Drosophila song as a species-specific mating signal and the behavioural importance of Kyriacou & Hall cycles in *D. melanogaster* song

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'Species-specific' mating signals may not make a significant contribution to sexual isolation if differences between species are not matched by narrowly tuned differences in female preferences. The courtship song of the *melanogaster* species group of *Drosophila* has been studied by several researchers as a potential factor in sexual isolation, but there are few clear demonstrations that species differences are behaviourally significant. We played synthetic song with typical pulse interval parameters for *D. melanogaster*, *D. simulans* or *D. sechellia* to *D. melanogaster* or *D. simulans* females in the presence of mute males. Females mated most quickly when stimulated by song typical of their own species, confirming that song can influence sexual isolation between these species. This effect was greater in *D. melanogaster* than *D. simulans*, which correlated with reduced variability in mean interpulse interval within *D. melanogaster*. We also examined the role of 'Kyriacou & Hall cycles' (periodic cycling of mean interpulse intervals) in *D. melanogaster*. Contrary to some predictions, the presence of the Kyriacou & Hall cycle was more important in increasing mating speed than the increased variability of interpulse intervals that is inevitably present in song containing a cycle. The large stimulatory effect of cycles on mating speed is curious, given that the periodicity of cycles would probably be difficult for females to detect during courtship in field conditions.

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Mating behaviour often distinguishes closely related species, implying that behavioural change can drive speciation in animals. However, a potential problem is distinguishing changes that contribute directly to speciation, usually by influencing sexual isolation, from those that are incidental to speciation. Many 'species-specific' behaviours may make little contribution to sexual isolation. For example, some túngara frogs, *Physalaemus pustulosus*, apparently elaborate changes in male calls between species are not accompanied by correspondingly attuned female preferences (Ryan & Rand 1995). Just because a signal differs between species does not necessarily mean that it makes a large contribution to the mate recognition system of either species.

Demonstrating that a signal plays a role in speciation requires a manipulative procedure to show that changes in the signal influence sexual isolation. An impressive example of such a study is provided by work on the role of cuticular hydrocarbons in mate recognition between species of the *melanogaster* group of *Drosophila*. The 7,11-

heptacosadiene (HD)/7-tricosene (T) polymorphism influences male recognition of females (Cobb & Jallon 1990; Coyne & Oyama 1995). A male *D. simulans* (whose cuticular hydrocarbon profile contains high levels of 7T) will not court *D. sechellia* females (who have high levels of 7,11-HD). If 7,11-HD is transferred from *D. sechellia* to *D. simulans* females by intimate confinement, *D. simulans* males will cease to court their conspecific females. Similarly, *D. simulans* males will court *D. sechellia* females (even dead ones) who have acquired 7-T (Coyne et al. 1994).

Once courtship is initiated in *Drosophila* species, one of the male signals upon which female preference can act is song produced by wing vibration. In some species male song is an obligatory requirement of courtship as females will not accept males made mute by wing removal (Liimatainen et al. 1992). A role of song in speciation is likely if male mating success is restored by playback of song of the appropriate species. This is indeed the case for some species (Tomaru et al. 1995; Ritchie et al. 1998).

There have been numerous studies of the courtship song of the *melanogaster* species group of *Drosophila*. Each species has a unique courtship song (Cowling & Burnet

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1981; Cobb et al. 1988) but little consensus seems to have been reached on the importance of these songs in sexual isolation. In contrast to the studies of female hydrocarbons, demonstrating a role of song in isolation has not been easy. There are two main song components, pulse song and sine song. Sine song is thought to 'prime' females (von Schilcher 1976a). Most of the species specificity occurs in aspects of pulse song. Bennet-Clark & Ewing (1969) showed that *D. melanogaster* females mated most quickly with wingless males when synthetic song with the species-specific mean interpulse interval (IPI) was replayed. However, the effects detected in this and another experiment (von Schilcher 1976a) were not statistically strong, and only wide variants of IPI were used (von Schilcher 1989). Using a particularly carefully designed experiment, van den Berg (1988) found that response to playback of song was species specific. However, he concluded that the importance of this to sexual isolation was probably only slight as environmental variation in mating speed was greater than the influence of the species-specific song model.

Kyriacou & Hall (1980) reported that the IPI of *D. melanogaster* did not vary in a random manner, but showed periodic, sinusoidal variation. The period of the cycle is determined by the clock gene *period* and varies between species. Kyriacou & Hall (1982, 1984, 1986) have shown that synthetic song with the correct combination of mean IPI and cycle length stimulates *D. melanogaster* and *D. simulans* females in a species-specific manner. However, their results were thrown into confusion by suggestions that incorrect statistics had been used to show that the cycles were present in *D. melanogaster* song, that cycles could not be detected by workers in other laboratories, and by arguments that cycles were unlikely to be of importance because of the nature of *D. melanogaster* courtship in the field (van den Berg 1988; Crossley 1988, 1989; Ewing 1988; Logan & Rosenberg 1989; von Schilcher 1989; Bennet-Clark 1990). Recently, Alt et al. (1998) have provided the first independent verification of the existence of cycles in the IPIs of *D. melanogaster* song, confirming that these exist. In addition, short periodic cycling of IPIs has been reported in *D. persimilis* (Noor & Aquadro 1998), and temporally patterned IPI variation found in *D. equinoxialis* (Ritchie & Gleason 1995).

As part of a study of the evolution of song in the *melanogaster* species group of *Drosophila*, here we report playback experiments examining whether artificial songs with mean IPI and Kyriacou & Hall cycles of *D. melanogaster*, *D. simulans* and *D. sechellia* act in a species-specific manner on mating speed (measured here as the cumulative number of copulations versus time) in *D. melanogaster* and *D. simulans*. We have also asked what aspects of Kyriacou & Hall cycles are important in influencing female preference. Previous studies have failed to distinguish between the importance of cycles and increased variability in IPI (Crossley et al. 1995). Kyriacou & Hall (1982, 1989) speculated that, if females vary in their preferred mean IPI, a male with a cycling song might achieve a greater mating success by stimulating more females. Variability could also prevent habituation of the neurological responses of individual females. Alter-

natively, females might have a preference specifically for song with a certain periodicity. We provide an independent verification of the species-specific stimulatory effect of song in these species, and we also show that females do indeed have preferences for song containing Kyriacou & Hall cycles.
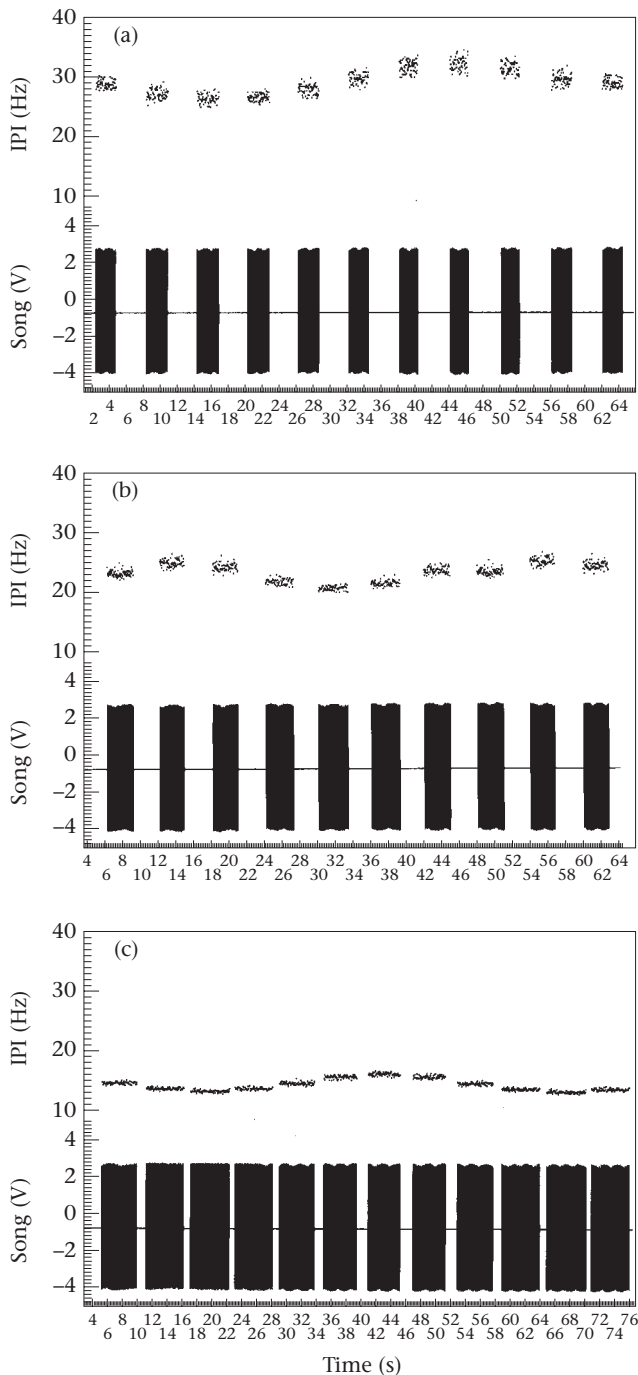
METHODS

Strains

The fly strains used were C80 (*D. melanogaster*) and Leticia (*D. simulans*). Both were collected in Europe in the late 1980s. A strain of *D. sechellia* was also examined initially, but the mating frequency was so low that we did not incorporate it into the study. Flies were reared in incubators at 25°C on a 12:12 h light:dark cycle. Virgins were collected twice daily under light carbon dioxide anaesthesia and kept at a maximum density of eight flies per vial before use in experiments. Experiments were carried out in a semianechoic room at a temperature of 25 ± 1°C. At least 24 h prior to experimentation, males had their wings removed under carbon dioxide anaesthesia.

Song Synthesis

We generated five artificial songs for two experiments. The first experiment re-examined the species-specific nature of song. Three song models were generated with pulse interval parameters (i.e. mean IPI at 25°C and Kyriacou & Hall cycle lengths) typical of *D. melanogaster* (mean IPI 35 ms, cycle length 55 s), *D. simulans* (mean IPI 45 ms, cycle length 35 s), and *D. sechellia* (mean IPI 70 ms, cycle length 48 s). Values were taken from the literature (Kyriacou & Hall 1986; Demetriades 1997). Songs were synthesized using the Signal 3.0 sound analysis system (Electronic Design, Belmont, MA, U.S.A.). The basic pulse was 10 ms long, and rapidly damped. The carrier frequency for all songs was 380 Hz, which is high for *D. melanogaster*, but typical for the other species. To generate the Kyriacou & Hall cycles, a sine wave of the appropriate period and amplitude was generated and the time of occurrence of pulses read off to produce one burst of 70 pulses every 6 s. To make the songs more realistic, we added a random term to the occurrence of each individual pulse ($\bar{X} \pm SE = 0 \pm 1$ ms). This has the effect of introducing some spread into the patterning of IPIs, without altering the overall periodicity. The amplitude of the Kyriacou & Hall cycles was set at 10% of the mean IPI (Kyriacou & Hall 1982). Figure 1 illustrates the patterning of IPIs for each song.

In a second experiment, we sought to disentangle the role of variability and cycling of IPIs with *D. melanogaster*. We used the '*D. melanogaster*' song (and silent control) from experiment 1, plus two additional songs. 'Constant IPI' had an invariant IPI (of 35 ms) and 'Random IPI' had the same IPI distribution as '*D. melanogaster*', but lacked the Kyriacou & Hall cycle. To produce the latter, the distribution of IPIs present in one complete cycle of the

Figure 1. The song models used in experiment 1. These represent song with typical mean interpulse interval (IPI) and Kyriacou & Hall cycle length for (a) *D. melanogaster* (mean IPI 35 ms, cycle length 55 s), (b) *D. simulans* (mean IPI 45 ms, cycle length 35 s) and (c) *D. sechellia* (mean IPI 70 ms, cycle length 48 s). The figure shows about 1 min of song (at this resolution individual pulses cannot be discerned clearly, only the burst structure is apparent) and the IPIs (with each dot representing a single IPI).

D. melanogaster song was resampled into a random sequence, and manipulated into the same burst structure as *D. melanogaster* song. Thus both songs had the same mean and variance of IPIs over the period of a cycle, but

the sequence in which they occurred was randomized in the Random IPI song model.
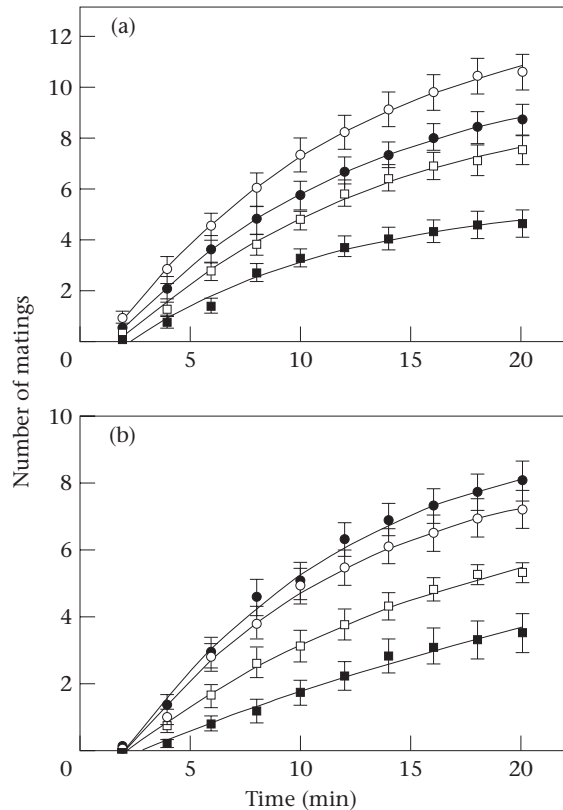
Test Procedure

We recorded songs on to reel to reel tape at 19 cm/s using a TASCAM 22-2 tape recorder. Another tape was recorded with only background electronic output from the PC and used for the 'Silent' control condition. Songs were played through the subwoofer of a MicroMedia PC sound system (Boston Acoustics Inc., Peabody, MA, U.S.A.), which reproduces the low-frequency pulses typical of fly song particularly well. Playback levels were standardized to 70 dB, measured using a Realistic 33-2050 sound level meter (calibrated with a Brüel & Kjær 2231M sound level meter and 4155 microphone).

To examine mating speeds, 20 virgin females were aspirated into the upper chamber of an acoustically transparent, Perspex and nylon, customized mating chamber measuring $50 \times 45 \times 30$ mm internally that was held over the speaker. We introduced 20 wingless males into a lower chamber and, after a 3-min period, removed a dividing partition and began playback. The number of mating pairs was recorded at 2-min intervals for 20 min (at which time early mating pairs began to dismount). Temperature was maintained at $25 \pm 1.5°$C during the playbacks.

A trial consisted of four playback tests (one for each model and the silent control). Each trial was completed on a single day. Male and female ages were not constant, but were equivalent across a single trial, for example on one day similar combinations of 2-, 3- or 5-day-old individuals would be used for each playback. Mean ages therefore varied between trials, and were examined as covariates as this could potentially influence mating speeds (average age in days: *D. melanogaster* females: 4.3, range 2–8; males: 4.18, 2–8; *D. simulans* females: 8.3, 6–14 days; males: 8.4, 6–12). The order of playback of each song and control was randomized for each trial. For experiment 1, 20 trials per species were completed, and for experiment 2, 21 trials were completed.
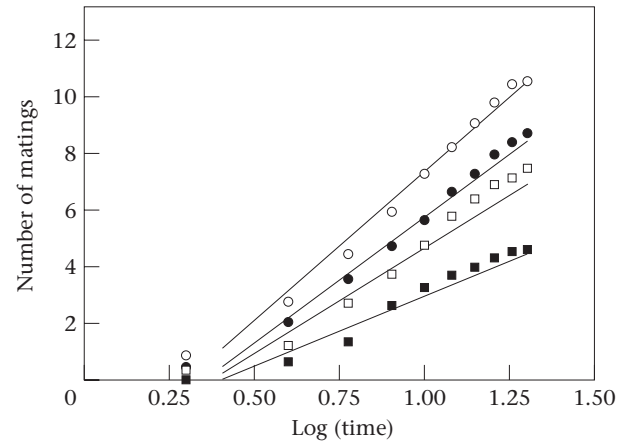
Statistical Analysis

Over the 20-min observation period, mating speed approximately followed a negative exponential function, reaching an apparent plateau before all females had mated (Fig. 2). The first statistical analysis carried out was an ANCOVA of the number of matings occurring at 20 min, with male and female age as covariates. The 'groups' term of all ANOVA analyses refers to the stimulus used for the playback, that is, the song models and silent control. Where the term 'song' is used, this refers only to the song models, that is, excluding the control group. Residual plots from this ANCOVA showed that all data conformed to normal assumptions. If a covariate was significant, residuals after adjusting for this covariate were used for further analysis. To explore the nature of significant differences in mating speed, we carried out

Figure 2. Mating speed (mean cumulative number of copulations per trial±SE versus time) of (a) *D. melanogaster* and (b) *D. simulans* females when stimulated with *D. melanogaster* (○), *D. sechellia* (□) or *D. simulans* (●), song models or silence (■). The fitted lines are negative exponential curves, for illustrative purposes only.

Figure 3. An example of the regression model fitted to the transformed data, in this case for *D. melanogaster* females. Note the lag before mating begins, which resulted in the use of models with variable intercept terms. Symbols as for Fig. 2.

one-way ANOVAS on the number of matings occurring at 20 min, followed by planned comparisons of these means across groups (using the procedures outlined in section 9.6 of Sokal & Rohlf 1981; one-way ANOVA after adjusting for covariates was chosen as it allows the use of this test). For experiment 1, there were two planned comparisons. First, was the number of matings greatest when females were stimulated with the homospecific song model (over the other songs and silent control)? Second, was the number of matings when females were stimulated with the homospecific song model greater than with the heterospecific songs (the first comparison could be significant simply because there were fewer matings in the silent control condition)? These comprise a nonorthogonal set of comparisons so, including the initial comparison across all song models and the control, the adjusted critical level of α is 0.017. For experiment 2, we carried out similar comparisons, except that the song model containing the Kyriacou & Hall cycle was compared with other songs and the silent control.

Only analysing the number of matings at one time point ignores information on the rate of mating during the playbacks (and, it could be argued, the choice of which time point to analyse is arbitrary and could influence the conclusions). Mating rate could be made linear by plotting against log (time), allowing relatively simple

linear regression analysis over the whole time course of the experiment. For each data set, a regression model was fitted with log (time), male age and female age as covariates, and song model (including silent control) as a grouping factor. All these analyses were carried out using the 'multiple linear regression with groups' routine of Genstat5 (1993). Omitting the intercept term from the model in order to constrain the lines to go through the origin, resulted in a poorer fit to the data than allowing a variable intercept. This occurs because there is a lag before mating commences, only after which is mating speed approximately log-linear. So the best statistical model for the data usually had a significantly negative intercept, which was related to the delay until initiation of mating. Figure 3 provides an example of the model-fitting procedure with the transformed data. In the resulting ANOVA tables, there are separate terms for each covariate (log (time), male and female age), the 'Groups' term tests whether the main, intercept, terms for the artificial song models (including silent control) were equal, and the 'Time*Groups' interaction term tests whether the slopes, that is, the mating rates over the whole experiment, are equal.

RESULTS

Experiment 1: Species-specific Songs

Figure 2 shows the cumulative mean mating speeds for both *D. melanogaster* and *D. simulans*. For both species, females mated most quickly when stimulated with the homospecific song model and the least effective song was that representing *D. sechellia*. Females mated in the absence of song, demonstrating that song is not obligatory for mating, but the silent control resulted in the slowest mating speeds.

Initial analyses showed that mating speeds were not influenced by male or female ages. Table 1 shows the result of an ANOVA of the number of matings occurring

Table 1. ANOVA of number of matings in experiment 1, examining the species specificity of song

Source of variance	*D. melanogaster*					*D. simulans*				
	df	SS	MS	F	P	df	SS	MS	F	P
Between groups	3	377.24	125.75	16.60	<0.001	3	247.64	82.55	15.19	<0.001
Homospecific song versus other groups										
(including control)	1	203.51	203.51	26.88	<0.001	1	110.71	110.71	20.39	<0.001
Homospecific song versus other songs	1	86.70	86.70	11.45	0.001	1	43.20	43.20	7.96	0.006
Within groups	76	575.65	7.57			76	413.05	5.43		
Total	79	952.89				79	660.69			

Table 2. Regression analysis of mating speeds in experiment 1, examining the species specificity of song

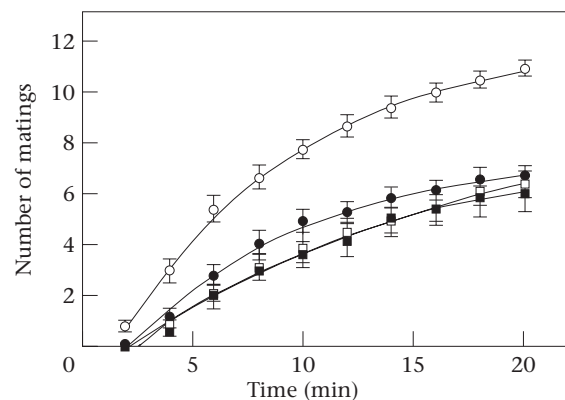| Source of variance	*D. melanogaster*				*D. simulans*			
	df	MS	F	P	df	MS	F	P
Time	1	4730.74	941.84	<0.001	1	3141.10	835.16	<0.001
Female age	1	67.58	13.45	<0.001	1	7.52	2.00	NS
Male age	1	3.77	0.75	NS	1	6.85	1.82	NS
Songs	3	571.67	113.81	<0.001	3	398.25	105.89	<0.001
Time*Songs	3	88.88	17.70	<0.001	3	80.58	21.426	<0.001
Residual	790	5.03			790	3.76		

at 20 min, including the planned comparisons. For both species, the number of matings varied between the groups and homospecific song resulted in significantly more matings than the other song models. However, heterospecific song still enhanced mating speed over the silent control. To confirm that *D. melanogaster* and *D. simulans* females respond differently to the songs of these two species, we carried out a combined two-way analysis of the number of matings of both species. The interaction term between female species and song model was significant ($F_{1,76}=4.71$, $P=0.03$), confirming that the response to these two song models differed between species.

Table 2 presents the results of the linear regression analysis. Female mating speeds differed significantly between the groups, with both main terms (related to the initiation of mating) and interactions (the slope of the regression, or overall rate of mating) differing significantly. *Drosophila melanogaster* females mated most quickly when stimulated with the *D. melanogaster* song model, and both the intercept ($F_{1,394}=29.61$, $P<0.001$) and slope ($F_{1,394}=4.14$, $P=0.043$) differed significantly between the *D. melanogaster* and *D. simulans* song models only. Younger *D. melanogaster* females mated more quickly. *Drosophila simulans* females mated most quickly when stimulated with the *D. simulans* song model and, as with *D. melanogaster* females, both intercepts and slopes varied significantly between groups (Table 2). Restricting the analysis to only the *D. melanogaster* and *D. simulans* song models did not produce a significantly different slope ($F_{1,394}=1.24$, NS), although the intercepts did differ ($F_{1,394}=31.31$, $P<0.001$). Neither male nor female age influenced mating speed in *D. simulans*.

Experiment 2: Influence of the Kyriacou & Hall Cycle

Figure 4 shows the results of experiment 2. Constant IPI was no more effective at increasing mating speed than the silent control, confirming the results of Kyriacou & Hall (1982). More surprisingly, song with variable yet non-cycling IPIs was hardly better. The presence of the Kyriacou & Hall cycle substantially increased female mating speed.

The initial ANCOVA showed that the ages of the individuals used in this experiment influenced mating speed (younger males mated more slowly, younger

Figure 4. Mating speed ($\bar{X}\pm SE$) of *D. melanogaster* females when stimulated with *D. melanogaster* song containing a Kyriacou & Hall cycle (○), one with randomly patterned IPIs (●), one with a constant IPI (■), or silence (□). The fitted lines are negative exponential curves, for illustrative purposes only.

Table 3. ANOVA of mating speeds in experiment 2, examining the role of Kyriacou & Hall cycles

Source of variance	df	SS	MS	F	P
Between groups	3	331.56	110.52	23.08	<0.001
Cycling song versus other groups (including control)	1	244.01	244.01	50.95	<0.001
Cycling song versus other songs	1	292.57	292.57	61.09	<0.001
Within groups	83	383.11	4.79		
Total	86	714.67			

females more quickly; $F_{2,78}=5.73$, $P=0.005$; and see the results of the regression analysis below). The comparisons between groups were therefore carried out using residuals from a multiple regression analysis of the number of females mating at 20 min against male and female ages. Table 3 shows that the song containing a Kyriacou & Hall cycle was significantly more effective in stimulating matings than the other songs were. The regression analysis confirms this result (Table 4); indeed, over the whole experiment more than 90% of the variance due to song models was contributed by the song containing the Kyriacou & Hall cycle.

DISCUSSION

The results of experiment 1 demonstrate that the courtship songs of *D. melanogaster* and *D. simulans* increase mating speed in a species-specific manner in that matings occurred most quickly when the homospecific song model was used for playback. Although male recognition of females is strongly determined by cuticular hydrocarbon blend, so that female pheromonal profile has a major influence on sexual isolation in this group (Jallon & David 1987; Cobb & Jallon 1990; Coyne et al. 1994), female preference is also important in at least some interactions between these species. *Drosophila simulans* females typically show persistent rejection behaviours towards the courtship of *D. melanogaster* males. Also, female preferences are strongly implicated in patterns of assortative mating within *D. melanogaster* (Wu et al. 1995). It is not clear if male pheromones have a large influence on female mating preferences (Scott 1994, 1996; Cobb & Jallon 1996; Ferveur et al. 1996; Coyne et al., in press). Our results suggest that, at least for the interspecific interactions, male song is a trait likely to influence sexual isolation. However, heterospecific song still stimulated mating, so song is not as important for

species discrimination as in some other *Drosophila* species, in which heterospecific song inhibits mating (Tomaru et al. 1995).

The fact that song is a sexually dimorphic male trait would strongly imply that its role is mediated via female preferences. However, male song can prime male sexual activity (von Schilcher 1976b; Crossley et al. 1995) and playback experiments such as ours potentially confound the effect of song on males and females (Ritchie et al. 1998; although it is not known if the stimulatory effect of songs on males is species specific). Playback experiments with males lacking aristae imply that female preferences are responsible for most of the effect (von Schilcher 1976b; Kyriacou & Hall 1982; but see Burnet et al. 1977; Averhoff et al. 1979). Whichever sex plays the major role, the potential for song to influence sexual isolation is evident.

A role of song in sexual isolation in this species group has come to be questioned recently for a variety of reasons. First, a major role for hydrocarbons has been unambiguously demonstrated (Coyne et al. 1994). Second, song playback experiments have proved notoriously variable in outcome. Although not quantifiable, it is certainly the case that published experiments are a biased sample of those attempted with *D. melanogaster*. Several attempts to replicate the results of Bennet-Clark & Ewing (1969) and Kyriacou & Hall (1982, 1984, 1986) have had only limited success (van den Berg 1988; Jones 1993; M. G. Ritchie, personal observations). The playback techniques typically used with *D. melanogaster* are of necessity rather crude, which militates against detecting weak preferences (Ritchie et al. 1998). We suspect that the choice of loudspeaker might have been a significant factor in the success of the experiments described here. Another reason to question the role of song involves how environmentally labile the trait is. Mean IPI shows high environmental variance (Ritchie & Kyriacou 1996; Noor & Aquadro 1998), with the major determinant being ambient temperature (Shorey 1962). It would seem logical to suppose that an important signal should not be environmentally labile (Noor & Aquadro 1998 question the role of song in sexual isolation between *D. persimilis* and *D. pseudoobscura* because of this). This may not be as significant a problem as it first appears. Female preferences might be equally influenced by temperature, maintaining a match between the sexes (e.g Bauer & von Helversen 1987), at least when courting individuals are at a similar temperature. This seems likely in poikilotherms. Alternatively, matings may occur only within a narrow temperature range in the

Table 4. Regression analysis of mating speeds in experiment 2, examining the role of Kyriacou & Hall cycles

Source of variance	df	MS	F	P
Time	1	4585.60	1167.92	<0.001
Female age	1	40.72	10.37	<0.001
Male age	1	294.79	75.08	<0.001
Songs	3	635.28	161.80	<0.001
Time*Songs	3	65.87	16.78	<0.001
Residual	830	3.926		

field. Also, it is important to note that Kyriacou & Hall cycles are not influenced by temperature (Kyriacou & Hall 1980; Costa et al. 1992). Because of this, Kyriacou & Hall cycles might be a more reliable signal component of songs, which might provide a reason for female preferences to become cued into this trait (Kyriacou & Hall 1982).

Experiment 1 suggests that the influence of song on sexual isolation would vary asymmetrically between *D. simulans* and *D. melanogaster*, with *D. melanogaster* females being more likely to discriminate against heterospecific song. Interestingly, this matches variation in mean IPI between strains; *D. simulans* strains often being more variable than *D. melanogaster* (Kawanishi & Watanabe 1980; Ritchie et al. 1994), perhaps reflecting a tighter song preference requirement among *D. melanogaster* females (*D. simulans* females do not mate in the dark, suggesting visual stimuli are important for this species). *Drosophila simulans* males are unlikely to court *D. melanogaster* females because of their hydrocarbon profile (Coyne et al. 1994), so the likelihood of heterospecific courtship would not seem to match the asymmetry in the strength of song preference.

Experiment 2 demonstrates that the presence of cycles in *D. melanogaster* song increases its effectiveness in playback experiments. Kyriacou & Hall (1982, 1988) speculated that cycling song is more effective than constant IPI because individual females vary in preferences for mean IPI. Males with a greater variability in IPI would stimulate more females. Our results show clearly that it is not variability per se that is important, but the presence of the Kyriacou & Hall cycle itself. We find this a surprising and remarkable result. One reason that Kyriacou & Hall cycles have proved controversial is that they are difficult to detect (Kyriacou et al. 1990; Alt et al. 1998). *Drosophila melanogaster* song typically contains short bursts of variable duration. Clear, unambiguous cycles in IPI are rarely obvious. Kyriacou & Hall (1989) recommended calculating mean IPI in 10-s time bins in order to reduce the variability, but even so, spectral or other time series analysis is usually required to detect the cycles (Kyriacou et al. 1990; Alt et al. 1998). If cycles in synthetic song have a strong stimulatory effect on mating speed (Fig. 4), why do males not produce more obvious cycles in their song? Also, how can female flies detect the cycles present in real song? Bouts of courtship in the field are usually brief, the great majority being less than 1 min long (Gromko & Markow 1993). Ewing (1988) argued that this implied that the cycle, with a period of around 1 min in *D. melanogaster*, was unlikely to have a significant behavioural role. Theoretically, females would in fact only need to hear between quarter to half a cycle in order to infer cycle length if it were clearly produced. Alternatively, the relative rate of increase or decrease in IPI rather than cycle length itself might play the key role in stimulation (Kyriacou & Hall 1982; Alt et al. 1998).

Because females have a preference for a trait, it does not necessarily follow that the trait is an important component of the natural variation in male mating success under field conditions. To give a trivial example, female guppies, *Poecilia reticulata*, might prefer bright male tails, but if males typically cannot display this because of water conditions (e.g. high turbidity), the preference may not be behaviourally significant. The recent expansion of interest in the sensory exploitation model of sexual selection relies on the observation that in some species females have preferences for traits lacking in males (Ryan 1990; Basolo 1995; Shaw 1995). Since male song does not show its cycling nature clearly, owing to the intermittent timing of courtship and its typical short duration, the preference female *Drosophila* have for Kyriacou & Hall cycles in song may not be a major force in song evolution. Males of a strain of *D. melanogaster* that had been genetically transformed with the *period* gene of *D. simulans*, and therefore sang with a Kyriacou & Hall cycle length of *D. simulans* (although were otherwise like *D. melanogaster*), did not have decreased mating success with *D. melanogaster* females (Ritchie & Kyriacou 1994). The mating system of *D. melanogaster* might therefore provide an example in which females have a preference for a trait that is present but not clearly expressed in males. The *period* gene, which determines the length of the song cycle in males, does not strongly influence female preference for this trait: females who carry different *period* mutant alleles that affect cycle length in males have normal preferences (Greenacre et al. 1993; Ritchie & Kyriacou 1994). So the preference does not arise as a pleiotropic by-product of the *period* gene. However, this does not rule out the possibility that the preference is an incidental product of the biological clock present in all organisms, as *period* plays only a part in this (Hall 1998).

In conclusion, we have confirmed that the courtship songs of *D. melanogaster* and *D. simulans* have the potential to influence sexual isolation between these species. We have also shown that the presence of Kyriacou & Hall cycles has a greater stimulatory effect on mating speed than the increased variability in IPI which inevitably results from these cycles. Whether Kyriacou & Hall cycles play an important role in mating success and sexual isolation in the field will require further dissection of the effect of IPI variability on mating speed by playback experiments. Study of courtship under 'natural' conditions would also be informative.

Acknowledgments

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