The Courtship of Patchwork Flies

Fruit flies composed of a mixture of male and female cells prove useful in the study of behavior

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Sex mosaic fly. The right eye and wing are of female cells and have normal colors. The left eye is male and white, due to a marker gene on the X chromosome. The left wing is male, and therefore smaller, and is marked with yellow cuticle.

That fruit fly with one red eye and one white eye, some straight and some forked bristles, and yellow and brown patches of body surface is no composite photograph, trick with mirrors or creation of science fiction. By taking advantage of a developmental quirk, Seymour Benzer and coworkers at the California Institute of Technology have raised thousands of seemingly patchwork flies. A collection of these flies is better than the most delicate scalpel, Benzer insists, for research on development, physiology and behavior of the fly *Drosophila melanogaster* and for describing the basic mechanisms flies share with other animals.

"The scalpel cleaves a biological system along anatomical lines," explain Benzer and Yoshiki Hotta, now at the University of Tokyo. "But genetics can also be used in a manner similar to the scalpel, by creating composite individuals."

Although the patchwork flies may appear to be assembled from a confused set of adult parts, they are the product of normal fly development after one early abnormal event. Some flies, geneticists have discovered, have a strange ring-shaped X chromosome that frequently gets lost during the first cell division. When that happens, the final adult fly is a composite of male and female cells. The researchers call these flies "mosaics."

Benzer and co-workers have produced flies with hundreds of distributions of male and female cells. The researchers can examine flies with a selected combination of parts. To put a male head on a female thorax, for example, selecting a mosaic fly is far more practical than performing a head transplant.

Mixed-sex, or gynandromorphic, flies should obviously be useful for investigating sex-related behaviors. "Is it head, heart or genitalia?" Benzer asked of the elaborate wing displays, the sweet love song of the fly.

A traditional first question to ask in approaching courtship might be: Is the lady attractive, or just what is fruit fly sex appeal? The final answer isn't in yet, but it clearly involves the posterior regions. Jean-Marc Jallon and Hotta spied on normal male flies encountering various flies of mixed sex. Only those with female structures in the posterior region attracted sexual advances. The provocative feature was not the genitalia, the researchers learned. Males were attracted to flies with male genitalia if other abdominal structures were female.

What part of the male fly responds to an alluring female? The head, Benzer and Hotta answered in the November PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES after watching the courtship behavior of 477 mosaic flies.

To link anatomy and behavior, the researchers scored each fly's behavior in relation to the sex of cells in different parts of the body. In Benzer's mosaics the male cuticle cells were yellow and the female's were brown. Thus, when a fly's head was entirely yellow, most cells in the head were likely to be male.

The data Hotta and Benzer tabulated indicate that following females and vibrating wings, male behavior typical of courtship, originates in the head. Of the flies with entirely male head cuticle, 99 percent followed the female and vibrated their wings. Only 4 percent of the flies with apparently female heads displayed such behavior. The correlation between male cuticle on other body segments and courtship behavior was much weaker.

Because the scalpel of genetics can divide internal structures as well as major body segments, the origin of courtship behavior could be located more precisely. Hotta and Benzer have worked out a chart of fruit-fly development to detect these finer divisions.

Benzer was originally a molecular geneticist and is known for his determination to apply the elegant techniques of molecular biology to studies of the nervous system. "Complex as it is, much of the vast network of cellular function has been successfully dissected, on a microscopic

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scale, by use of mutants in which one element is altered at a time," Benzer has written. "A similar approach may be fruitful in tackling the complex structures and events underlying behavior."

Benzer and Hotta adopted a strategy for mapping the developing fly that is similar to that used by geneticists in order to locate specific genetic information on chromosomes. The gene locations are determined by the frequency with which breaks in the chromosome separate two genes. The closer the genes, the less likely they are to be divided by a break.

"Fate" mapping with mosaic flies provides a parallel. The future parts of the adult fly are represented on the blastoderm, a hollow ball of embryonic cells. The boundary on the blastoderm between the male and female cells of a mosaic embryo is randomly determined by the orientation of the fertilized egg nucleus as it divides for the first time. The closer the precursor cells for two adult parts are on the blastoderm, the less likely the boundary will fall between them.

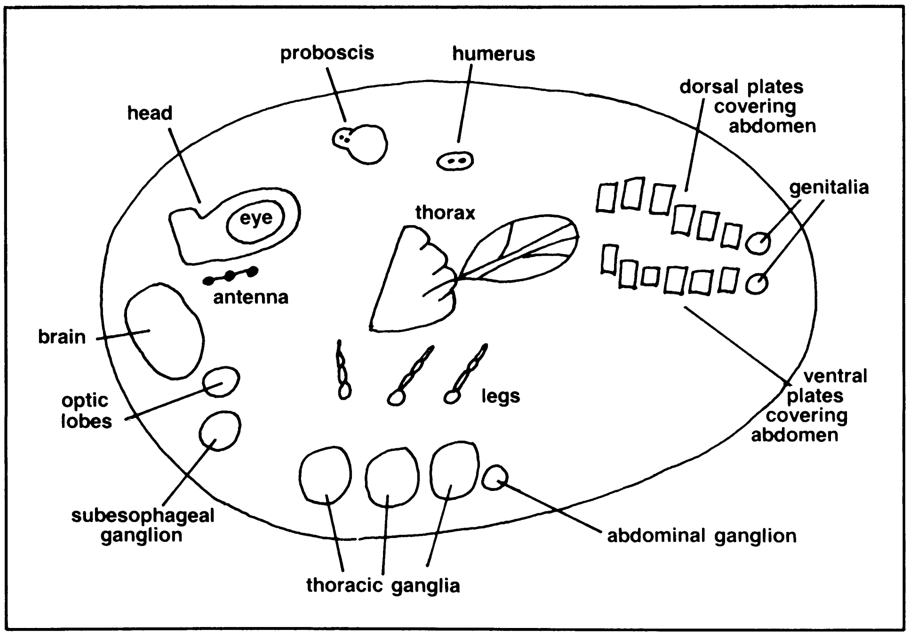
By looking at which adult cells are of different sex in many mosaic flies, Antonio Garcia-Bellido and John Merriam, working at California Institute of Technology, constructed a diagram they call the fate map, because it designates the destiny of embryonic parts. "The arrangement of adult body parts arising from the various sites is a sort of cubist collage; later developments will involve extensive movement and reorientation of the parts," Hotta and Benzer explain.

The usefulness of the fate map comes in linking new traits to previously mapped structures. In the case of courtship, Hotta and Benzer calculated the map distances between wing-vibrating behavior and each of 24 landmarks on the fly. "The focus is localized at the anterior [front] end, close to the midline, near the head, but not in the head cuticle," they concluded. "It is in this general area of the fate map that the brain is located."

Applying a further technique for determining the genetic content of internal tissues, Jeffrey Hall, now at Brandeis University, confirmed the brain as the source of courting behavior. By using an X chromosome gene that causes the absence of a cellular enzyme, and then staining frozen slices of the flies with a reagent that interacts with that enzyme, Hall could tell which nerve cells were of which sex. When the brain cells were male, the fly began courting.

Although the brain seems to originate male courtship behavior, mating isn't all in the head. In the next phase of courtship, Hotta and Benzer learned, the brain no longer directs the action alone.

After a decent interval of following and wing-vibrating behavior, the male attempts copulation by curling its abdomen under while following close behind a female. Of 130 flies that vibrated their wings, only 99 actually got around to attempting copulation. The persistent group included flies with male, female and mixed-sex genitalia.



Fate map of the fruit fly embryo resembles a cubist collage.

Attempted copulation correlated most closely with the sex of cuticle cells on the thorax, the middle segment of the fly. Mapping the behavior in reference to seven surface landmarks indicated that attempted copulation requires a group of nerve cells called the thoracic ganglion. These cells control various muscle activities. "Thus the focus for this courtship step is in a very different location from the ones for following and wing vibration," Hotta and Benzer note. First comes the impulse and next the coordination.

Finally, actual copulation with a female did require male genitalia. Of the flies that attempted to copulate, none of those with female or mixed genitalia, but almost all with male genitalia, were successful. Mating, from the male fly point of view, requires in turn structures at the head, thorax and abdomen.

It takes two to tango, so Hotta and Benzer also more briefly examined the female component of courtship. They introduced a variety of gynandromorphs to normal male flies and observed subsequent behavior. Not all mosaics with female genitalia would copulate with a male. Mating seemed to take a female head as well. Only 5 percent of mosaics with male head landmarks were receptive to male attentions, but 80 percent of those with female head landmarks and female genitalia participated in copulation.

"These results indicate that receptivity (or lack of rejection) is controlled by an anterior focus distinct from the posterior structures that provoke courtship," the researchers say.

Mosaic flies have a further characteristic that Benzer feels should make psychologists jealous. "The split brain is standard in *Drosophila*," Benzer says. Mosaics are sometimes divided straight down the middle, with male cells on one side and female on the other.

For male courtship behavior, Hotta and Benzer found, half a brain is definitely better than none. When the head cuticle was split along the midline into male and female halves, most of the flies acted as males, following females and vibrating both wings. So in all the different ambiguous situations where partly male, partly female flies meet, their behavior depends on the exact distribution of male and female parts.

Sex behavior is certainly not the only use for mosaic flies. "Looking at mosaics has become a standard technique for studying any behavior," Benzer says.

Ronald Konopka, also at the California Institute of Technology, has analyzed the daily rhythm of fruit-fly activity. A gene on the X chromosome controls that trait, so male and female cells can contain different genetic programming for circadian rhythm. By examining mosaic flies with cells programmed for 19- and 28-hour cycles, Konopka mapped the circadian rhythm to the brain. Unlike courtship activity, one hemisphere does not dominate the biological clock. When the two halves of the brain have intrinsically different rhythms, the fly's activity is the sum of the two. "Both sides can tick independently," Benzer says.

Further work on mosaic flies may soon contribute to understanding a variety of problems, such as the molecular events underlying nerve-muscle communication, development of the complex eye, and the $64,000 question, the mechanism of learning. But even Benzer, still a molecular biologist at heart, admits, "The nucleotide sequence of consciousness will have to wait." □