in the t(2;14) provides a means for studying a previously uncharacterized region of chromosome 2. Although band 2p13 has not been implicated in other translocations, nor is it a constitutional (inherited) fragile site, Yunis and Soreng report that this area is a constitutive fragile site in normal human lymphoid cells (*9*). They found that the position of other such sites correlates with the cytogenetic location of proto-oncogenes and chromosomal breakpoints associated with human malignancies. These sites may represent single-stranded breaks which provide free ends suitable for interchromosomal ligation by an enzyme involved in switch recombination. Therefore, the information derived from further studies of this translocation could provide valuable insights into the interrelations of somatic recombination, chromosomal translocation, and the unusual development of CLL in children.

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Interspecific Genetic Control of Courtship Song Production and Reception in *Drosophila*

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The genetic control of courtship song differences between *Drosophila melanogaster* and *Drosophila simulans* males was investigated by producing hybrids from reciprocal crosses. The song rhythm difference between the parental species appears to be due to sex-linked genes, whereas the basic interpulse-interval difference is autosomally inherited. Hybrid females show selective preferences for artificially generated songs carrying intermediate "hybrid" characteristics.

THE STUDY OF ACOUSTIC COMMUNICATION in hybrids has provided insight into the genetic mechanisms controlling this social interaction. In crickets, certain features of the male's calling song are autosomally inherited, whereas others are sex-linked (*1*). In both tree frogs and crickets, hybrid females respond more favorably to the songs of hybrid males than to the parentals (*2*). The possibility that the same set of genes influences both the male transmission and the female reception of song has long been discussed (*3*).

We have genetically analyzed the male lovesong in hybrids between the sibling species *Drosophila melanogaster* and *Drosophila simulans*. The song, which is produced by the male's wing vibration, stimulates the female to mate. A major component of this signal is a series of pulses with interpulse intervals (IPI's) that are typically 30 to 40 msec in *D. melanogaster* and 45 to 55 msec in *D. simulans* (*4*). The IPI's fluctuate rhythmically as the male sings, with the former species having an IPI cycle of approximately 50 to 60 seconds and the latter a 30- to 40-second cycle (*5*). We have produced two types of hybrid males between these two species, both of which have an autosome from each parent, but whose X chromosome is derived solely from one or other parental species. The well-known unisexuality of progeny produced by reciprocal crosses between the two species (*6*) usually makes it impossible to produce male hybrids with *D. melanogaster* X chromosomes. We overcame this difficulty by using a mutation in *D. simulans* which gives viable male hybrids carrying the *D. melanogaster* X chromosome. To produce a hybrid male with a *D. simulans* X chromosome, we crossed *D. melanogaster* females carrying attached-X chromosomes to males from our *D. simulans* strain. The reciprocal male carrying the *D. melanogaster* X chromosome was generated by crossing *melanogaster* females homozygous for the *yellow* body color mutation, to *D. simulans* males carrying the autosomal *Lhr* mutation which rescues the otherwise lethal male genotype from such a *melanogaster/simulans* pairing (*7*). Males from the *D. simulans Lhr* stock were also mated to *D. melanogaster* females homozygous for the *per* mutations, per^S, per^1, and per^o which, respectively, shorten, lengthen, and obliterate both circadian and song cycles (*5, 8*).

Figure 1 illustrates some of the song profiles produced by parental and hybrid males and Table 1 summarizes the data. Males from the *yellow D. melanogaster* strain show the typical *D. melanogaster* song pattern with an IPI value of 34 msec and a song oscillation period of 58 seconds. Our *D. simulans* males sang with characteristically higher IPI's of 49 msec and the shorter song cycle of 33 seconds. Compared with this *D. simulans* line, the *D. simulans Lhr* males gave a much higher IPI of 79 msec ($P < 0.01$) and a slightly longer song cycle of 39 seconds ($P < 0.05$). The large variation in the IPI among *D. simulans* strains has been documented (*9*). The male hybrid carrying

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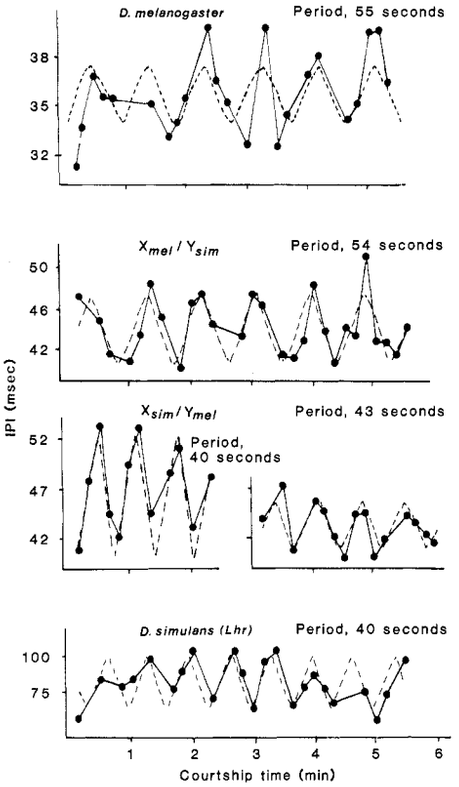

Fig. 1. Song profiles of males of *yellow D. melanogaster*, *D. simulans*, and reciprocal hybrids between the two species. Songs were recorded and analyzed as described previously (*5*). Briefly, a 4- to 6-day-old male and a 2-day-old virgin female were placed in a small mating chamber, which was then suspended above the ribbon of a Reslo ribbon microphone. After suitable amplification and filtering, the song of the male was recorded on magnetic tape, until copulation had occurred or 5 to 6 minutes of courtship had elapsed. Hard copy of the song was obtained by oscillographing the records onto light-sensitive paper. The mean IPI for every consecutive 10 seconds of courtship time was fed into a computer. The best-fitting sine wave passing through these means was calculated from a succession of iterations by the methods of least-squares nonlinear regression. An F ratio was computed to assess the significance of the fit. From the equation describing the best-fitting sine wave, the parameters for the period of the sine wave, the value of IPI around which the sine wave cycled, and the amplitude of the oscillation were extracted. Any sine wave whose F ratio did not reach the 5 percent probability level of significance for goodness of fit to the data points was considered arrhythmic. A minimum of ten IPI's were used to compute a mean for each 10-second fraction of time. For *D. melanogaster*, we used minimum and maximum IPI cut-off points of 15 and 65 msec, respectively; for *D. simulans* and the reciprocal hybrids, 20 and 90 msec; and for the *Lhr D. simulans* strain, 25 and 150 msec. Analysis of variance of the various song parameters was followed by the Newman-Keuls a posteriori procedure for multiple comparisons (*16*). The *D. melanogaster* and *D. simulans* females were paired with conspecific females. Hybrid males carrying the *melanogaster* X were paired with *melanogaster* females, and the reciprocal males were paired with hybrid females because these females elicited the most vigorous courtship behavior from such males. All recordings were made at 25° ± 1°C.

the *D. simulans* X chromosome has an IPI of 44 msec, which is intermediate to the parental values of 34 and 49 msec; the hybrid IPI is significantly different from both the *D. melanogaster* ($P < 0.01$) and *D. simulans* IPI's ($P < 0.05$). The male hybrid also has a *D. simulans*–like song rhythm of 39 seconds, compared with the parental values of 54 ($P < 0.01$) and 33 seconds ($P < 0.05$). The reciprocal hybrid carrying the *D. melanogaster* X chromosome also has an IPI of 44 msec, which is intermediate and statistically different ($P < 0.01$) from each parental value of 34 and 79 msec although now falling nearer the *D. melanogaster* IPI (*9*). The song rhythm period of 56.5 seconds in this hybrid is clearly *D. melanogaster*–like. The amplitude of the song cycle is intermediate between the parental values in both reciprocal hybrids. Because the reciprocal hybrid males have the same cytoplasmic origin (*D. melanogaster* female parents), we conclude that the difference in song rhythm between *D. simulans* and *D. melanogaster* is largely due to genes on the X chromosome, and that both the overall IPI difference and the song cycle amplitude are inherited autosomally. With respect to the IPI, the X chromosome may have some influence because, even though each hybrid's IPI is intermediate between the parental values, it does fall closer to that of the maternal species. Hybrids hemizygous for the sex-linked per^s and per^l mutations show significant differences from the per^+ hybrid males in the period of their song oscillation ($P < 0.01$). Per^o hybrid males' songs are arrhythmic, demonstrating that the *D. melanogaster per* gene also influences song rhythms on a hybrid genetic background.

We previously reported that *D. melanogaster* and *D. simulans* females mate with significantly shorter latency when they are stimulated with artificial songs carrying both the conspecific male's IPI and song rhythm (*10*). Songs that do not carry both of these critical song features are relatively ineffective. We now asked to which kinds of songs do hybrid females respond most favorably. Hybrid females were generated by crossing *yellow D. melanogaster* females to *D. simulans* males. Unlike hybrid males, they carry both the *simulans* and *melanogaster* X chromosomes. Groups of females were stimulated with artificial songs generated from an electronic song simulator (*10, 11*). Each group of females was stimulated in the presence of hybrid males (carrying the *D. simulans* X chromosome), whose wings and aristae had been surgically removed, rendering them deaf and dumb (*10*). Despite these handicaps, the males court the hybrid females vigorously. The number of matings after 5 minutes of song stimulation was taken as a measure of mating latency and therefore as a quantitative index of the females' song preferences (Fig. 2). Twelve different songs were used, each of which combined one of three different IPI values—34, 41, or 48 msec—with one of four different rhythms—periods of 35, 45, or 55 seconds or pulses generated with invariant intervals. Analysis of variance revealed a significant interaction between IPI and song rhythm ($P < 0.01$). There were no significant differences in the number of pairs mating when the female hybrids were stimulated with nonrhythmic IPI's of 34 (*D. melanogaster*), 48 (*D. simulans*), or 41 msec (hybrid, intermediate). When presented with songs having a *D. simulans*–like rhythm of 35 seconds, the hybrid females began mating significantly sooner with the *D. simulans* 48-msec IPI than with the *D. melanogaster*

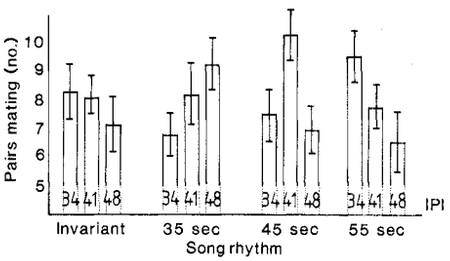

Fig. 2. Responses of hybrid females to simulated courtship songs. Hybrid females were generated by crossing *yellow D. melanogaster* females to *D. simulans* males. Virgin hybrid females ($n = 25$) 4 to 5 days old were placed in the lower portion of a mating chamber (*10, 11*), which was suspended over a loudspeaker connected to an electronic simulator. Hybrid males ($n = 25$) of the same age whose wings and aristae had been surgically removed were placed in the upper portion of the chamber. The males were produced by crossing *D. melanogaster* females carrying the attached-X chromosomes to *D. simulans* males. The flies were given 3 minutes to accustom themselves to their new environment, and then the partition between them was removed, allowing them to mix. The simulator was switched on, and the flies were stimulated with 90 dB of artificial song. The number of pairs mating was recorded at 1-minute intervals for 10 minutes. Twelve songs representing combinations of one of three different IPI's—34, 41, or 48 msec, with one of four different song rhythms: invariant, 35, 45, or 55 seconds—were used. The amplitude of the electronically generated rhythm was set to ±10 percent of the IPI value, giving a peak-to-trough amplitude of 20 percent in individual IPI's. The song was played in an alternating pattern of 2 seconds song, 3 seconds silence (*10, 11*). The figure shows the mean number of pairs mating after 5 minutes of stimulation and the SEM based on 15 to 18 replications of each song condition. Analysis of variance was performed on the number of pairs mating and was followed by the Newman-Keuls a posteriori method for multiple comparisons (*16*). The results are grouped by song rhythm. All experiments were performed at 25°C between 10 a.m. and 4 p.m., and all stocks were maintained in a light:dark cycle of 12:12, with lights turned on at 8 a.m.

Table 1. Analysis of songs of *D. melanogaster*, *D. simulans*, and hybrid males. The genotypes are shown with reference to X and Y chromosomes, X^m, Y^m (*melanogaster*) X^s, Y^s (*simulans*). Amplitude is measured from the best-fitting sine wave and represents the displacement of the cycle from the mean IPI value to either the peak or the trough (half the total amplitude peak-to-trough). In the hybrids, the *per* allele carried by the *D. melanogaster* chromosome X^m is also specified.

Species	Rhythmic males (*n*)	Mean IPI ± SEM (msec)	Amplitude ± SEM (msec)	Period ± SEM (seconds)	Arrhythmic males (*n*)
D. melanogaster					
yellow $X^m(y)Y^m$	9	34.1 ± 0.6	1.6 ± 0.2	58.5 ± 3.0	2
Canton-S (XX/Y) X^mY^m*	9	33.8 ± 0.7	1.3 ± 0.1	54.0 ± 0.8	0
D. simulans					
D. simulans X^sY^s*	5	48.9 ± 0.9	3.4 ± 0.7	33.2 ± 1.0	0
Lhr X^sY^s	7	78.8 ± 1.9	9.4 ± 0.6	38.7 ± 0.8	2
Hybrids					
X^sY^m	8	44.15 ± 1.4	2.6 ± 0.5	39.0 ± 0.8	2
$X^m(per^+)Y^s$	6	44.5 ± 1.0	2.8 ± 0.4	56.5 ± 1.9	2
$X^m(per^s)Y^s$	3	46.1 ± 1.7	1.9 ± 0.4	36.0 ± 3.5	2
$X^m(per^l)Y^s$	5	43.1 ± 2.1	2.7 ± 0.8	75.4 ± 3.9	0
$X^m(per^o)Y^s$	1†	45.3 ± 0.5‡	2.53†	141.6†	5

*Kyriacou and Hall (*5*). The males from the attached-X stock were made nearly isogenic to the Canton-S wild-type males (*5*). The *D. simulans* values are also from (*5*), as this is the same strain we used in the present study. †One of the per^o hybrids gave a rhythmic song. ‡Arithmetic mean of the IPI's for each arrhythmic per^o hybrid; the value for the rhythmic fly is included.

34-msec IPI ($P < 0.05$). When offered songs carrying the *D. melanogaster* rhythm of 55 seconds, mating was enhanced with the *D. melanogaster* 34-msec IPI, but not with the 48-msec IPI ($P < 0.05$). Similar preferences have been observed with nonhybrid *D. simulans* and *D. melanogaster* females (*10*). However, when females are stimulated with a "hybrid" rhythm of 45 seconds, they exhibit significantly shorter latencies when the IPI is also intermediate (41 msec), than when it matches either parental IPI value of 48 msec ($P < 0.01$) or 34 msec ($P < 0.05$). Thus the discrimination of IPI by hybrid females is most pronounced in tests in which the songs all have a 45-second rhythm. The 41-msec–45-second song also gives the greatest number of matings in the entire experiment (Fig. 2), although this value is not significantly different from the responses of the females to the 34-msec–55-second and 48-msec–35-second songs. Hybrid females therefore retain the song preferences of the parental species females, but they also show a unique response when presented with song characteristics intermediate between the two species.

The genetic control of the song differences between males of the two species seems clear. The difference in song rhythm appears to be sex-linked, and both the species differences in basic IPI and song rhythm amplitude seem to be autosomally inherited (*9, 12*). Interpretation of the responses of the females, however, is more difficult. Hybrid frogs and crickets prefer the calls of the hybrid males (*2*), which suggests that some kind of "genetic coupling" of the communication system is at work, with genes controlling the temporal patterns of the male song also serving the complementary function of programming the temporal recognition properties of the female reception apparatus. Although this may not be a general phenomenon [for example, grasshopper hybrids do not show this type of coupling (*13*)], Alexander has pointed out how such behavioral and genetic matching between the caller and recipient could lead to the rapid evolution of a communication system (*3*).

For genetic coupling to operate, behavioral coupling must occur. In our experiments, reciprocal hybrid males sing two different types of songs because of the sex-linkage of the song rhythm. If behavioral coupling of the male and female is involved, hybrid females might be expected to respond selectively to hybrid songs and therefore respond most rapidly when the simulated song carries the hybrid male characteristics—either intermediate IPI and 35-second song rhythm or intermediate IPI and 55-second song rhythm. In fact, when we compare all songs with the intermediate hybrid 41-msec IPI, it is the 45-second song cycle that significantly reduces latencies to mating. The case for any kind of coupling, be it behavioral or genetic, therefore seems weak.

Further consideration of the sex-determining system in *Drosophila* gives a different perspective. For genetic coupling to be evident, the same set of genes controlling male song output should control female song "input." *Drosophila* females are diplo-X and therefore inherit an X chromosome from each parent. Males are haplo-X, taking their X chromosome from their mothers. If any characteristic of the song is X-linked, for a rigorous test of genetic coupling the male and female must be genetically identical and carry the same set of putative "song" genes. Thus an important test to determine whether genetic coupling is occurring in our experiments would be to record songs of diplo-X hybrid males. If such hybrid males were to produce intermediate rhythm periods and intermediate IPI's (that is, the *D. melanogaster* and *D. simulans* song rhythm gene or genes are not dominant to each other), we could argue for behavioral coupling of the output system with that of the input system of the female, because this type of song enhances female mating behavior (Fig. 2). Therefore it is desirable to demonstrate behavioral coupling of the diplo-X male's song to the response of the corresponding hybrid female. Consequently we attempted to generate such diplo-X hybrid males by crossing *D. simulans* females to *D. melanogaster* males carrying either the sex transforming genes dsx^{Mas} *(doublesex-Masculiniser)* or dsx^D *(doublesex-Dominant)*. These dominant mutations transform diplo-X females into intersexes (*14*), which might be "male enough" to court. Unfortunately, none of our diplo-X intersexes showed any inclination for performing any male behavior. Therefore we are left with the tantalizing result that hybrid females prefer songs with characteristics intermediate between the parental species, and yet we are unable to generate "pseudo-males" that might naturally produce such courtship songs.

By using more elaborate genetic techniques to produce partial hybrids, we hope to be able to deduce which chromosomes are involved in both the male production of IPI and the female's selective responses to songs. The sex-linkage of the species difference in the song rhythm could conceivably include the involvement of the X chromosomal *per* locus. This gene has been molecularly cloned from *D. melanogaster*, and fragments of DNA encoding per^+ gene function have been identified (*15*). It will be possible to use these per^+ clones to find the homologous gene in *D. simulans*, and then to transduce this *simulans* per^+ gene into *D. melanogaster* flies deleted of the gene (*15*). Thus a

partial hybrid could be made that would be nearly all *D. melanogaster*, but would carry the *D. simulans per* gene. Such flies would be valuable for experiments on courtship song. Would such a *D. melanogaster* male fly, carrying *per*$^+$ DNA from *D. simulans*, sing with a *D. melanogaster* or *D. simulans* song rhythm? What types of songs would enhance the mating behavior of the corresponding partially hybrid females produced by transformation? These experiments will allow sophisticated molecular techniques to be applied toward answering more refined questions about the genetic control of song production and reception in *Drosophila*.

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Bioavailability of Dioxin in Soil from a 2,4,5-T Manufacturing Site

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Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) is a highly toxic contaminant produced in the manufacture of phenoxy herbicides. Despite its high TCDD content, soil from a contaminated area associated with a 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) manufacturing site in Newark, New Jersey, did not induce acute toxicity when administered to guinea pigs (the most sensitive species) by gavage. Analysis of liver samples demonstrated low bioavailability of TCDD from this soil. A comparative analysis of soils showed that Soxhlet extraction was necessary for the determination of TCDD on Newark soil, whereas solvent extraction was sufficient for soil from Times Beach, Missouri. The difference in the bioavailability of TCDD from these soils is correlated with TCDD extractability and may be related to the different compositions of the soils.

DIOXIN (2,3,7,8-TETRACHLORODIbenzo-*p*-dioxin, TCDD) is one of the most toxic man-made compounds known. The effects of an acute dose vary with species and include liver and kidney damage, chloracne, reduction in weight, wasting, thymic atrophy, immunotoxicity, and death (TCDD syndrome). TCDD promotes liver tumors in rats and skin tumors in HRS/J hairless mice (*1*), and it is also an anti-initiator of benzo[*a*]pyrene skin tumor carcinogenesis in Sencar mice (*2*).

Although TCDD is not manufactured commercially, it is a contaminant in the manufacture of several chlorinated phenolic products including the herbicide 2,4,5-trichlorophenoxyocitic acid (2,4,5-T) and hexachlorophene. Humans have been exposed to TCDD in several industrial accidents (Nitro, West Virginia; Seveso, Italy) and through environmental contamination (Times Beach, Missouri; Newark, New Jersey; and Vietnam). Low levels of TCDD have been found in many populations around the world. Combustion and fly ash have been suggested as other possible sources of TCDD, and may be responsible for continued human exposure (*3*).

The toxicity of complex environmental mixtures contaminated with TCDD has received little study. Such materials, including soils, natural waters, foliage, fly ash, soots, and hazardous wastes may differ in toxicity by comparison with the pure compound because of the influence of the matrix on the bioavailability of TCDD. Adsorption of TCDD to the surface of matrix particles may alter the animal absorption of the compound. Poiger and Schlatter (*4*) found that rats treated by gavage with a mixture of TCDD and activated charcoal did not absorb the TCDD to any appreciable extent; TCDD from a soil-TCDD mixture was taken up into the liver to a lesser extent than pure TCDD, and this uptake could be decreased if the time of contact between the TCDD and soil was increased.

Studies on the toxicity of materials environmentally contaminated with TCDD have been reported. Heida and Olie (*5*) demonstrated the presence of polychlorinated dibenzodioxins and polychlorinated dibenzofurans in terrestrial and aquatic wildlife living in a contaminated refuse dump in the Netherlands. Other studies (*6, 7*) have demonstrated varying bioavailability of TCDD from fly ash. Silkworth *et al.* (*8*) showed substantial uptake and acute toxicity of soots from a polychlorinated biphenyl fire where TCDD and other chlorinated dioxins and dibenzofurans were present. McConnell *et al.* (*9*) reported acute toxicity, aryl hydrocarbon hydroxylase induction, and tissue accumulations of TCDD from soils from the Times Beach area of Missouri. On the basis of the positive control values reported by McConnell *et al.*, one can calculate a bioavailability of TCDD from these soils of ~85%. The results presented here, however, show very low acute toxicity resulting from the ingestion of soil from a heavily contaminated 2,4,5-T manufacturing site in Newark, New Jersey.

We examined two soils. One was from the vicinity of a Newark plant that manufactured several chlorinated phenol products,

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