Genet. Res., Camb. (1999), **74**, *pp.* 121–128. *With* 2 *figures.* Printed in the United Kingdom © 1999 Cambridge University Press

Genetic variation affecting heart rate in *Drosophila melanogaster*

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(*Received 27 September 1998 and in revised form 22 February 1999*)

Summary

Heart rate in pre-pupae of *Drosophila melanogaster* is shown to vary over a wide range from 2·5 to 3·7 beats per second. Quantitative genetic analysis of a sample of 11 highly inbred lines indicates that approaching one-quarter of the total variance in natural populations can be attributed to genetic differences between flies. A hypomorphic allele of the potassium channel gene *ether-a-gogo*, which is homologous to a human long-QT syndrome susceptibility gene (*HERG*), has a heart rate at the low end of the wild-type range, but this effect can be suppressed in certain wild-type genetic backgrounds. This study provides a baseline for investigation of pharmacological and other physiological influences on heart rate in the model organism, and implies that quantitative genetic dissection will provide insight into the molecular basis for variation in normal and arrhythmic heart function.

1. Introduction

The ability of a heart to beat at a constant rate over the lifetime of an organism is a remarkable genetic phenomenon. Mammalian hearts skip just a handful of approaching one hundred thousand beats in any given day, and yet heart rate responds in a controlled manner to numerous variables such as circadian rhythm, stress and exercise, ageing, and genetic differences among individuals. Invertebrate hearts also must respond to a range of variables, including changes in temperature and developmental differences associated with moulting and metamorphosis. These observations suggest that the genetic regulation of heart rate is likely to be complex, and raise interesting issues concerning the homeostatic mechanisms that maintain the constancy of the heart beat.

The *Drosophila* heart is a simple muscular dorsal vessel that is used to pump haemolymph around the body throughout embryogenesis, and in larvae, pupae and adults (Rizki, 1978). A caudally located pacemaker responds to injection of the neurotransmitters serotonin, dopamine and acetylcholine, among others (Johnson *et al*., 1997), while a rostrally located pacemaker is innervated by neurons that express the cardioinhibitory SDNFMRFamide peptide (Nichols *et al*., 1999). In addition, differences in basal heart rate and in the pharmacology of cardiac excitation between larvae and adults (Zornik *et al*., 1999) imply that different genetic factors regulate heart rate at successive stages of the life cycle.

Despite the relative simplicity of the structure of the dorsal vessel, it has been established that vertebrate and invertebrate hearts have a common evolutionary origin and share numerous developmental and pharmacological properties. Homologues of the homeobox gene *tinman*, which is absolutely required for *Drosophila* heart development, are expressed and required in the early stages of mouse cardiac development, and aspects of heart muscle structure are also likely to be conserved (Bodmer & Venkatesh, 1998). There is good evidence for involvement of potassium and calcium channels in the regulation of ion currents in the fly heart muscle (Gu & Singh, 1995) as in vertebrates, but blockers of sodium channels do not appear to affect the *Drosophila* pupal heart (Johnson *et al*., 1998). Another pharmacological difference is

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that acetylcholine is cardioinhibitory in vertebrates and in *Drosophila* adults (Zornik *et al*., 1999) but cardio-acceleratory in insect pupae. Consequently, quantitative study of the regulation of heart rate in *Drosophila* is likely to illuminate general principles of genetic interactions that affect cardiac arrhythmia (Dowse *et al*., 1995), and to some extent may even provide a direct model for genetic modulation of human heart rate.

The power of genetic analysis to identify the molecular basis of aberrant heart rates is demonstrated by the recent cloning of two major susceptibility loci for hereditary long-QT syndrome in humans (Curran *et al*., 1995; Russell & Dick, 1996). The two genes encode components of the heart muscle sodium and potassium channels that regulate the rate of contraction. One of these, *HERG*, is structurally related to *Drosophila ether-a-gogo* (*eag*), mutations of which cause a significant lengthening of each heart beat of the fly (Johnson *et al*., 1998). Some of the properties of long-QT syndrome that are not well understood include the age of onset of severe arrhythmia, causes of sudden cardiac arrest, and modulation of syncope. It is interesting to ask whether genetic factors might modulate these properties and, if they do, what population genetic factors control the frequency of mutations or polymorphisms affecting heart rate.

In this paper we present an initial analysis of the distribution of genetic variation affecting heart rate in wild-type and eag^1 mutant *Drosophila*. We show that there is extensive genetic variation affecting basal heart rate in pupae, the effects of which become particularly apparent in inbred lines. Recently caught flies can have heart rates as low as those seen in mutant *eag* stocks, while some genetic backgrounds can render the *eag* phenotype indistinguishable from wild-type. The results suggest both that there is a strong buffering capacity modulating heart rate in flies, and that polymorphisms that have a strong influence on heart rate are prevalent in natural populations.

2. Materials and methods

(i) *Lines and crosses*

Wild-type lines designated W1, W3, etc., were obtained from the Bowling Green stock centre and have been in the laboratory for up to 20 years. Localities of individual lines (which were established from one or a few isofemales) are listed in Table 1 of Gibson & van Helden (1997), and include Bermuda, Columbia, Kenya, Australia, South Africa, southern Europe, and various localities in the United States. Lines designated A1, A2, etc., derive from isofemales trapped in the Kerrytown fruit market, Ann Arbor, Michigan, in summer 1996. Near-isogenic lines were established by between 12 and 15 generations of sib pair-mating of a subset of 11 of the isofemale lines.

A laboratory stock carrying the X-linked eag^1 homozygous viable, recessive loss of function, EMS-induced allele was obtained from Dr B. Ganetzky (University of Wisconsin). It was inbred by pairwise sib-mating for 10 generations to generate a series of near-isogenic lines, two of which (renamed *eag*-2 and *eag*-6) were chosen for further analysis as they have slightly different basal heart rates but relatively constant rhythms.

Genetic crosses were performed by mixing approximately 50 virgin female *eag* flies with 10 or more wild-type males, and allowing them to lay eggs in bottles for periods of 2 or 3 days. All progeny are consequently either heterozygous females or hemizygous mutant males in a constant heterozygous autosomal background. Typically, four or five crosses were analysed per week, and the replicate blocks were performed 2 months apart. Humidity was not controlled in the laboratory, and may be a source of environmental variation contributing to replicate effects.

(ii) *Heart rate assays*

Larvae were reared in small plastic bottles on standard cornmeal/agar medium supplemented with live yeast, and grown at 25 °C. To record the heart rate, white pre-pupae were placed dorsal-side up on a glass slide, and examined at $\times 10$ magnification until a strong heart beat was detected at the posterior end of the animal. Recordings were taken after refocusing at $\times 40$ magnification. Fluctuation in light intensity due to movement of the dorsal vessel tissue was detected by a phototransducer mounted on one of the two binocular eyepieces and amplified electronically using a small hand-made circuit board. For the preliminary analyses, the output was printed directly on a simple polygraph after manual modulation of the voltage to produce discrete beats of approximately 2 cm amplitude on graph paper.

For the more precise analyses of isogenic lines and *eag* crosses, the amplified signal was fed into a Power Macintosh 7100 computer by way of a MacLab hardware system (AD Instruments), and analysed using MacLab Chart software, which allows the distance between successive peaks to be measured. In this case, the heart rate of each individual fly was calculated as the mean value of 100 successive beats, after removal of occasional skipped beats that were indicated by a gap between successive peaks equivalent to the length of two or more typical beats for the line. Within-individual variance in beat length was measured as the standard deviation of the 100 beat lengths. Comparison of the five lines in common between the isofemale and isogenic studies indicated that measurements made with the chart recorder were

consistently 10–15% lower than with the computer. This difference can be attributed to errors associated with manual reading of the charts, the fact that skipped beats were not deleted from the preliminary study, and mis-calibration of the rate of paper-feeding of the polygraph. To allow qualitative comparison of the two data sets, the raw data from the preliminary survey were multiplied by 1·11 to obtain the values in Table 1. No statistical comparisons of the two data sets were made.

(iii) *Statistical analyses*

The effect of sampling error on estimation of the range of phenotype means among lines can be calculated by regressing the observed line means onto the population mean using the coefficient $V_B/(V_B+V_W)$ where V_B is the between-line variance (equivalent to the variance component for lines in Table 3*a*) and V_W is the within-line variance (equivalent to the common environmental replicate variance component, plus the residual variance component divided by the number of individuals scored per line). For the isogenic lines, this value is $0{\cdot}0945/(0{\cdot}0945+0{\cdot}0109+(0{\cdot}0976/40)) \sim 0{\cdot}92$. For the isofemale lines, the variation in number of replicates and sample size per line leads to some variation in the estimate of this coefficient according to how variance components are estimated, but conservatively takes a value of 0·75. Consequently, the true range of phenotypes is overestimated by about 10% in the isogenic lines and 25% in the isofemale lines.

Analysis of variance was performed using PROC GLM in SAS for Windows Version 6.12 software (SAS Institute, 1989), assuming random effects of line and replicate, but a fixed effect of cross. Error mean squares for *F* value calculations for the two-way ANOVA in Table 3*b* were MS(Line × Cross) for the Line and Cross terms, MS (Replicate(Line × Cross)) for the Line × Cross interaction, and MS(Error) for the replicate within-line and Cross term. Variance components were calculated using the restricted maximum likelihood (REML) option of PROC VARCOMP. Raw data were essentially normally distributed (Fig. 1), so no scale transformation was performed. The correlation coefficient (Robertson, 1959) was calculated using mean square (MS) values given in Table 3 as $r=(MS_L-MS_{C\times L})/(MS_L+MS_{C\times L}-2.MS_{error})$.

3. Results

(i) *Preliminary survey of variation in 24 isofemale lines*

In order to gain an appreciation of the amount and distribution of variation in pre-pupal heart rate, we first performed a survey of the phenotype in 24 near-isofemale lines using a simple detection system. Heart rate was picked up as a fluctuation in light intensity that was received by a phototransistor connected to the eyepiece of a compound microscope, the signal was amplified, and then plotted using a polygraph/chart-recorder. The heart rate in each individual was calculated by manually counting the number of recorded peaks in 10 consecutive 10 s intervals, and taking the mean of these. No systematic change in heart rate over the 2 min of recording was indicated in the total data set. Initially, measurements were made from 10 pre-pupae of each line, and subsequently replicate measures were taken for some lines from animals reared in separate bottles. In all but one case, replicate means were statistically similar, and the results are summarized in Table 1.

Table 1. *Heart rate in 24 isofemale lines*[a]

Line	Rate	SD	*n*
W1	2·3	0·5	20
W16	2·4	0·3	20
W10	2·5	0·4	20
W9	2·6	0·3	20
A2	2·6	0·3	19
A4	2·7	0·2	10
W6	2·8	0·2	10
W5	2·8	0·2	10
A17	2·8	0·3	10
W25	2·8	0·4	20
A5	2·9	0·4	9
A1	3·0	0·3	16
A21	3·0	0·2	10
W17	3·1	0·3	10
A13	3·1	0·3	10
A3	3·1	0·3	19
W23	3·2	0·2	10
W3	3·2	0·3	20
W4	3·2	0·4	30
A6	3·2	0·3	30
W14	3·2	0·3	30
W13	3·2	0·3	30
A10	3·3	0·3	10
W7	3·3	0·3	10

[a] Heart rates in hertz (beats per second) calculated manually from a chart-recorder and re-calibrated by multiplying raw values by 1·11.

It is immediately apparent that the mean heart rates of each line differ widely. Due to sampling effects, the observed range of mean phenotypes is likely to be an overestimate of the true range by between 10% and 25%, as described in the Section 2. Nevertheless, it is equivalent to approximately 3 standard deviation units of the typical within-line variation, as the line with the lowest mean heart rate (W1, 2·3 Hz) is one full beat per second slower than the lines with the highest heart rates (A10 and W7, 3·3 Hz), whereas the standard deviation in heart rate within most lines is

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Table 2. *Heart rate in 11 near-isogenic lines*[a]

Line	Rate	SD	*eag*[b]
A8	2·52	0·36	2·59
W6	2·85	0·33	2·55
A3	2·90	0·35	2·60
A1	3·00	0·29	2·56
W23	3·16	0·37	2·90
W14	3·19	0·33	2·58
A6	3·24	0·30	2·79
W22	3·17	0·30	2·74
W29	3·19	0·35	3·06
W11	3·51	0·27	3·12
A20	3·72	0·28	3·05

[a] Rates in hertz (beats per second) calculated from both replicates ($n = 40$ per line). The SD in this table is the standard deviation of heart rate among individuals within each line, *not* the mean standard deviation of beat length within individuals that is shown in Table 3 and in Figs. 1 and 2.
[b] Average rate measured in F1 of both replicate crosses to *eag*-2 ($n = 40$ per cross).

0·3 Hz. Ten of the lines were derived from an Ann Arbor fruit market, and 14 from localities around the world, but there was no difference in ranking of phenotypes of these two groups (Kruskal–Wallis test, $P = 0{\cdot}86$). The distribution was skewed towards the high end, with 13 lines having mean heart rates between 3·0 and 3·3 beats per second and just five lines having rates of 2·6 beats per second or lower.

(ii) *Estimate of heritability of heart rate in* Drosophila melanogaster

To estimate the relative contributions of genetic and random factors to the high phenotypic variance, we next obtained data from near-isogenic lines using a more precise detection system. Flies from 11 isofemale lines (five from Ann Arbor and six from the world-wide group) were inbred by pairwise sib-mating for between 12 and 15 generations. This is expected to lead to an average homozygosity exceeding 95% throughout the genome of each line, and analysis of molecular markers in a subset of lines confirmed that they are nearly isogenic (G. Gibson, unpublished data). Heart rate was detected by feeding the amplified light signal from the microscope into a computer with software (MacLab, version 3.1; AD Instruments, 1997) designed to calculate the time in milliseconds separating each successive peak. Twenty pre-pupae were examined in each of two replicates from different generations of each line, with all measurements at 25 °C (Table 2).

The heart rate for each of 440 individuals was calculated as the inverse of the mean length of 100 consecutive beats. These had an average of 3·13 Hz (beats per second), and were normally distributed

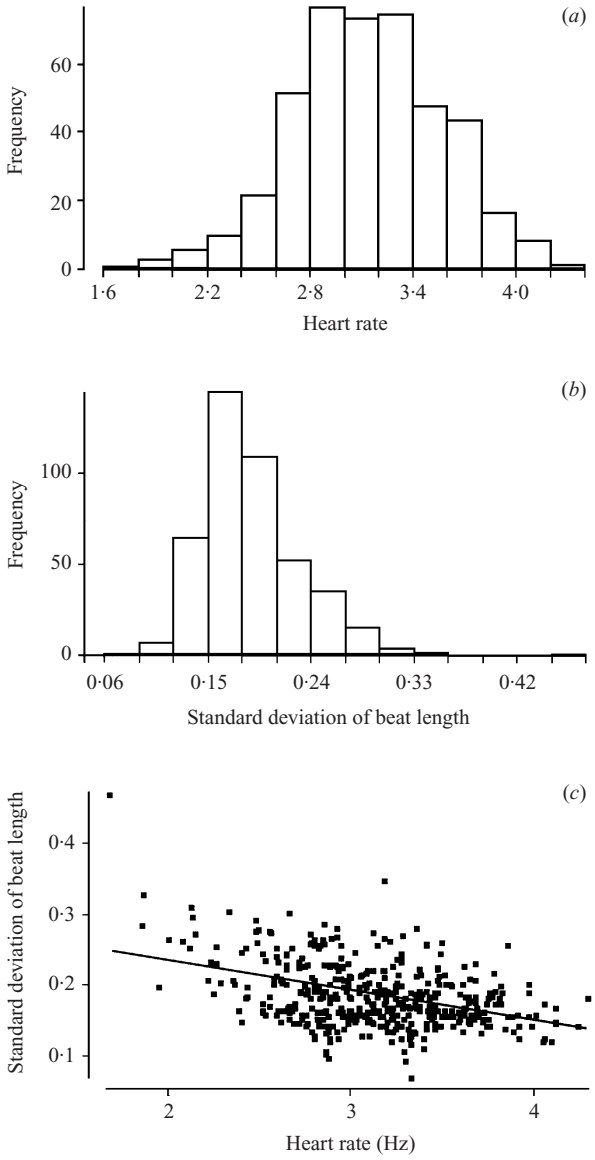

Fig. 1. Variation affecting heart rate and rhythmicity in 11 inbred wild type lines. (*a*) Frequency of individuals with the indicated heart rate in beats per second. (*b*) Frequency of individuals with the indicated standard deviation of the beat length in seconds. (*c*) Regression of standard deviation of within-individual beat length against heart rate for 440 pupae (40 individuals × 11 lines listed in Table 2).

(Fig. 1*a*). The within-individual standard deviation in beat length (Fig. 1*b*), which can be considered as a simple measure of arrhythmia, was negatively correlated with heart rate (Fig. 3*c*: $R^2 = 0{\cdot}16$, $P < 0{\cdot}001$), but scaled positively with beat length. Consequently, although flies with faster heart rates appear to have a tendency to be more rhythmic, this can be attributed to the expected increased variance associated with longer beats, since the coefficient of variance (the standard deviation divided by the mean) was actually positively correlated with heart rate ($R^2 = 0{\cdot}07$, $P < 0{\cdot}001$).

https://doi.org/10.1017/S0016672399003924 Published online by Cambridge University Press

Table 3. *Analysis of variance of heart rate*

Source	d.f.	Effect MS	*F* value	Probability	REML Varcomp
(*a*) Near-isogenic wild-type lines					
Line	10	4·0952	12·96	0·0001***	0·0945
R(Line)	11	0·3159	3·24	0·0003***	0·0109
Error	418	0·0976	—	—	—
(b) Wild-type by *eag-2* interaction					
Line	10	5·1974	6·03	0·0044**	0·0542
Cross	1	27·6010	32·04	0·0002***	Fixed
Line × Cross	10	0·8616	2·59	0·0303*	0·0132
R(Line × Cross)	22	0·03332	3·34	0·0001***	0·0117
Error	836	0·0997			

d.f., degrees of freedom of effect mean square (MS); REML Varcomp, variance component calculated using restricted maximum likelihood method. * $0{\cdot}01 < P < 0{\cdot}05$; ** $0{\cdot}001 < P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

Genetic parameters were calculated from an analysis of variance of the distribution of variation within and among lines (Table 3*a*). Although the replicate within-line term is significant, presumably due to uncontrolled environmental parameters such as humidity, its variance component is much smaller than that of the highly significant ($P < 0{\cdot}0001$) main effect of line. Assuming the lines to be isogenic, the genetic variance in the sample is estimated as twice the variance component for the line effect, $2V_G = 0{\cdot}0945$ Hz, where the factor of 2 accounts for the effect of inbreeding (Falconer & Mackay, 1996, p. 265). The environmental component of variance in this experiment is mostly due to random within-line effects, and is estimated as the sum of the error variance components, $V_E = 0{\cdot}1085$, whence the ratio $V_G/(V_E + V_G) = 0{\cdot}30$. Further assuming that non-additive components account for a small proportion of the between-line differences, this value is indicative of the heritability in natural populations, and implies that around one-quarter of the variation in pupal heart rate in wild-type *D. melanogaster* raised at 25 °C is attributable to genetic factors. This estimate is unlikely to be greatly affected by residual genetic variation segregating within lines, but is sensitive to the presence in the sample of one extreme line, A20, which has a heart rate over 10% faster than that in any other line. Removal of A20 from the sample reduces the estimate of V_G/V_P to 0·21, which nevertheless still indicates a very strong genetic component to the overall phenotypic variance.

(iii) *Modification of the* ether-a-gogo *mutant phenotype*

Ether-a-gogo mutant flies have disrupted potassium channel function, one effect of which is a marked reduction in heart rate (Johnson *et al.*, 1998). Ten generations of inbreeding of a laboratory stock of *eag*^{1} hypomorphs resulted in two lines, *eag*-2 and *eag*-6, with slightly different heart rates of 2·3 and 2·5 beats per second, respectively. These lines were crossed to each of the near-isogenic lines described above to determine whether the genetic background can modify the mutant phenotype. Virgin females carrying the X-linked *eag* mutation were crossed with wild-type males to generate hemizygous *eag*^{1} males and heterozygous *eag*^{1}/*eag*$^+$ females. We were unable to sex pre-pupae with confidence, and survival rate to adulthood following the heart beat assay was only around 20%, so the data are not separated according to sex. Nevertheless, most phenotypic distributions in the F1 were slightly bimodal (data not shown), consistent with a difference between hemizygotes and heterozygotes of between 0·3 and 0·6 Hz. For purposes of analysis the F1 data are treated as a single homogeneous group, noting that in no cases were the phenotypic distributions significantly different from normality. Twenty pre-pupae of each F1 genotype were examined, and the *eag*-2 crosses were replicated 2 months after the first collection of data. The line mean heart rates and within-individual standard deviation of beat lengths are plotted in Fig. 2.

Heart rates of *eag*^{1} heterozygous F1 flies are more similar to one another than are wild-type lines, as expected, since all F1 flies share half the genes derived from the inbred *eag* parent. Mean heart rates per line were generally the same in both the *eag*-2 and *eag*-6 inbred backgrounds (Fig. 2*a*), although one line, W6, may carry a modifier that suppresses the mutant phenotype only in the *eag*-2 background. Several lines have heart rates in all F1 individuals that are greater than the *eag*^{1} rate of 2·5 Hz, which implies that the mutant phenotype in males can be suppressed at least partially.

The interaction between genetic backgrounds and *eag*^{1} was examined by analysis of variance of the replicate wild-type and *eag*-2 crosses (Table 3*b*). The Line × Cross term is only marginally significant, but

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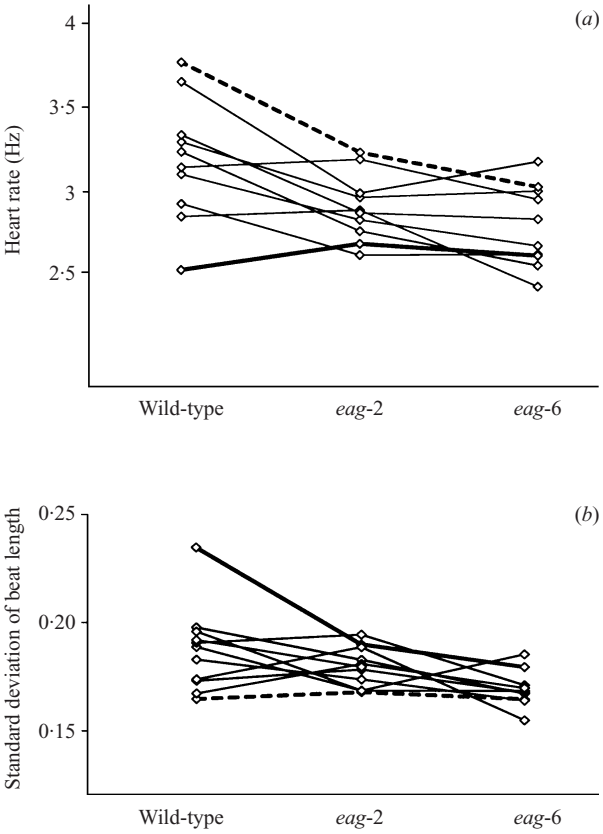

Fig. 2. Variation among lines and genotypes. (*a*) Line means for heart rate in beats per second in wild-type (left) and F1 progeny of crosses to two eag^1 mutant backgrounds. Broken heavy line, A20; continuous heavy line, A8. Crossing of line means suggests non-additive genetic interactions. (*b*) Line means for standard deviation of beat length.

comparison of variance components indicates that the interaction contributes close to one-quarter of the magnitude of the between-line genetic variance. This can be seen visually from the crossing of lines joining means on the left-hand side of Fig. 2*a*. An estimate of the genetic correlation (Robertson, 1959) between the wild-type and *eag*-2 genotypes yielded $r_{\mathrm{wt},\,eag} = 0{\cdot}74$, confirming that the effect of the potassium channel mutation is largely but not solely additive. The wild-type lines with the most extreme heart rates (A20 and A8) are also extreme in the *eag*-2 background. Interestingly, the heart rate in *eag* animals crossed to the slow wild-type lines is not further reduced below that observed in the parental *eag* lines.

Within-individual variance of beat length was also considered, as a possible measure of arrhythmia. Although there was no overall significant difference among lines in this measure, nor any interaction effect between wild-type and *eag* backgrounds, the plots of line means in Fig. 2*b* suggest that line A 5 (continuous heavy line) may have an unusually variable heart beat. The difference in variance is significant by independent contrasts, but is not extraordinary when scaled by the mean beat length. The results do, however, highlight how inbreeding can impair both heart rate and rhythmicity.

4. Discussion

(i) *Existence of genetic variation affecting heart rate*

The data presented above document the existence of considerable genetic variation affecting heart rate in *D. melanogaster*. In a recent twin study, Russell *et al.* (1998) also found substantial heritable components, comparable to those documented here, for the length of aspects of human heart beat, including the QT interval. Heritabilities between 0·25 and 0·5 are not uncommon for morphological traits in *Drosophila*, but can be obtained over a wide range of genetic and residual variances. Houle (1992) has suggested that scaling of the variance by the trait mean provides a more appropriate way to compare traits. For example, the coefficient of genetic variation, CV_G, is the standard deviation of a trait divided by the trait mean and expressed as a percentage. The value for heart rate lies between 8 and 10, similar to that described for sternopleural bristle number in *D. melanogaster*, towards the high end of the values typically observed for morphological traits. Similarly, it is unusual for inbred lines generated without selection to differ from one another by several standard deviation units of the residual variance. With respect to heart rate, the fastest line beats almost twice as fast as the slowest.

There may be several reasons for a high coefficient of genetic variation. In the case of sternopleural bristles, it is usually taken as evidence that only weak stabilizing selection acts on the trait (Nuzhdin *et al.*, 1995; Mackay, 1996). There is also no *a priori* reason to believe that pre-pupal heart rate is closely related to fitness, so a high proportion of the variance may be maintained passively as a consequence of not being removed by purifying selection. This process would be enhanced if there is a relatively high mutation rate producing variation. Life history traits also tend to have high CV_G values that are attributable to their complexity and the likely involvement of many genes that present a large mutation target (Houle *et al.*, 1996). Certainly heart rate is potentially affected by many genes, including those encoding channel proteins, neuropeptides and enzymes involved in neurotransmitter biosynthesis, but nothing is yet known about the mutational variance of heart rate.

Genetic variation might alternatively be maintained actively, for example as a result of antagonistic pleiotropy or genotype × environment interactions (Barton, 1990; Gillespie & Turelli, 1989). It is possible that some genes have opposite effects on heart rate in larvae and adults (Zornik *et al.*, 1999), or on other physiological traits, and if these are more closely

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related to fitness, variation could be maintained. Similarly, change in heart rate with environmental variables need not be additive, as preliminary studies suggest that some lines respond to increases in temperature more dramatically than others. If so, selection in a fluctuating environment could maintain variation. It should be considered that the rate at which the heart beats is not the sole determinant of the volume of haemolymph pumped through the animal. There is certainly qualitative variation among lines for the amplitude of heart beats, possibly indicating variation in their power. Amplitude is unlikely to be a good measure of power, but the point is that pulse rate interacts with other components of heart function that can modify the 'marginal fitness' of genotypes. In any case, it is clear that heart rate is an easily measured quantitative trait that should be amenable to genetic dissection.

Our data also suggest that the genetic variation is distributed across populations. More extensive comparison of variation among lines derived from several different populations may conceivably provide evidence for local differentiation with respect to heart rate, but at the level of a comparison of one local population (from a fruit market in Ann Arbor, Michigan) with isofemale lines trapped throughout the world, there is no partitioning of the variation. That is, the range of phenotypes observed in the Ann Arbor sample is as great as that observed in the worldwide sample. These flies were trapped just a few months before the measurements presented above, so this result also indicates that the genetic variation is found in nature and is not restricted to long-term laboratory cultures.

(ii) *Mutational and genetic background effects on heart rate*

Several of the wild-type lines have heart rates that are indistinguishable from those of flies that carry mutations that are thought to affect heart rate. This is most notable in the case of bradycardia: the mean heart rates of W1, W16, W10 and A8 are all equivalent to the rate observed in *eag* mutants, and described by others for *Ddc* (Johnson *et al*., 1997). This result can be interpreted as a caution that mutant phenotypes may actually be due to the genetic background, so the effects of particular genes on heart rate should be confirmed in a variety of different genetic backgrounds. However, it does seem that alleles segregate in natural populations that singly or in combination can reduce the heart rate to levels similar to those caused by loss-of-function of key components of the regulation of heart beat. The tachycardia observed in lines W11 and A20 indicates that mutations should also be expected that increase the heart rate, a possible example being the *Shaker* potassium channel subunit gene (Johnson *et al*., 1998). Preliminary analysis of a cross between the two most divergent lines in this study, A8 and A20, suggests that the differences in heart rate are due to just a handful of loci as there was a substantial increase in the variance of the F2 relative to the F1 progeny, with some pupae showing parental-type rates.

The crosses of wild-type to *eag* mutant stocks also attest to the buffering capacity of the regulation of heart rate. Even accounting for the fact that male hemizygotes are likely to have heart rates up to 0·3 Hz slower than those indicated in Table 2, the rates in these flies are brought close to the normal range of 2·7–3·3 Hz in most crosses. This observation highlights the importance of consideration of the genetic background when studying traits with variable penetrance. A major current effort in clinical human genetics is to screen candidate genes for polymorphisms that may affect heart disease susceptibility (Clark *et al*., 1998) using association tests in known pedigrees. Despite differences from humans in the pharmacology of heart rate, similarities in aspects of the underlying genetics imply that *Drosophila* could be an effective model system for dissecting disease-like heart function using the complementary strategies of specific genetic manipulation and high-throughput genotyping.

Mel Dickerson and Jen McCormick's help in establishing the assay in our laboratories is gratefully acknowledged. We also thank Drs Rich Hume for suggesting the use of the MacLab system and Marc Ammerlan for loan of the hardware. This work was supported by a fellowship to G.G. from the David and Lucille Packard Foundation, and a Beginning Grant in Aid from the North Carolina Affiliate of the American Heart Association (B98418N).

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