

Genetic variability of the interpulse interval of courtship song among some European populations of *Drosophila melanogaster*

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The interpulse interval of the courtship song of *Drosophila melanogaster* is a character which may play a significant role in mating success and reproductive isolation. Here we examine the variability of interpulse interval among replicated laboratory strains of *D. melanogaster*. There is no significant variation among populations of different geographical origin. This suggests that interpulse interval is subject to strong selection, as the populations are known to differ for other characters. One population, however, was sufficiently different to allow a genetic analysis. Reciprocal F₁s and backcrosses implied that the variance was predominantly additive and autosomal. Possible sources of selection on interpulse interval are discussed.

Keywords: courtship song, *Drosophila melanogaster*, genetics, intraspecific variation, mating behaviour, speciation.

Introduction

Premating isolation between closely related animal species often arises following the evolution of new forms of mating signal and preferences (Butlin & Ritchie, 1994). Identifying populations of a species which show divergent mating signals may help distinguish factors which contribute to this process and genetic analysis of such populations can tell us the nature and extent of genetic differences contributing to the new phenotype. Finding suitable populations for such analyses may be difficult if species-specific mating signals demonstrate low variability among populations, as might be expected. However, there is an increasing number of examples where thorough studies have identified variation (e.g. Henry & Wells, 1990; Ritchie, 1991; Maksymovich & Verrell, 1993; see Butlin, 1994, for a review). Genetic analyses of these populations have been limited, precluding general conclusions about their genetic architecture. For example, are new forms of mating signal or preference likely to be polygenic or due to single genes, and is sex-linkage involved? It has been argued that knowledge of the genetic changes involved may allow us to infer aspects

of the evolutionary process. Polygenic control may suggest that new forms have evolved gradually whereas single genes may imply that they can appear more suddenly (Templeton, 1981; Carson & Templeton, 1984; Coyne, 1985, 1992a). The degree of additive, dominance and epistatic effects may indicate the likely strength and even the direction of selection (Broadhurst, 1979; Falconer, 1981; Mather & Jinks, 1982).

Broadly speaking, there have been three approaches to studying the variability of mating behaviour in species of the melanogaster group of *Drosophila*. These are studies of patterns of assortative mating in the laboratory, of cuticular pheromonal variation and of courtship song. Assortative mating studies (within or between species) have detected some variation among populations (van den Berg, 1988; Welbergen *et al.*, 1992) but the largest systematic studies of natural populations have indicated that such variation is limited (Petit *et al.*, 1976; Henderson & Lambert, 1982; Lambert & Henderson, 1986). Studies of pheromonal variation have identified two major pheromonal races within *Drosophila melanogaster* and these have the potential to influence mating patterns between races (Jallon, 1984; Scott & Richmond, 1988). The courtship song of *D. melanogaster* seems to be another important mating signal because all courtships include vigorous singing by males and some experiments have shown that female mating propensity can be enhanced by playback of artificial song (e.g.

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Bennet-Clark & Ewing, 1969; von Schilcher, 1976; but see Kyriacou & Hall, 1982; van den Berg, 1988; von Schilcher, 1989; Greenacre *et al.*, 1993). Song may have influenced speciation within the subgroup as song varies substantially among species (Cowling & Burnet, 1981) and some playback experiments show that female mating speed increases most with the correct species-specific song pattern, at least for *D. melanogaster* and *D. simulans* (Kyriacou & Hall, 1982, 1986). Two distinct types of song are produced during courtship, pulse and hum (or 'sine') song, and most studies have concentrated on characteristics of the interpulse interval of pulse song. Ritchie & Kyriacou (1994) found that the heritability of characteristics of both song types was low within a large laboratory collection of *D. melanogaster*, suggesting strong selection from some source. Here we examine variation in mean interpulse interval between one North African and five European populations of *D. melanogaster*.

Materials and methods

Flies were maintained in the laboratory at 18° or 25°C using standard techniques (stocks were maintained at 18°C but moved to 25°C during periods of experimental work). Details of how the populations were collected can be found in Costa *et al.* (1992). Each population was sublined into at least two independent stocks within two generations of arriving in the laboratory. Each separate stock was maintained by simple mass transfer of a sample of flies to a fresh bottle each generation. We estimate that at least 40 flies were used for each transfer.

After around 20 generations of culture in the laboratory, the songs of males from two stocks of each population were recorded using standard procedures (Kyriacou & Hall, 1980; Ritchie & Kyriacou, 1994). Briefly, virgin male flies were collected and maintained individually until recorded at an age of 2–5 days using an 'Insectavox' microphone (Gorzycza & Hall, 1987) and Revox tape recorder. All the recordings analysed here were made in a period of a few weeks under similar conditions and flies from the different stocks were interspersed in the recording sequence. We have found that interpulse interval can change slightly between different periods of recording (e.g. Ritchie & Kyriacou, 1994) although differences between strains remain. Such environmental effects can confuse comparisons between recordings from different experimental periods; therefore we report only results from flies recorded within a single such period. This limits the number of populations that can be included in such a survey.

For each fly, at least 2 min of song was digitized using a Cambridge Electronic Design 1401 A/D con-

verter (at a minimum of 2 kHz after bandpass filtering at around 150 Hz to 1 kHz). Mean interpulse intervals were measured for each fly using the 'SPIKE2' software package (Copyright C.E.D.) and custom written programs. No mean based on fewer than 100 individual interpulse intervals was included in the analysis and typically around 400 would contribute to the mean per male. This analytical technique has been checked for accuracy (Ritchie & Kyriacou, 1994).

Variation in interpulse intervals within an individual *D. melanogaster* song is not random but cycles periodically with a periodicity of around 1 min (Kyriacou *et al.*, 1990). Analysing a minimum of 2 min of song per fly will have ensured that all stages of these cycles were sampled. Cycle length itself was not measured during these studies. Recordings were made over the temperature range 20–28°C (mean = 24.1°C, s.d. = 1.4°C). Temperature has a large influence on interpulse interval, therefore each mean interpulse interval was corrected to 25°C using a highly significant linear regression coefficient prepared from a detailed study of song from a single population from N. Italy (Ritchie & Kyriacou, 1994) which was not included in the present survey. If the relationship between interpulse interval and temperature varies substantially between populations this could introduce some error into our estimates of interpulse interval but this is not likely to be a problem as the variability in recording temperature is low.

Results

Table 1 shows the overall mean interpulse interval for each population. Variation among populations was greater than variation among stocks within a population, possibly suggesting that genetic drift within laboratory stocks reared by routine techniques does not have a major effect on mean interpulse interval. Variation among populations was not statistically significant when tested against the variation among stocks.

Apart from one population, Rethimnon, from the Greek islands, the mean interpulse interval per population seems remarkably stable. The mean interpulse interval of Rethimnon is 2–3 ms different from the other populations, which themselves vary by only around 0.5 ms. For comparison, the mean difference between *D. melanogaster* and *D. simulans* is approximately 15 ms and no pair of species from the melanogaster group differs by less than this (Cowling & Burnet, 1981). While it is noticeable that the range of interpulse intervals within a population is large (Table 1), the coefficients of variation are all low (averaging 7 per cent). Thus interpulse interval seems somewhat stereotyped.

Table 1 Overall mean interpulse interval (IPI) per population†

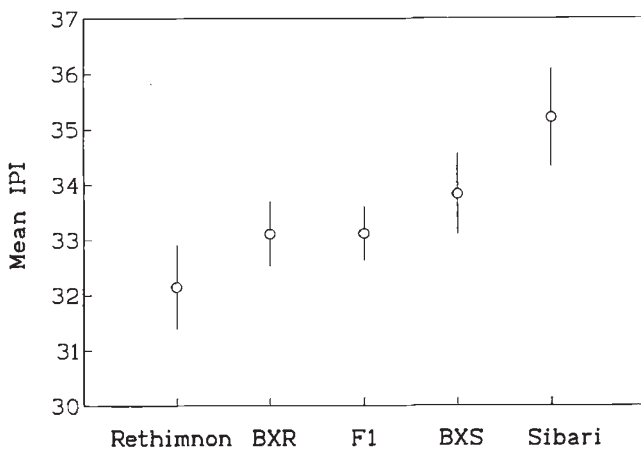
Population	N	Mean IPI	S.E.	CV (%)	Range
Casablanca (Morocco)	12	35.995	0.672	6.47	32.201–39.525
Leiden (Holland)	7	35.630	1.090	8.11	30.470–39.500
Bologna (Italy)	16	35.779	0.572	6.39	31.212–38.424
Sibari (Italy)	17	35.375	0.529	6.17	30.620–39.013
Andros (Greece)	19	35.557	0.663	8.13	31.895–44.198
Rethimnon (Greece)	22	32.907	0.506	7.21	30.062–40.041

Analysis of variance:

Source of variance	Mean square	d.f.	F	P
Populations	25.808	5	3.34	0.087
Stocks (populations)	7.721	6	1.28	0.274
Error	6.011	81		

†Calculated by averaging the mean IPIs of each male (column N gives number of males per population).

CV is the coefficient of variation of these means, S.E. the standard error.

**Fig. 1** Overall mean interpulse interval amongst Rethimnon, Sibari, F₁ and backcross genotypes (F₁ males × Rethimnon or Sibari females). Error bars are 95 per cent confidence intervals.

To examine the genetic basis of the unusual phenotype of flies from Rethimnon, these were crossed to the Sibari population. Figure 1 shows the mean interpulse interval of F₁ and backcross genotypes. The difference between these populations was clearly genetic in origin. Table 2 presents estimates of the additive and dominance components and summarizes tests of an

Table 2 Genetic components of interpulse interval for Rethimnon and Sibari populations

Genotype	N	Vx	Weight	Observed	Expected
<i>Sibari</i>	12	0.149	6.711	32.149	32.441
<i>Rethimnon</i>	9	0.199	5.025	35.210	35.049
F ₁	24	0.062	16.129	33.115	33.189
BXS	19	0.087	11.494	33.107	32.815
BXR	16	0.137	7.299	33.834	34.119

Parameter	Fitted value	S.E.
m (midpoint)	33.745	0.258
[d] (additive)	-1.304	0.250
[h] (dominance)	-0.556	0.384

Scaling tests
A = 0.950 ± 0.748
B = -0.657 ± 0.899
Joint: $\chi^2_{[2]} = 2.363$, n.s.

additive-dominance model (after Mather & Jinks, 1977), which fitted the data well. Only the additive component was significant (Table 2), there was no difference between reciprocal F₁s (R × S = 33.000 ms, N = 12, S.E. = 0.401; S × R = 33.230 ms, N = 12, S.E. = 0.307; *t* = 0.455, n.s.) and the backcross genotypes were continuously distributed (Fig. 2). It therefore seems most probable that the Rethimnon song difference is an autosomal polygenic additive character, although these data do not allow us to reject the possibility of ambidirectional dominance.

Discussion

Henderson & Lambert (1982) found no significant assortative mating between stocks of *D. melanogaster* taken from world-wide populations (see also Lambert & Henderson, 1986) and attributed this to stabilizing selection acting on the 'Specific Mate Recognition System' (Paterson, 1985). It does seem likely that behaviours important to mating success will be subject to strong selection and coevolution between signals and preferences would be expected to generate a degree of stabilizing selection which would reduce the genetic variability within populations. However, in the absence of external sources of selection on the communication system (e.g. environmental selection on signal propagation), there is no reason to suppose that truly disjunct geographical populations should evolve to any single particular phenotype as long as preferences and signals are in balance within a population. Indeed, theory shows that a direct viability optimum on a male mating signal imposed by the environment can still result in isolated populations (with the same environment) showing a range of combinations of trait and preferences (Lande, 1981). Stable equilibria occur

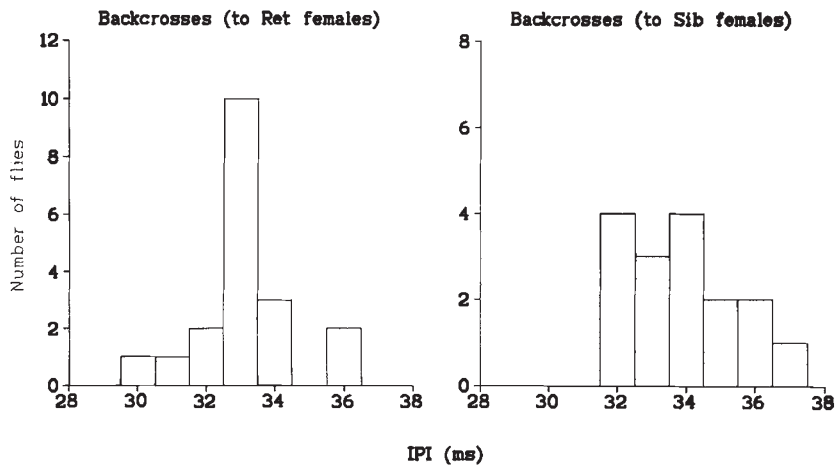


Fig. 2 Distribution of mean interpulse interval within the backcross genotypes (F_1 males \times Rethimnon or Sibari females).

where selection from viability and preference counterbalance.

The relatively low variability of interpulse interval in *D. melanogaster* is therefore something of a surprise. The present study found little variation among stocks recently originating from six European populations. Kawanishi & Watanabe (1980) obtained similar results comparing 11 Japanese and three non-Japanese laboratory strains. Van den Berg (1988) found a difference of almost 3 ms in the mean interpulse intervals of single strains originating from Africa and South America. Comparisons between different studies are not valid due to variation in techniques and environmental effects.

Limited variability could occur if distinct geographical populations are genetically homogeneous due to extensive contemporary gene flow or were all recently colonized from a common source. This seems unlikely as *D. melanogaster* is typically relatively variable for morphological and genetic markers (Singh & Long, 1992) and the populations studied here are known to vary for at least one genetic marker (Costa *et al.*, 1992). Nevertheless, the combination of relatively recent colonization from Africa (Lachaise *et al.*, 1988) and large migration rates within Europe could contribute to the results found here. The fact that *D. melanogaster* normally shows more variation among populations than *D. simulans* (Singh & Long, 1992), yet the opposite pattern may be found for interpulse interval is extremely interesting (Kawanishi & Watanabe, 1980; Kyriacou & Hall, 1986). Selection on song must differ between the species.

Low variation amongst populations of *D. melanogaster* would be less surprising if selection is directional, despite the prediction of stabilizing selection for mating signals. This could arise if, for example, female mating propensity were simply a product of the

amount of song heard. Males would then be under strong directional selection for reduced interpulse interval, maximizing song output. Interpulse interval may then reflect a physiologically determined upper limit of song production which might be expected to vary less among populations than signal-preference equilibrium states. An artificial selection experiment has been more successful at lengthening than shortening mean interpulse interval (M. G. Ritchie, unpublished data). However, the predominantly additive genetic variation among populations found here would not be predicted under such a selection scheme as strong directional selection is expected to produce a directionally dominant genetic architecture (Broadhurst, 1979; Mather & Jinks, 1982). Cowling (1980) also found only additive genetic variation for *D. melanogaster* interpulse interval in a diallel analysis of variability among laboratory strains (one originally from Africa showed a low interpulse interval but the history of the stocks studied was not clear).

Another possibility is that the strongest source of selection on male song is indirect, i.e. from sources other than female mating preferences. Environmental sources of selection on muscle physiology will presumably be quite stable in the laboratory which could reduce variation among stocks. Artificial song playback experiments with *D. melanogaster* typically show low resolution of female preferences and seem unlikely to reveal whether selection from female preferences is directional or stabilizing. It is difficult to distinguish if this is because female song preferences are truly weak, because of poor experimentation (e.g. song synthesis), or if the species is simply not well suited to such experiments. We suspect the latter may be likely.

That the slightly divergent song found here was due to quantitative additive genetic variation suggests that

this new form has arisen gradually. However, relatively few genes could be involved. The lack of sex-linkage does not support the contention that new mating signals may be predominantly sex-linked (Ewing, 1969). Charlesworth *et al.* (1987) found little evidence to support this when reviewing behavioural data. In general, sex-linkage may be more prevalent for sources of postmating than premating isolation (Coyne, 1992b).

It is not known if flies from Rethimnon show any premating isolation from the other *D. melanogaster* populations. White *et al.* (1994) have recently argued that Specific Mate Recognition Systems may often retain their cohesive qualities across a species despite showing variability in their components. This is of course true; many aspects of mating behaviour may vary widely without constituting new 'systems' of mate recognition and song is only one of many such factors in *Drosophila*. However, White *et al.* (1994) take this argument further when they argue that the concept of variability cannot be applied to mate recognition systems [e.g. 'the concept of variation cannot be used in relation to the systems (SMRSs) themselves']. This is incompatible with the observation that many species are composed of clearly differentiated subforms including significant components of mating behaviours, often evidenced by strong assortative mating between forms (e.g. Ritchie, 1991; Maksymovich & Verrell, 1993). These forms represent the raw material of evolutionary change and speciation must have often originated from such races. They therefore deserve our special attention.

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