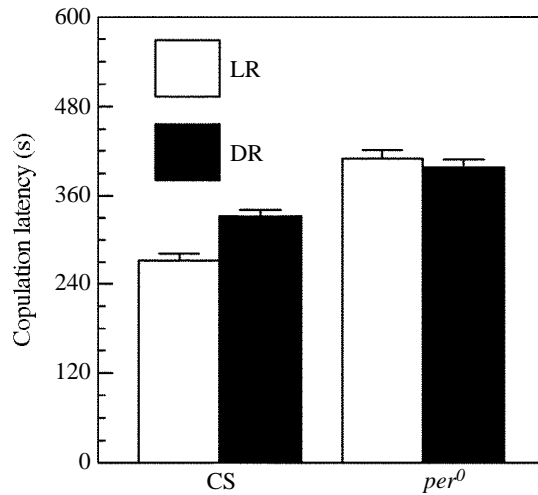


Fig. 6. Mean copulation latencies for two strains (Canton-S wild-type, CS, and mutant strain *per*<sup>0</sup>) of light- and dark-reared males, tested with light-reared females from the same strain. For Canton-S, but not for *per*<sup>0</sup>, mean copulation latencies of light-reared (LR) males were significantly shorter than for dark-reared (DR) flies. There was no difference in the mean copulation latencies of light- and dark-reared *per*<sup>0</sup> flies; moreover, both groups of *per*<sup>0</sup> flies had longer latencies than did either group of Canton-S flies. Error bars indicate S.E.M.,  $N=738$ , CS;  $N=621$ , *per*<sup>0</sup>.

*per*<sup>0</sup>, was motivated by the discovery that there is a 1h window, about 6h after 'sunrise', during which there is a sharp increase in the proportion of pairs that copulates rapidly (D. Possidente, B. Possidente and H. V. B. Hirsch, in preparation). To determine whether this rhythm was driven by the cycling illumination or by an internal clock, one group of male flies was kept in darkness throughout the first 4 days of adult life ('dark-rearing') and copulation latencies were measured (using light-reared females) at hourly intervals throughout the day and night. The rhythm persisted despite the dark-rearing of the male flies, although their copulation latencies were significantly longer than for light-reared male flies courting light-reared females. There is thus a circadian rhythm of courtship which persists despite dark-rearing of the males. It will be necessary to dark-rear the females to determine whether they are responsible for maintaining the rhythm.

As a first step in determining whether this rhythm was controlled by the *period* gene, copulation latencies of *per*<sup>0</sup> males raised in cycling illumination and of *per*<sup>0</sup> males raised in constant darkness during the first 4 days of adult life, then tested with light-reared females, were determined. The circadian rhythm of copulation observed in Canton-S flies was not evident in the *per*<sup>0</sup> flies, nor was there a difference in the mean copulation latencies of light- and dark-reared *per*<sup>0</sup> flies (Fig. 6). Both groups of *per*<sup>0</sup> flies had longer latencies than either group of Canton-S flies. Thus, the developmental plasticity of courtship was reduced in the *per*<sup>0</sup> strain. Whether this is due to the absence of a functional *period* gene product or to some other background characteristics of the specific strain that was tested remains to be determined.

Are these strain differences in developmental plasticity of courtship behavior accompanied by differences in developmental plasticity of responses to vertical stripes? Preliminary results of a comparison of responses to vertical stripes in light- and dark-reared *raised* and *per<sup>0</sup>* flies revealed no difference between the two strains, in that both dark-reared *raised* flies and dark-reared *per<sup>0</sup>* flies responded to wider bars than did light-reared controls of either strain (H. Epstein and H. V. B. Hirsch, unpublished results). This result suggests that the two manifestations of visual plasticity may be mediated, in part, by different gene products.

*Effects of early experience on use of sensory cues in courtship*

Visual deprivation affects courtship behavior in *Drosophila melanogaster*. Do the neuronal changes produced by visual stimulation in invertebrates help account for this behavioral plasticity? The electroretinogram is reduced in visually deprived locusts (Bloom and Atwood, 1980) and in bees exposed to selected wavelengths of light (Hertel, 1983). In dark-reared locusts, a major visual interneuron (the descending contralateral movement detector, DCMD) is less responsive to stimuli and fatigues more rapidly than the corresponding neuron in light-reared controls (Bloom and Atwood, 1980). Moreover, light exposure increases synaptic frequencies at an excitatory synapse in the visual system of the housefly, while decreasing synaptic frequencies at an inhibitory synapse whose activity also exhibits circadian rhythmicity (reviewed by Meinertzhagen and Hanson, 1993).

In general, then, visual deprivation reduces responsiveness. Activity-dependent modulation of nervous system function could thus enhance the salience of stimuli to which the fly was exposed during its critical period and thereby facilitate behavioral responsiveness to the sensory channels carrying these stimuli. This modulation could influence the extent to which flies 'attend to' (make use of) the many different cues provided by other individuals and could thereby have an indirect impact on communication. For example, dark-reared flies may pay less attention to visual information and more attention to non-visual information than do their light-reared controls. This might adversely affect reproductive behavior unless a common early exposure history has led both the male and the female to make comparable adjustments in their attention to different sensory modalities; courting pairs would perform best when both members have been reared in the same environment. Early experience would thus ensure that males use 'language' in the same manner as do females.

*The role of tutoring: homosexual courtship in flies and song-learning in birds*

Our observation that adult male flies subjected to homosexual courtship during early adult life perform better than 'untutored' males when courting females has striking similarities to observations on the development of bird song. In both cases, the immature male receives the stimulation during early life, before he expresses the behaviors that are influenced by the exposure. In birds, the effect of early exposure to the adult song has been explained by postulating that the young male lays down an auditory template of what he has heard and later compares his own output with that template, modifying the output until there is an adequate match. Male *Drosophila* may be engaged in a similar

process. During the episodes of homosexual courtship, they may be storing something analogous to a neural template representing certain key features of courtship; when they mature, this template may in some way facilitate development of appropriate behaviors.

One prediction of this model is that the effects of homosexual experience should be very specific, since a young male is exposed to particular behaviors that he will later have to mimic when he starts courting. For example, if the courting males are unable to sing because their wings have been removed, the young males would be expected to sing abnormally when they become adults. Similarly, if the homosexual courtship occurs in darkness and the young males do not receive visual information about courtship, they would be less able to use vision in courtship later. Experimental tests of these predictions would involve a detailed analysis of the component behaviors of 'tutored' and 'untutored' males, perhaps using a sequence analysis procedure such as that developed by Markow and Hanson (1981).

Since elicitation of homosexual courtship benefits males, why do males gradually lose their sex appeal and, thus, their ability to elicit vigorous homosexual courtship as they become sexually mature? One possibility is suggested by the observation that females courted by two mature males, which occasionally perform brief bouts of homosexual courtship in response to each other (Tompkins *et al.* 1980), are less likely to mate during a 1 h observation period than females courted by one male (Tompkins and Hall, 1983). Thus, elicitation of vigorous homosexual courtship by sexually mature males may have been selected against because a male that elicited vigorous homosexual courtship while courting a female would take longer to mate than a male that did not elicit much homosexual courtship.

#### *Sexual selection and mate choice plasticity*

Mate choice, which has an important role in mediating sexual selection (Halliday, 1983), is influenced both by genotype (Tebb and Thoday, 1956; O'Donald, 1978; Partridge, 1983) and by early experience (D'Udine and Alleva, 1983; Bateson, 1983; H. V. B. Hirsch, M. Barth, L. Sun, H. Sambaziotis, M. Huber, D. Possidente, H. Ghiradella and L. Tompkins, in preparation). Female *Drosophila* raised in a cycling illumination (comparable to that of their natural habitat) discriminate against males that have been deprived of all visual stimulation during the first 4 days of adult life. Furthermore, light-reared flies are less likely to mate with dark-reared flies; in some test situations, light/dark-reared couples also take longer to initiate copulation when they do mate. The effects of visual deprivation are not likely to involve simply debilitation of the flies or reductions in their overall responsiveness to stimuli; in this case, one would expect dark-reared flies to have low copulation frequencies and high copulation latencies regardless of the exposure history of the flies with which they are paired. Rather, light deprivation in our experimental conditions does not have deleterious effects on flies' reproductive behavior unless only one fly is light-deprived.

Experience-dependence of mate choice may have implications for the natural history of these insects. A *Drosophila melanogaster* female typically lays her eggs in the food source on which she copulated (Spieth and Ringo, 1983), making it likely that her offspring will eclose in the environment in which she chose a mate. Presumably, if both

parents matured in this same environment, they were well adapted to it, so that preference for a mate from a similar background (in our case similar environmental exposure history) might enhance the fitness of the resulting offspring in that particular environment. The ability to identify potential mates from similar backgrounds and/or with the same exposure history may provide a selective advantage, enabling flies whose genotype is compatible with survival in a given environment to identify others that can also survive there. This strategy would maximize the probability that progeny would inherit, from both parents, alleles that facilitate survival in the same conditions. Thus, what we have learned about developmental plasticity of mate choice may help us to understand better the factors affecting fitness in this species.

#### *Genetic dissection of complex behaviors*

The discovery of a possible link between plasticity of mate choice and circadian rhythmicity may help us to understand survival within an ecological community of different *Drosophila* species that share a common food source. Since males approach any nearby *Drosophila*, sexual interactions involving different species often occur (Spieth and Ringo, 1983). While inappropriate pheromonal cues or rejection responses by the female will eventually discourage the courting male, interspecific differences in circadian courtship rhythms could reinforce species isolation by reducing the extent to which males court non-conspecific flies. The possibility that the *period* gene product could be involved both in the regulation of courtship rhythms and of developmental plasticity affecting mate choice is thus intriguing. This relatively simple system provides us with an analytical probe that may allow us to gain new insights into the genetic control of two important behavioral processes, rhythmicity and developmental plasticity. Once again *Drosophila* can open a window – this time into the behavioral world.

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#### References

- BARTH, M. (1994). Die Auswirkungen visueller Deprivation auf das Balzverhalten männlicher Fruchtfliegen *Drosophila melanogaster*. Diplomarbeit. Bayerische Julius-Maximilians-Universität, Würzburg, Theodor-Boveri-Institut für Biowissenschaften, Fachbereich Genetik.
- BATESON, P. (1983). Optimal outbreeding. In *Mate Choice* (ed. P. Bateson), pp. 257–277. Cambridge, UK: Cambridge University Press.
- BLOOM, J. W. AND ATWOOD, H. L. (1980). Effects of altered sensory experience on the responsiveness of the locust descending contralateral movement detector neuron. *J. comp. Physiol.* **135**, 191–199.
- BRADLEY, P. AND HORN, G. (1987). Neural consequences of imprinting. In *Imprinting and Cortical Plasticity: Comparative Aspects of Sensitive Periods* (ed. J. P. Rauschecker and P. Marler), pp. 137–149. New York: John Wiley and Sons.
- CAMPAN, R., BEUGNON, G. AND LAMBIN, M. (1987). Ontogenetic development of behaviour: the cricket visual world. *Adv. Study Behav.* **17**, 165–212.



- COLLETT, T. S. (1988). How ladybirds approach nearby stalks: a study of visual selectivity and attention. *J. comp. Physiol.* **163**, 355–363.
- CURCILLO, P. G. AND TOMPKINS, L. (1987). The ontogeny of sex appeal in *Drosophila melanogaster* males. *Behav. Genet.* **17**, 81–86.
- DENNIS, W. (1973). *Children of the Crèche*. New York, NY: Appleton-Century-Crofts.
- D'UDINE, B. AND ALLEVA, E. (1983). Early experience and sexual preferences in rodents. In *Mate Choice* (ed. P. Bateson), pp. 311–327. Cambridge, UK: Cambridge University Press.
- FORD, S. C., NAPOLITANO, L. M., MCROBERT, S. P. AND TOMPKINS, L. (1989). Development of behavioral competence in young *Drosophila melanogaster* adults. *J. Insect Behav.* **2**, 575–588.
- FRÉGNAC, Y. AND SHULZ, D. (1994). Models of synaptic plasticity and cellular analogs of learning in the developing and adult vertebrate visual cortex. In *Advances in Neural and Behavioral Development* (ed. V. A. Casagrande), pp. 149–235. Norwood, NJ: Ablex Publishing Corporation.
- GREENOUGH, W. T. (1976). Enduring brain effects of differential experience and training. In *Neural Mechanisms of Learning and Memory* (ed. M. R. Rosenzweig and E. L. Bennett), pp. 255–278. Cambridge, MA: MIT Press.
- GREENOUGH, W. T. (1988). The turned-on brain: Developmental and adult responses to the demands of information storage. In *From Message to Mind* (ed. S. S. Easter, Jr, K. F. Barald and B. M. Carlson), pp. 288–302. Sunderland, MA: Sinauer Associates, Inc.
- HALL, J. C. (1977). Portions of the central nervous system controlling reproductive behavior in *Drosophila melanogaster*. *Behav. Genet.* **7**, 291–312.
- HALL, J. C. (1979). Control of male reproductive behavior by the central nervous system of *Drosophila*: dissection of a courtship pathway by genetic mosaics. *Genet.* **92**, 437–457.
- HALL, J. C. (1982). Genetics of the nervous system in *Drosophila*. *Q. Rev. Biophys.* **15**, 223–479.
- HALLIDAY, T. R. (1983). The study of mate choice. In *Mate Choice* (ed. P. Bateson), pp. 3–32. Cambridge, UK: Cambridge University Press.
- HECHT, S. AND WALD, G. (1934). The visual acuity and intensity discrimination of *Drosophila*. *J. gen. Physiol.* **17**, 517–547.
- HEISENBERG, M. AND WOLF, R. (1984). *Vision in Drosophila*. Berlin: Springer-Verlag.
- HERTEL, H. (1983). Change of synapse frequency in certain photoreceptors of the honeybee after chromatic deprivation. *J. comp. Physiol.* **151**, 477–482.
- HIRSCH, H. V. B. (1985). The tunable seer: activity-dependent development of vision. In *Handbook of Behavioral Neurobiology* (ed. E. M. Blass), pp. 237–295. New York: Plenum Publishing Corporation.
- HIRSCH, H. V. B., POTTER, D., ZAWIERUCHA, D., CHOUDHRI, T., GLASSER, A., MURPHEY, R. K. AND BYERS, D. (1990). Rearing in darkness changes visually-guided choice behavior in *Drosophila*. *Visual Neurosci.* **5**, 281–289.
- HIRSCH, H. V. B. AND TIEMAN, S. B. (1987). Perceptual development and experience-dependent changes in cat visual cortex. In *Sensitive Periods in Development: Interdisciplinary Perspectives* (ed. M. H. Bornstein), pp. 39–79. Hillsdale, NJ: Lawrence Erlbaum Associates.
- HORN, E. AND WEHNER, R. (1975). The mechanism of visual pattern fixation in the walking fly, *Drosophila melanogaster*. *J. comp. Physiol.* **101**, 39–56.
- IMMELMANN, K. (1972). Sexual and other long-term aspects of imprinting in birds and other species. *Adv. Study Behav.* **4**, 147–174.
- KNUDSEN, E. I. (1987). Early experience shapes auditory localization behavior and the spatial tuning of auditory units in the barn owl. In *Imprinting and Cortical Plasticity: Comparative Aspects of Sensitive Periods* (ed. J. P. Rauschecker and P. Marler), pp. 7–21. New York: John Wiley and Sons.
- KONOPKA, R. J. AND BENZER, S. (1971). Clock mutants of *Drosophila melanogaster*. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2112–2116.
- KRAL, K. AND MEINERTZHAGEN, I. A. (1989). Anatomical plasticity of synapses in the lamina of the optic lobe of the fly. *Phil. Trans. R. Soc. Lond.* **323**, 155–183.
- LAUGHLIN, S. (1981). A simple coding procedure enhances a neuron's information capacity. *Z. Naturforsch.* **36c**, 910–912.
- LAUGHLIN, S. (1984). The roles of parallel channels in early visual processing by the arthropod compound eye. In *NATO Advanced Science Institutes Series, series A, vol. 74, Photoreception and Vision in Invertebrates* (ed. M. A. Ali), pp. 457–481. New York, London: Springer Verlag.
- LINDSLEY, D. L. AND ZIMM, G. G. (1992). *The Genome of Drosophila melanogaster*. Academic Press: San Diego.

- LNENICKA, G. A. (1991). The role of activity in the development of phasic and tonic synaptic terminals. *Ann. N.Y. Acad. Sci.* **627**, 197–211.
- LNENICKA, G. A., HONG, S. J., COMBATTI, M. AND LEPAGE, S. (1991). Activity-dependent development of synaptic varicosities at crayfish motor terminals. *J. Neurosci.* **11**, 1040–1048.
- MAHAFFEY, J. W., COUTU, M. D., FYRBERG, E. A. AND INWOOD, W. (1985). The flightless *Drosophila* mutant *raised* has two distinct genetic lesions affecting accumulation of myofibrillar proteins in flight muscles. *Cell* **40**, 101–110.
- MANNING, A. (1967). The control of sexual receptivity in female *Drosophila*. *Anim. Behav.* **15**, 239–250.
- MARKOW, T. A. (1987). Behavioral and sensory basis of courtship success in *Drosophila melanogaster*. *Proc. natn. Acad. Sci. U.S.A.* **84**, 6200–6204.
- MARKOW, T. A. AND HANSON, S. J. (1981). Multivariate analysis of *Drosophila* courtship. *Proc. natn. Acad. Sci. U.S.A.* **78**, 430–434.
- MARLER, P. (1987). Sensitive periods and the roles of specific and general sensory stimulation in birdsong learning. In *Imprinting and Cortical Plasticity: Comparative Aspects of Sensitive Periods* (ed. J. P. Rauschecker and P. Marler), pp. 99–135. New York: John Wiley and Sons.
- MARLER, P. (1991). Song-learning behavior: the interface with neuroethology. *Trends Neurosci.* **14**, 199–206.
- MCRROBERT, S. P. AND TOMPKINS, L. (1983). Courtship of young males is ubiquitous in *Drosophila melanogaster*. *Behav. Genet.* **13**, 517–523.
- MCRROBERT, S. P. AND TOMPKINS, L. (1988). Two consequences of homosexual courtship performed by *Drosophila melanogaster* and *Drosophila affinis* males. *Evol.* **42**, 1093–1097.
- MEILLE, O., CAMPAN, R. AND LAMBIN, M. (1994). Effects of light deprivation on visually guided behavior early in the life of *Gryllus bimaculatus* (Orthoptera: Gryllidae). *Ann. ent. Soc. Am.* **87**, 133–142.
- MEINERTZHAGEN, I. A. (1989). Fly photoreceptor synapses: their development, evolution and plasticity. *J. Neurobiol.* **20**, 276–294.
- MEINERTZHAGEN, I. A. AND HANSON, T. E. (1993). The development of the optic lobe. In *The Development of Drosophila melanogaster* (ed. M. Bate and A. Martinez-Arias), pp. 1363–1491. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- MIMURA, K. (1986). Development of visual pattern discrimination in the fly depends on light experience. *Science* **232**, 83–85.
- MIMURA, K. (1987). Persistence and extinction of the effect of visual pattern deprivation in the fly. *Exp. Biol.* **46**, 155–162.
- MURPHEY, R. K. (1986). The myth of the inflexible invertebrate: competition and synaptic remodelling in the development of invertebrate nervous systems. *J. Neurobiol.* **17**, 585–591.
- NOTTEBOHM, F. (1991). Reassessing the mechanisms and origins of vocal learning in birds. *Trends Neurosci.* **14**, 206–211.
- O'DONALD, P. (1978). Reply to Spiess and Ehrman. *Nature* **272**, 189.
- OSORIO, D., SRINIVASAN, M. V. AND PINTER, R. B. (1990). What causes edge fixation in walking flies? *J. exp. Biol.* **149**, 281–292.
- PARTRIDGE, L. (1983). Non-random mating and offspring fitness. In *Mate Choice* (ed. P. Bateson), pp. 227–255. Cambridge, UK: Cambridge University Press.
- SCHMIDT, J. T. (1994). The roles of activity, competition and continued growth in the formation and stabilization of retinotectal connections in fish and frog. In *Advances in Neural and Behavioral Development* (ed. V. A. Casagrande), pp. 69–122. Norwood, NJ: Ablex Publishing Corporation.
- SHUVALOV, V. F. (1990). Plasticity of phonotaxis specificity in crickets. In *Sensory Systems and Communication in Arthropods* (ed. F. G. Gribakin, K. Wiese and A. V. Popov), pp. 341–344. Basel: Birkhäuser Verlag.
- SPIETH, H. T. AND RINGO, J. M. (1983). Mating behavior and sexual isolation in *Drosophila*. In *The Genetics and Biology of Drosophila*, vol. 3c (ed. M. Ashburner, H. L. Carson and J. N. Thompson, Jr), pp. 223–284. New York: Academic Press.
- STURTEVANT, A. H. (1915). Experiments on sex recognition and the problem of sexual selection in *Drosophila*. *J. Anim. Behav.* **5**, 351–366.
- TAMIS-LEMONDA, C. AND BORNSTEIN, M. H. (1987). Is there a 'sensitive period' in human mental development? In *Sensitive Periods in Development: Interdisciplinary Perspectives* (ed. M. H. Bornstein), pp. 163–181. Hillsdale, NJ: Lawrence Erlbaum Associates.

- TEBB, G. AND THODAY, J. M. (1956). Reversal of mating preference by crossing strains of *Drosophila melanogaster*. *Nature* **177**, 707.
- TEES, R. C., MIDGLEY, G. AND BRUINSMA, Y. (1980). Effect of controlled rearing on the development of stimulus-seeking behavior in rats. *J. comp. Physiol. Psychol.* **94**, 1003–1018.
- TIMNEY, B., MITCHELL, D. E. AND GIFFIN, F. (1978). The development of vision in cats after extended periods of dark-rearing. *Expl Brain Res.* **31**, 547–560.
- TOMPKINS, L. (1984). Genetic analysis of sex appeal in *Drosophila*. *Behav. Genet.* **14**, 411–440.
- TOMPKINS, L., GROSS, A. C., HALL, J. C., GAILEY, D. A. AND SIEGEL, R. W. (1982). The role of female movement in the sexual behavior of *Drosophila melanogaster*. *Behav. Genet.* **12**, 295–307.
- TOMPKINS, L. AND HALL, J. C. (1983). Identification of brain sites controlling female receptivity in mosaics of *Drosophila melanogaster*. *Genet.* **103**, 179–195.
- TOMPKINS, L., HALL, J. C. AND HALL, L. M. (1980). Courtship-stimulating volatile compounds from normal and mutant *Drosophila*. *J. Insect Physiol.* **26**, 689–697.
- VON SCHILCHER, F. (1976). The role of auditory stimuli in the courtship of *Drosophila melanogaster*. *Anim. Behav.* **24**, 18–26.
- VON SCHILCHER, F. AND HALL, J. C. (1979). Neural topography of courtship song in sex mosaics of *Drosophila melanogaster*. *J. comp. Physiol.* **129**, 85–95.
- WEHNER, R. (1972). Spontaneous pattern preferences of *Drosophila melanogaster* to black areas in various parts of the visual field. *J. Insect Physiol.* **18**, 1531–1543.
- WEHNER, R., GARTENMANN, G. AND JUNGLI, T. (1969). Contrast perception in eye color mutants of *Drosophila melanogaster* and *Drosophila subobscura*. *J. Insect Physiol.* **15**, 815–823.
- WEHNER, R. AND WEHNER-VON SEGESSER, S. (1973). Calculation of visual receptor spacing in *Drosophila melanogaster* by pattern recognition experiments. *J. comp. Physiol.* **82**, 165–177.