

## Circadian rhythm mutations in *Drosophila melanogaster* affect short-term fluctuations in the male's courtship song

(interpulse interval/*per* mutations/diplo-X transformed males)

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**ABSTRACT** Courtship song in *Drosophila* is produced by the male's wing vibration and consists of pulses of tone produced at intervals of approximately 34 msec in *D. melanogaster* and 48 msec in *D. simulans*. We have observed that the intervals between these pulses are not constant but fluctuate rhythmically with periods of approximately 1 min in *D. melanogaster* and 0.5 min in *D. simulans*. In *D. melanogaster*, three allelic *per* mutations have been isolated which affect the periodicity of the circadian oscillators affecting both eclosion and locomotor activity [Konopka, R. & Benzer S. (1971) *Proc. Natl. Acad. Sci. USA* 68, 2112–2116]. Each of the *per* alleles—*per<sup>s</sup>*, which shortens the circadian period, *per<sup>l</sup>*, which lengthens it, and *per<sup>o</sup>*, which abolishes it—strikingly affects the 60-sec song rhythm in a parallel fashion. Therefore, both circadian rhythms and a very short, noncircadian oscillation appear to be influenced by the same gene.

Courtship in *Drosophila melanogaster* consists of a series of highly stereotyped behavioral patterns, the most conspicuous of which is the male's wing display (1, 2). The male extends and vibrates his wing, producing a patterned acoustic signal or "song" (3). The song is illustrated in Fig. 1 and has several acoustic components, including a train of pulses with an interpulse interval (ipi) of approximately 34 msec together with a sinusoidal hum, which has a frequency of 160 Hz (4). The ipi is species specific; males of *D. simulans*, a closely related species, sing with an ipi of approximately 48 msec (5). Von Schilcher (6) has reported that *D. melanogaster* females mate faster with wingless, deafened conspecific males when they are exposed to an artificial song with an ipi of 34 msec compared to one having an ipi of 48 msec. Therefore, the ipi of the courtship song may play an important role as a recognition factor in preserving the sexual isolation between the two species.

This paper describes some experiments that demonstrate that the variation in the ipi values produced by males of both *D. melanogaster* and *D. simulans* is greater than previously believed. However, we will show that this variation conforms to a highly structured temporal pattern which is dramatically affected in *D. melanogaster* by three circadian rhythm mutations isolated by behavioral criteria unrelated to courtship (7).

### MATERIALS AND METHODS

*Drosophila* stocks were cultured on cornmeal/agar/molasses/yeast medium. Unless stated otherwise, all were reared at 25°C in a light-dark cycle of 16 hr in the light and 8 hr in the dark. Two wild-type strains were used, Canton-S and Oregon-K.

The courtship song was recorded by placing a single 3- to

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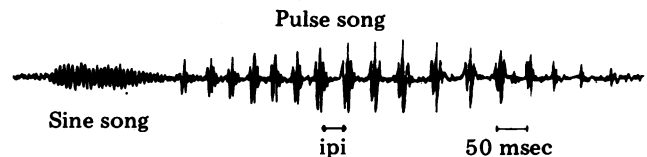


FIG. 1. Courtship song of *D. melanogaster* males.

5-day-old male with a 3-day-old virgin attached-X female inside a transparent mating cell 2 × 1 × 0.3 cm high. The floor of the chamber was composed of Nytex (stiff nylon mesh), and the unit was placed 2 mm above the ribbon of a Reslo (Sheffield, England) ribbon microphone, which was then encased within a screened aluminum box (12 × 8 × 8 inches) which served as an anechoic chamber. A porthole was cut in the box to enable the experimenter to observe the flies. The song of the male was amplified and recorded on a Tandberg 10X reel-to-reel tape recorder. A visual record of the song was obtained by tracing the recording, together with a time reference, on light-sensitive paper (Dupont, Type 5B) with a multichannel oscillograph (Consolidated Electroynamics, Newark, NJ, type 5-124). The paper was exposed, and ipi were measured peak to peak with a ruler.

The courtship was divided into 10-sec fractions, and the mean and SEM of all ipi falling within each successive time period was computed. The minimum number of ipi used to obtain a mean was 10, and most means were based on between 25 and 70 individual ipi. These means were then used to compute a nonlinear regression of ipi against the successive time periods for each courtship. The data were fitted to a variety of nonlinear mathematical functions, and the regression line of best fit was estimated by the method of least squares. The BMDP series of computer programs was used for this purpose (8). The residual variation after fitting a particular function was then used to compute an *F* ratio for the goodness-of-fit (9). Various parameters of the song were extracted for each courtship with these analyses, and when comparisons were made between different genotypes, analysis of variance was used together with the Newman-Keuls procedure for making *a posteriori* tests between means (10).

### RESULTS

The courtship songs of wild-type Canton-S and Oregon-K males were recorded for 5–6 min or until copulation had occurred. Fig. 2 illustrates typical ipi profiles for males of each genotype. As the courtship proceeds, the ipi produced by the males follow a sinusoidal path. The standard errors of the points are such that the ipi produced at the crest of the sine wave are nearly always significantly longer than those generated at the trough. When these points were fitted to a sine function, regression analysis

Abbreviation: ipi, interpulse interval(s).

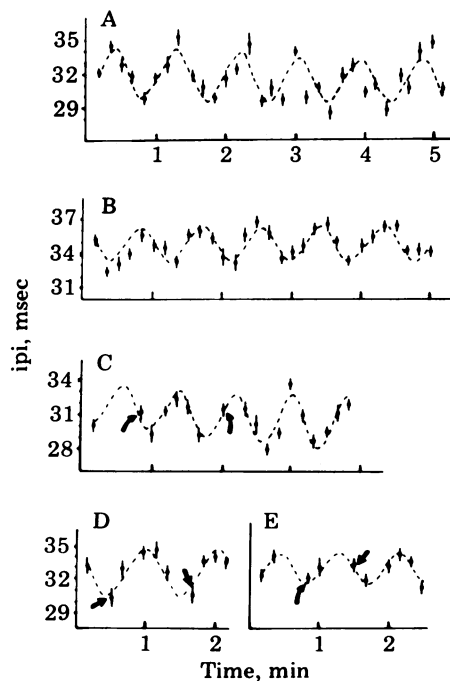


FIG. 2. Courtship song profiles of Oregon-K (A) and Canton-S (B-E) wild-type males. The means  $\pm$  SEM of all ipi falling within each consecutive 10-sec period of courtship are plotted together with the best least-squares solution of the regression analysis and the period  $p$  of the ipi oscillation. Arrows in C-E indicate where a male resumed his courtship song after interruptions of between 20 and 40 sec. A,  $p = 53$  sec; B,  $p = 53$  sec; C,  $p = 49$  sec; D,  $p = 63$  sec; E,  $p = 52$  sec.

revealed highly significant reductions in the total variation. Consequently, the ipi produced by the males can be considered to be oscillating. From the equation describing the best least-squares fit (see legend in Table 1), we can directly obtain the period of the oscillation ( $p$ ), the amplitude ( $a$ ), and the value around which the ipi oscillate (mean ipi). The courtships illustrated in Fig. 2 all show  $p$  values around 55 sec,  $a$  values of approximately 2-3 msec (or 4-6 msec peak to trough), and mean ipi of approximately 32 msec.

Table 1 summarizes the results of the song analyses performed with the Canton-S and Oregon-K strains, together with similar analyses of *yellow* males (made nearly isogenic with the Canton-S strain); males carrying the cuticular markers *yellow*, *singed*<sup>3</sup>, and *miniature*; diplo-X flies, which were transformed to phenotypic males with the third chromosome *tra* mutation (11); and haplo-X males homozygous for *tra* or heterozygous with *tra* and the *TM6* balancer chromosome. The reasons for studying these mutant males will become clear in the following sections. The results in Table 1 show that even though the males express a wide variety of mutant genes, they all show similar song patterns with respect to the ipi period  $p$ , which is always close to 55 sec, and the amplitude  $a$ , which is within the range 1.3-2 msec. Interestingly, the most variable character of the song appears to be the value around which the ipi oscillate (mean ipi), which varies from 30 to 42 msec. Only 1 of the 44 males analyzed failed to show a statistically significant oscillation of ipi; in 30 males it was significant beyond the 1% level.

There are two striking features of these ipi profiles. First, males do not all initiate their courtship song at the same phase of the sine wave. This can clearly be seen in the song profiles of Fig. 2. Second, if a male ceases to sing for one or more of the 10-sec courtship periods, as often is the case, when he resumes his song he remains in phase with the ipi oscillation previously

Table 1. Temporal analysis of ipi of the courtship song

Genotype	No. of males tested	$\bar{p} \pm \text{SEM}$ , sec	$\bar{a} \pm \text{SEM}$ , msec	Mean ipi $\pm \text{SEM}$ , msec
Canton-S	9	54.0 $\pm$ 0.8	1.3 $\pm$ 0.1	33.8 $\pm$ 0.7
<i>yellow</i>	8	56.7 $\pm$ 2.1	1.8 $\pm$ 0.2	35.9 $\pm$ 0.3
Oregon-K	9	54.1 $\pm$ 1.8	1.5 $\pm$ 0.1	31.4 $\pm$ 0.5
<i>ysn</i> <sup>3</sup> <i>m</i>	6	54.0 $\pm$ 2.4	1.6 $\pm$ 0.4	31.0 $\pm$ 1.0
XX/Y; <i>tra/tra</i>	4	53.4 $\pm$ 1.9	2.0 $\pm$ 0.1	40.9 $\pm$ 0.4
X/Y; <i>tra/tra</i>	4	53.2 $\pm$ 1.0	1.4 $\pm$ 0.1	41.6 $\pm$ 1.2
X/Y; <i>tra/TM6</i>	4	53.1 $\pm$ 1.1	1.8 $\pm$ 0.1	38.8 $\pm$ 1.2

See text for description of genotypes. The raw data consisted of the mean ipi for each consecutive 10-sec period from the initiation of the courtship song (see Fig. 1). A least-squares nonlinear regression was computed by using the sine function

$$f(x) = b_1 + b_2e^{b_3x} + (b_4 + b_5e^{b_6x}) \sin(b_7 + b_8x),$$

in which  $b_1$  is the value of ipi around which the sine wave oscillates and which can ascend or descend by incorporating the  $b_2e^{b_3x}$  parameter (mean ipi =  $b_1 + b_2e^{b_3x}$ );  $b_4$  is the amplitude, which can similarly increase or decrease by using the term  $b_5e^{b_6x}$  ( $a = b_4 + b_5e^{b_6x}$ );  $b_7$  is the angular displacement of the sine wave at the first data point (i.e., when  $X = 10$  sec); and  $b_8$  is the period of the oscillation  $p$ . Of the 44 song profiles examined, 30 showed significant reduction of the total variation on fitting the sine function beyond the 1% level, 12 beyond the 5% level, 1 beyond the 10% level (showed two successive oscillations out of a possible four), and only 1 was not significant (i.e., considered arrhythmic).

initiated. The arrows superimposed on the three courtships in Fig 2 C-E indicate where a male continued his song after interruptions of 20-40 sec. These two characteristics of the song rhythm suggest that the ipi oscillation is coupled to some ongoing temporal process.

**Effects of the *per* Gene on Song Rhythm.** Konopka and Benzer (7) isolated three X-linked mutations in *D. melanogaster* that alter circadian behavior with respect to two characteristics, eclosion and locomotor activity. These mutations appear to affect one functional gene termed the *per* locus. One mutation, *per*<sup>s</sup>, shortens the normal 24-h rhythm to 19 h, whereas a second, *per*<sup>l</sup>, lengthens it to 28 h. The third mutant, *per*<sup>o</sup>, is arrhythmic. Because oscillations are involved in both circadian behavior and the song rhythm, we wondered whether the *per* mutations might also affect the expression of the ipi pattern.

We recorded the songs of males carrying each of the *per* alleles, with each individual mutant gene being tested on two different genetic backgrounds expressing various combinations of the cuticular markers *yellow*, *singed*<sup>3</sup>, and *miniature*. Fig. 3 shows the ipi profiles for typical mutant songs together with a *per*<sup>+</sup> male for comparison. The difference between the *per*<sup>+</sup> and mutant males is clearly illustrated. The period of the ipi oscillation was reduced to 40 sec in the *per*<sup>s</sup> male and lengthened to 76 sec with *per*<sup>l</sup>. The *per*<sup>o</sup> male revealed no systematic sinusoidal patterning in his song. Regression analysis of 12 *per*<sup>o</sup> songs failed to find a mathematical function, linear or nonlinear, that could significantly reduce the total variation. We therefore conclude that the ipi profile for *per*<sup>o</sup> is arrhythmic. A summary of these results is presented in Table 2. The two genetic backgrounds on which each of the mutant genes was studied have been pooled because the results with each were essentially identical. The mean periodicity of the ipi oscillation in *per*<sup>s</sup> males is 41 sec and that of *per*<sup>l</sup> is 82 sec. Analysis of variance revealed that these values are highly significantly different from those of the Canton-S wild-type strain ( $P < 0.001$ ). No significant differences were observed in the other song parameters. Therefore, the *per* mutations have similar effects on the periodicities of both the 55-sec courtship song rhythm and the 24-h circadian phenotype.

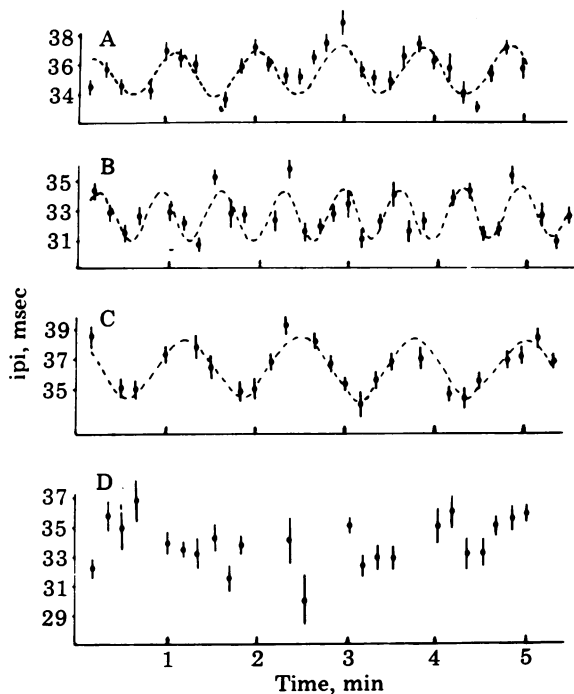


FIG. 3. Courtship song profiles of the *per* mutants and a *per*<sup>+</sup> male (yellow on a Canton-S genetic background). (A) *per*<sup>+</sup>,  $p = 56$  sec; (B) *per*<sup>s</sup>,  $p = 40$  sec; (C) *per*<sup>l</sup>,  $p = 76$  sec; (D) *per*<sup>o</sup>,  $p = ?$ .

#### Effects of Light and Temperature Shifts on Song Rhythm.

Constant light conditions cause arrhythmia in many circadian rhythms (12). Having uncovered an unexpected relationship between the circadian and courtship song rhythms, we imagined that if the circadian oscillator "drives" the one mediating the song, then rearing males in constant light might disrupt the song pattern. We raised wild-type flies in constant light conditions for five generations, then counted the number eclosing every 8 hr. We observed the characteristic arrhythmic pattern when compared to the same stocks reared for 16 hr in the light and 8 hr in the dark. The songs of 10 wild-type males raised in constant light were then recorded and analyzed. There was no evidence of any change in the normal pattern of the song rhythm and, consequently, the oscillation in the courtship song appears to be insensitive to changes in the light-dark cycle.

One of the most prominent features of circadian rhythms is that they are temperature compensated in that changes in temperature do not significantly affect their periodic characteristic (13). We were therefore interested in seeing whether rearing males and recording their songs at relatively high and low temperatures would affect their ipi pattern. Canton-S flies were reared at 29°C and the male's courtship song was recorded

Table 2. Temporal analysis of the courtship song of *per*<sup>s</sup>, *per*<sup>l</sup>, and *per*<sup>o</sup> males

Genotype	$\bar{p} \pm \text{SEM}$ , sec	$\bar{a} \pm \text{SEM}$ , msec	Mean ipi $\pm \text{SEM}$ , msec
<i>per</i> <sup>s</sup>	41.5 $\pm$ 1.0	1.7 $\pm$ 0.1	33.2 $\pm$ 1.5
<i>per</i> <sup>l</sup>	82.1 $\pm$ 3.3	1.6 $\pm$ 0.1	34.6 $\pm$ 0.6
<i>per</i> <sup>o</sup>	?	?	35.4 $\pm$ 1.1

Twelve males of each genotype were tested. Of the 24 *per*<sup>s</sup> and *per*<sup>l</sup> song profiles, 17 showed significant reductions of the total variation on fitting the sine function beyond the 1% level, 5 beyond the 5% level, and 2 beyond the 10% level, with all *per*<sup>o</sup> males not significant in this respect. See Table 1 for explanation of symbols.

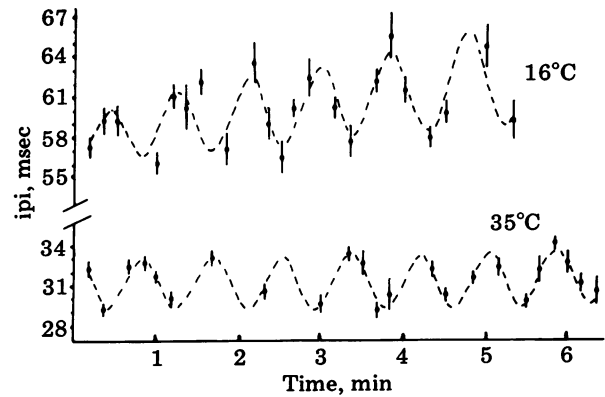


FIG. 4. Courtship song profiles of two Canton-S wild-type males, one reared at 29°C and recorded at 35°C ( $p = 51$  sec) and the other reared and recorded at 16°C ( $p = 53$  sec).

in the usual way at 35°C after 1 hr of acclimation at this temperature. Males were also reared at and their songs recorded at 16°C. The song profile of one male at each temperature is illustrated in Fig. 4.

Although low and high temperatures have the effect of, respectively, raising and slightly lowering the overall mean ipi (see ref. 14), the periodicity of the song oscillation remains essentially the same. Although significant differences were obtained between the mean ipi values at the high and low temperatures (32.2  $\pm$  1.0 compared with 56.4  $\pm$  1.4 msec, respectively;  $P < 0.001$ ) and amplitude  $a$  (1.9  $\pm$  0.3 compared with 3.0  $\pm$  0.3 msec, respectively;  $P < 0.05$ ), no significant differences could be detected in the period of ipi oscillation between the two groups (high-temperature  $\bar{p} = 56.1 \pm 1.8$  sec; low-temperature  $\bar{p} = 52.6 \pm 2.2$  sec). These results are based on nonlinear regression analyses for 7 songs at 16°C and 6 songs at 35°C, of which 8 showed significant reductions in total variation on fitting the sine function beyond the 1% level, 4 beyond the 5% level, and 1 beyond the 10% level.

**Heterozygosis for the *per* Locus.** Males heterozygous for the various *per* alleles were constructed by making diplo-X flies that were transformed into phenotypic males by the *tra* mutation. Transformed males are sterile, but they show normal courtship behavior and song rhythm (see Table 1). All heterozygous combinations of the different *per* alleles were generated, together with transformed males carrying a *per* allele and a small deletion of the *per* locus, *Df(1)w*<sup>258+242</sup> (7). Each het-

Table 3. Temporal analysis of courtship songs for transformed males heterozygous for *per* alleles or *Df(1)w*<sup>258-242</sup>

Genotype	No. of males tested	$\bar{p} \pm \text{SEM}$ , sec	$\bar{a} \pm \text{SEM}$ , msec	Mean ipi $\pm \text{SEM}$ , msec
<i>per</i> <sup>s</sup> / <i>per</i> <sup>+</sup>	12	43.1 $\pm$ 1.4	1.5 $\pm$ 0.1	35.3 $\pm$ 0.7
<i>per</i> <sup>l</sup> / <i>per</i> <sup>+</sup>	14	53.2 $\pm$ 1.1	1.5 $\pm$ 0.1	37.7 $\pm$ 0.4
<i>per</i> <sup>s</sup> / <i>per</i> <sup>l</sup>	13	52.2 $\pm$ 1.2	1.6 $\pm$ 0.1	36.9 $\pm$ 0.3
<i>per</i> <sup>o</sup> / <i>per</i> <sup>+</sup>	14	39.7 $\pm$ 0.7	1.6 $\pm$ 0.1	38.4 $\pm$ 0.5
<i>per</i> <sup>o</sup> / <i>per</i> <sup>s</sup>	12	32.4 $\pm$ 1.2	1.7 $\pm$ 0.3	37.3 $\pm$ 0.8
<i>per</i> <sup>o</sup> / <i>per</i> <sup>l</sup>	12	54.7 $\pm$ 1.4	1.3 $\pm$ 0.1	36.5 $\pm$ 0.5
<i>per</i> <sup>+</sup> / <i>Df</i>	7	39.4 $\pm$ 0.7	1.5 $\pm$ 0.2	36.6 $\pm$ 0.8
<i>per</i> <sup>s</sup> / <i>Df</i>	8	33.7 $\pm$ 1.2	1.5 $\pm$ 0.2	38.2 $\pm$ 0.7
<i>per</i> <sup>l</sup> / <i>Df</i>	6	51.7 $\pm$ 1.9	1.9 $\pm$ 0.3	37.1 $\pm$ 0.5

Of the 98 song profiles examined, 66 showed significant reductions of the total variation of fitting the sine function beyond the 1% level, 23 beyond the 5% level, and 9 beyond the 10% level. See Table 1 for explanation of symbols.

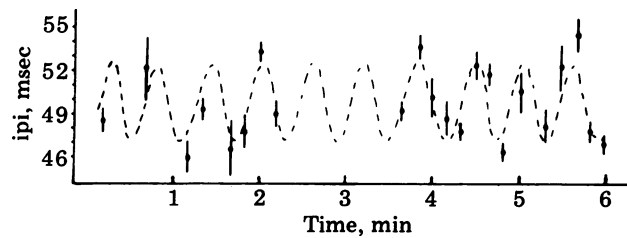


FIG. 5. Courtship song profile of a *D. simulans* male ( $p = 36$  sec). Note how this male resumes his courtship song in phase with the ipi oscillation he had initiated, even after a comparatively long lull of 110 sec. Of the five songs examined, four showed significant reduction of the total variation in fitting the sine function beyond the 1% level and one showed reduction beyond the 10% level.

erozygous combination of *per* alleles was studied with respect to the courtship song on two different genetic backgrounds, and the results are presented in Table 3.

The mean period length  $\bar{p}$  for the song oscillation in *per<sup>s</sup>/per<sup>+</sup>* heterozygotes is 43 sec, which is not significantly different from that of *per<sup>s</sup>* males. Thus, the *per<sup>s</sup>* mutation shows a dominant phenotype with respect to this character. In contrast, the *per<sup>l</sup>* allele is recessive to the wild-type allele, with *per<sup>l</sup>/per<sup>+</sup>* heterozygotes having an ipi oscillation period of 53 sec. When *per<sup>s</sup>* and *per<sup>l</sup>* are placed together, a wild-type phenotype ( $\bar{p} = 52$  sec) is obtained. These three results are similar to those obtained by Konopka and Benzer (7) with respect to circadian variation of locomotor activity in heterozygous *per* females. They observed that *per<sup>s</sup>* was semidominant and *per<sup>l</sup>* recessive to the wild-type allele. However, when the *per<sup>o</sup>* allele is made heterozygous with *per<sup>+</sup>*, the period length of the ipi oscillation is significantly reduced to 40 sec compared with the wild type ( $P < 0.01$ ). Similarly, the *per<sup>o</sup>* allele significantly reduces the song period of both the *per<sup>s</sup>* and *per<sup>l</sup>* alleles ( $P < 0.01$ ). This result is not parallel to the findings of Konopka and Benzer (7) with circadian rhythms in females, in which the *per<sup>o</sup>* mutant allele was recessive to the other alleles. We also observed that placing the deficiency *Df(1)w<sup>258-242</sup>* with each of the other *per* alleles simply mimicked the effect of *per<sup>o</sup>* heterozygosis. Evidently *per<sup>o</sup>* is a null allele (see ref. 7) but appears to have different effects on circadian and song rhythms.

**Song of *D. simulans* Males.** We asked if the courtship song of *D. simulans* males also showed oscillation of the ipi. The songs of five males were recorded, and Fig. 5 illustrates one of the ipi profiles obtained. The ipi of this male also showed an oscillation which is superimposed upon the longer ipi of *D. simulans*. The period of the oscillation in this case is 36 sec, much shorter than that for wild-type *D. melanogaster* males (see Table 1). The mean oscillation period for this group of five males was  $33.2 \pm 1.0$  sec, and the amplitude of the oscillation was  $3.4 \pm 0.7$  msec. Both of these values are significantly different from the corresponding ones of Canton-S wild-type males of *D. melanogaster* ( $P < 0.01$ ). Therefore, *D. simulans* males also exhibit an oscillation of the ipi of the courtship song, but it has a significantly shorter period and larger amplitude than that of the closely related *D. melanogaster* males.

## DISCUSSION

The effects of circadian rhythm mutations on short-term oscillations in *Drosophila* courtship behavior illustrate how mutant genes isolated with respect to one system can have profound influence on another. Indeed, it might have been extremely difficult to demonstrate a relationship between the song oscillation and circadian rhythm without using a genetic approach. The fact that we have uncovered such a relationship

suggests the possibility that the *per* gene may specify a product that controls a fundamental property of temporal regulation in this species. There are several examples of short-period oscillating processes; e.g., the rapid, 0.5-min glycolytic oscillation observed in yeast (15, 16) and the 5-min oscillation of ATP levels in *Acetabularia* cells (17). The question of whether such short-period oscillations may share common features with the more universal circadian variation has been previously discussed (18), but until now there has been no evidence to support this notion.

The genetic analysis of the song rhythms of *per* heterozygotes is parallel in most respect to the results obtained by Konopka and Benzer (7) with the circadian rhythm of locomotor activity. The *per<sup>s</sup>* allele is dominant with respect to the song rhythm and semidominant in circadian locomotor activity variation. Similarly, the *per<sup>l</sup>* allele is recessive in relation to both characters. However, we observed that placing *per<sup>o</sup>* with any of the other *per* alleles always resulted in a significant shortening of the period of the song rhythm. This can be interpreted as if *per<sup>o</sup>* is a semidominant acting in the same direction as *per<sup>s</sup>*. Because the same effect is observed when each *per* allele is made heterozygous with the deficiency *Df(1)w<sup>258+242</sup>*, this also suggests that a dosage effect is occurring. In contrast, Konopka and Benzer (7) observed that *per<sup>o</sup>* was always recessive in circadian rhythms of heterozygous females. However, a recessive X-linked allele in females could have a different effect in transformed males than in regular heterozygous females due to the possible influences of sex *per se* on X-chromosome dosage compensation in *Drosophila* (e.g., see refs. 19 and 20). Our results with the *per* heterozygotes (e.g., *per<sup>s</sup>/per<sup>o</sup>*, *per<sup>s</sup>/Df*) also show that the effect on the song rhythm maps to the *per* locus and is not the result of some other mutation or multigene combination fortuitously present in our *Drosophila* strains.

We are currently interested in searching for the specific region of the nervous system that is responsible for the production of the mutant song rhythms. Stocks are being prepared which will enable us to generate genetic mosaics, which are *Drosophila* individuals composed of both haplo and diplo-X tissue. The diplo-X tissue will, for example, express the heterozygous combination *per<sup>l</sup>/per<sup>+</sup>* and the haplo-X tissue will express only the *per<sup>l</sup>* allele. The mutant and heterozygous tissue will be identified by the presence of an internal histological marker (21). These mosaics will be transformed to phenotypic males by the *tra* mutation so that they will exhibit normal courtship behavior. Because the mutant song can be unambiguously distinguished from that of the heterozygote (see Tables 2 and 3), we can correlate the internal distribution of haplo-X and diplo-X tissue with the song phenotype expressed by the fly.

Mosaic analysis of the *per* locus has been performed with respect to circadian variation in locomotor activity (22). The results suggest that the mutant focus for the *per<sup>o</sup>* allele is in the brain. Transplanting the brain of one *per* allele into the abdomen of another and observing that the recipient may take on the circadian characteristics of the donor has also raised the possibility that a neurohumoral factor may be involved (23). The song oscillation may also have a focus in the brain. However, some genetic mosaic data implicate the thoracic nervous system as an important component of the song production mechanism (24).

In addition to being studied neurobiologically, the song rhythm can also be studied from an evolutionary perspective. The finding that *D. simulans* males produce songs that are markedly different from those of *D. melanogaster* both in the amplitude and period of the ipi oscillation suggests that the rate of change of ipi in the two species may have some functional significance. Perhaps it is a factor involved in species recogni-

tion which complements the basic difference in ipi between the two species.

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