ORIGINAL RESEARCH Larks, owls, swifts, and woodcocks among fruit flies: differential responses of four heritable chronotypes to long and hot summer days

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Results: Despite the identity of such experimental conditions for four chronotypes, their circadian rhythms and sleep timing showed significantly distinct patterns of response to exposure to heat and/or long days. All two-way repeated measures analysis of variances yielded a significant interaction between chronotype and time of the day (P < 0.001).

Conclusion: An experimental study of heritable chronotypes in the fruit fly can facilitate a search for genetic underpinnings of individual variation in vulnerability to circadian misalignment, maladaptive sleep-wake behavior, and sleep disorders.

Keywords: sleep-wake pattern, morning-evening preference, circadian rhythm, photoperiod, temperature, locomotor activity

Introduction

Drosophila melanogaster and our own species (Homo sapiens) share the history of relatively rapid out-of-Africa dispersal.¹ Eurasian populations had faced the novel adaptive problem of adjustment of the circadian rhythms and night sleep episode to seasonal variation in day length. Such photoperiodic effects on the circadian rhythms of locomotor activity and sleep in D. melanogaster were intensively studied.²⁻⁸ It was, in particular, shown that, to some extent, the circadian rhythms are capable of adjusting to seasonal change in day length by shifting the evening peak of activity and lengthening

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of daytime "siesta." For instance, this adaptive response was always observed after exposure to the light–dark cycle consisting of 16 h of light and 8 h of darkness (abbreviated as LD16:8). However, further day lengthening (eg, LD20:4) resulted in a response of the circadian clock mechanism that might be regarded as maladaptive, due to a failure of additional delay of the evening peak for keeping it in synch with the clock time of transition from light to darkness.^{2,3,5}

It is also enticing to draw parallels between sleep–wake behavior of humans and fruit flies exposed to thermal stress.⁹ The fly's sleep pattern is too sensitive to heat. In particular, it might be reorganized by an increase of ambient temperature in a way that is very similar to the human sleep response, that is, nighttime sleep and daytime activity might decrease, whereas "siesta" and early night activity might increase.^{4,9-12}

Therefore, the study of the circadian rhythms in *D. melanogaster* can provide a powerful and rapid platform to uncovering the mechanisms responsible for sleep–wake disturbances associated with environmental stresses. To further explore the similarity between two species in respect of their circadian adaptations, maladaptations, and disorders, we examined whether four chronotypes (diurnal types), nicknamed "larks", "owls", "swifts", and "woodcocks"¹³ might be distinguished among fruit flies, and whether they differentially respond to light and heat stressors.

The reason for asking such questions in the present study was that, to our surprise, we did not find in the *Drosophila* literature any reports exploring a possibility to divide the flies on the basis of their sleep–wake pattern into even only two rather than four chronotypes, such as just "larks" and "owls". Although the division into "larks" and "owls" was previously explored, it was only in the studies of early and late eclosion chronotypes.¹⁴ As for the studies of sleep–wake typology in *D. melanogaster*, the previously published reports focused on the distinction between the types of short and long sleepers¹⁵ rather than between the birds of different feathers.

Consequently, in four of our *Drosophila* experiments, we tested the following four hypotheses.

 Can we identify, among the strains from wild populations, the representatives of four distinct chronotypes, nicknamed¹³ "larks" (early morning and evening activity peaks), "owls" (late morning and evening peaks), "swifts" (early morning and late evening peaks), and "woodcocks" (late morning and early evening peaks)?

- 2. Can this division into chronotypes persist, despite seasonal changes in the sleep-wake pattern associated with circadian phase adjustment, such as the shift of the evening activity peak that is proportional to change in day length at moderate latitudes of Eurasia?
- 3. Can these chronotypes differ one from another in a pattern of their maladaptive response to extreme lengthening of day length, such as occurring at high latitudes of Eurasia?
- 4. Can these chronotypes also differ one from another in their maladaptive response to combination of such an extremely lengthened day with hot air temperature?

Materials and methods

Prior to the circadian rhythms' recordings, flies were always kept under natural photoperiod and room temperature, ranging between 20°C and 24°C. Only males aged between 4 and 10 days were recorded. For the circadian rhythms' recording, flies were placed individually for 5 days in glass locomotormonitoring tubes with standard cornmeal agar medium (ie, 50 g of maize meal, 5.6 g of agar, 18 g of dry yeast, and 60 g of sugar per liter of water). Locomotor activity was monitored in 1-min bins using the Drosophila Activity Monitoring System ("Trikinetics". Waltham, MA, USA). The same data sets were also used to measure sleep events, defined as 6 consecutive minutes of absence of any locomotor activity.¹⁶ Parameters of locomotor activity and sleep events were calculated using a data acquisition software package downloaded from the TriKinetics website (www.trikinetics.com).

Under air temperature of 20°C and in constant darkness (DD), the flies were recorded for the first time in June (Experiment 1). The recordings were obtained from flies originating from eight strains (eight flies per each strain). From these eight strains, four strains were selected as the representatives of four distinct chronotypes,^{17,18} nicknamed "larks," "owls," "swifts," and "woodcocks" (early morning and evening activity peaks, late morning and evening peaks, early morning and late evening peaks, and late morning and early evening peaks, respectively).¹³ These selected strains originated from wild populations of the USA (U28), Africa (G10, G15), and Europe (F30). The strain (#28265) abbreviated here as U28 was initially maintained in the Bloomington Drosophila Stock Center (its ancestors were collected in Raleigh, NC, USA). The flies (#10, #15, and #30), abbreviated here as G10, G15, and F30, originated from African and French populations. Their ancestors were collected by P. Haddrill in Ghana (G10, G15) and Montpellier (F30), respectively.

In February, the recordings of winter generation of flies from these four selected strains were obtained again in the same photoperiodic and temperature condition (Experiment 2). Prior and after these recordings (DD at 20°C), the same four strains were also recorded during 5-day exposure to a long photoperiod (LD20:4 with 4-h darkness interval between 23:00 and 3:00 of local clock time) and constant temperature of 20°C in Experiment 3 and 29°C in Experiment 4. These experimental conditions essentially resemble hot and/or long summer days in several Eurasian regions above 60°North, that is, at the boundaries of modern distribution of wild populations of this species.

For each fly, the number of beam breaks and sleep episodes were summed on 30-min intervals. Data for the first day were excluded. The following 30-min estimates of activity or sleep were further averaged over 4 consecutive days to obtain mean values for each of 49 time points of the 24-h cycle (Figures 1–3).

The SPSS statistical software package (IBM, Armonk, NY, USA, version 22.0) was used to perform two-way repeated measure ANOVAs (rANOVAs) and one-way multivariate analysis of variances (mANOVAs). Locomotor activity and sleep were analyzed using data of each of four experiments and each of four strains exposed to LD20:4 at 20°C and 29°C. The repeated measure in rANOVAs was "Time point" (n=49), and the independent factors were "Strain" (G10, G15, U28, and F30) and "Temperature"

(20°C and 29°C). Degrees of freedom were corrected using Greenhouse-Geisser correction, controlling for type 1 error associated with violation of the sphericity assumption, but the original degrees of freedom are reported in Tables 1 and 2.

Moreover, the estimates of activity and sleep were further averaged on six 4-h intervals, corresponding to clock times of early morning, late morning, middle of the day, early evening, late evening, and middle of the Night (EM, LM, MD, EE, LE, and MN; 3:00–7:00, 7:00–11:00, 11:00–15:00, 15:00–19:00, 19:00–23:00, and 23:00–3:00, respectively, Figure 4). MANOVAs for each of four experiments and for each of four strains exposed to LD20:4 at 20°C and 29°C were run with the independent factor "Strain" (G10, G15, U28, and F30) and "Temperature" (20°C and 29°C), respectively. The multiple variables were either activity or sleep on the 4-h intervals of EM, LM, MD, EE, LE, and MN (Table 3 reports results on the factor "Temperature").

Results

Figure 1 illustrates the ways by which the circadian rhythms of locomotor activity and sleep (upper and lower graphs, respectively) in the fruit flies were modified by season of birth (left graphs) and exposure to hot temperature and/or extremely long day (right graphs).



Figure I Twenty-four h time courses in the fruit fly under four experimental conditions. Data of each of four experiments were averaged within and across four strains. Notes: Upper and lower graphs: Locomotor activity and sleep, respectively. Left and right graphs: Exposure to constant darkness (DD in winter and summer) and long photoperiod (LD20:4 at 20°C and at 29°C), respectively. Black line on the x-axis indicates the interval of darkness, either 24 h for DD or 4 h in the middle of the night (between 23:00 and 3:00 of local clock time).

Abbreviations: SEM, standard error of mean; h, clock hour; DD, constant darkness.



Figure 2 Twenty-four h time courses in four chronotypes under different lighting conditions. Data of experiments under constant darkness (DD) and long photoperiod (LD20:4) were averaged within each of four strains (G10, U28, G15, and F30). Upper and lower graphs: Locomotor activity and sleep, respectively. Left and right graphs: Exposure to DD and LD20:4, respectively.

Note: Black line on the x-axis indicates the interval of darkness, either 24 h for DD or 4 h in the middle of the night (between 23:00 and 3:00 of local clock time). Abbreviations: SEM, standard error of mean; h, clock hour; DD, constant darkness; EM, early morning; LM, late morning; MD, middle of the day; EE, early evening; LE, late evening; MN, middle of the night.



Figure 3 Twenty-four h time courses in four chronotypes at 20°C and 29°C. Data of flies from each of two experiments with exposure to long photoperiod were averaged within each of two strains (G10 and U28). Upper and lower graphs: Locomotor activity and sleep in G10 (left graphs) and U28 (right graphs) and in G15 (left graphs) and F30 (right graphs), respectively.

Note: Black line on the x-axis indicates 4 h interval of darkness in the middle of the night (between 23:00 and 3:00 of local clock time). Abbreviations: SEM, Standard error of mean; h, clock hour.

Response of flies to the season in Experiments 1 and 2

As for the influence of season of birth (photoperiodic history of flies' generation), those flies that were born and studied in constant darkness in summer went to sleep relatively later and had rather short but more consolidated sleep at night compared with the flies of the same strains that were born and studied in winter (Figure 1, left).

Irrespective of the season, the timing of the evening peak of locomotor activity shown prior to the experiment remained adjusted to the time of naturally occurring sunset, for example, the evening peak observed in constant darkness Table I Results of two-way rANOVAs with repeated measure "Time" and independent factor "Strain"

Factor	Measure	DD				Light-dark cycle (LD20:4)				
		Winter		Summer		20°C		29°C		
		Activity	Sleep	Activity	Sleep	Activity	Sleep	Activity	Sleep	
"Strain" (G10, G15,	F	39.202	38.245	4.181	21.593	6.891	8.149	14.656	18.818	
U28, F30)	Df	I/48	1/48	1/26	1/26	1/53	1/53	1/38	1/38	
	Р	<0.001	<0.001	0.015	<0.001	0.001	<0.001	<0.001	<0.001	
"Time" (49 time	F	33.940	44.490	31.480	51.650	33.940	44.490	26.068	20.035	
points)	Df	48/2,304	48/2,304	48/1,248	48/1,248	48/2,544	48/2,544	48/1,824	48/1,824	
	Р	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Interaction: "Strain"	F	6.407	4.011	7.910	9.500	7.886	7.798	4.121	4.084	
by "Time"	Df	144/2,304	144/2,304	144/1,248	144/1,248	144/2,544	144/2,544	144/1,824	144/1,824	
	Р	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Notes: The repeated measure "Time" includes 49 time points, and the independent factor "Strain" includes strains abbreviated as G10, G15, U28, and F30. Data on each of four experiments (DD in two seasons and LD20:4 at 20°C and 29°C) were analyzed separately. Activity Measure: Locomotor Activity per 30 min; Sleep Measure: Sleep duration per 30 min; F, Df, P: F-ratio, degree of freedom, and level of significance for the main effects and interaction.

Abbreviations: rANOVA, repeated measure ANOVA; DD, constant darkness.

 Table 2 Results of two-way rANOVAs with repeated measure "Time" and independent factor "Temperature"

Factor	Measure	G10		GI5		U28		F30	
		Activity	Sleep	Activity	Sleep	Activity	Sleep	Activity	Sleep
"Temperature" (20°C vs 29°C)	F	0.206	0.002	0.453	3.811	9.097	4.175	2.289	ty Sleep 6.319 1/25 0.019 19.421 10 48/1,200 <0.001 16.153 10 49/1,200
	Df	1/20	1/20	1/24	1/24	1/22	1/22	1/25	1/25
	Р	0.655	0.967	0.507	0.063	0.006	0.053	0.143	0.019
"Time" (49 time points)	F	5.704	6.575	15.572	14.913	23.783	20.316	20.223	19.421
	Df	48/960	48/960	48/1,152	48/1,152	48/1,056	48/1,056	48/1,200	48/1,200
	Р	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction: "Temperature" by	F	1.278	2.361	11.785	8.799	3.918	5.122	9.081	16.153
"Time"	Df	48/960	48/960	48/1,152	48/1,152	48/1,056	48/1,056	48/1,200	48/1,200
	Р	0.274	0.028	<0.001	<0.001	0.008	<0.001	<0.001	<0.001

Notes: The repeated measure "Time" (49 time points) and the independent factor "Temperature" (20°C and 29°C). Data on each of four stains in two long day experiments (20°C vs 29°C) were analyzed. Activity Measure: Locomotor Activity per 30 min; Sleep Measure: Sleep duration per 30 min. F, Df, P: F-ratio, degree of freedom, and level of significance for the main effects and interaction.

Abbreviation: rANOVA, repeated measure ANOVA.

in the winter season was phase-advanced relative to that observed in summer (Figure 1, left).

Response of flies to conditions of Experiment 3

However, such an adjustment was completely lost in any of four strains under a extremely long photoperiod when the interval of darkness was further shifted to 23:00 local time. Consequently, the position of evening peak was determined exclusively by the internal clocks and it was advanced relative to the time of transition from light to darkness (Figure 1, right).

Instead, the clear masking effects on locomotor activity and sleep were caused by the transition from light to darkness and back from darkness to light (ie, a rapid but short-lasting rise of locomotor activity and, consequently, a dramatic reduction of sleep episodes during these transitions).

Response of flies to conditions of Experiment 4

When an exposure to hot air temperature was combined with such an abnormal lighting regimen, further disturbance of the sleep–wake cycle was revealed. Namely, flies exposed to 29°C used to sleep more during "siesta" than during the shortened night interval (Figure 1, right).

The results indicated a partial loss of recovery function of night sleep due to the heat-induced sleep disturbances, the failure of the evening peak to delay to remain in synch with the time of light–dark transition, the replacement of this peak by the masking effects of such a transition, the other masking effect of the transition back to light in the early morning, etc.

Selection of chronotypes and their persistence over the seasons in Experiments I and 2

Surprisingly, we succeeded in identification of the strains that can represent all four chronotypes by testing only eight strains in the summer experiment (Figure 4). Two-way rANOVAs yielded a significant interaction term (Table 1), suggesting the difference between the strains (the independent factor) in the patterns of locomotor activity and sleep (repeated measure "Time").

The second exposure of the flies from these four selected strains to constant darkness suggested the persistence of the



Figure 4 Locomotor activity and sleep in four chronotypes under four experimental conditions. Data of each of four experiments under constant darkness (DD in summer and winter) and long photoperiod (LD20:4 at 20°C and at 29°C) were averaged within each of four strains (G10, U28, G15, and F30).

Notes: Summer, Winter, 20°C, and 29°C: Four experiments. Upper and lower graphs: Exposure to DD and LD20:4, respectively. Left and right graphs: Locomotor activity and sleep for summer and winter season in constant darkness (upper graphs) and for LD20:4 at 20°C and at 29°C (lower graphs), respectively. Black line on the x-axis indicates the interval of darkness, either 24 h for DD or 4 h on MN interval (between 23:00 and 3:00 of local clock time)

Abbreviations: SEM, standard error of mean; DD, constant darkness; EM, early morning (3:00–7:00); LM, late morning (7:00–11:00); MD, middle of the day (11:00–15:00); EE, early evening (15:00–19:00); LE, late evening (19:00–23:00); MN, middle of the night (23:00–3:00).

Interval	Measure	G10		G15		U28	U28		F30	
		Activity	Sleep	Activity	Sleep	Activity	Sleep	Activity	Sleep	
EM	F	1.401	3.496	3.796	9.291	10.561	8.074	5.861	9.599	
	Df	1/20	1/20	1/24	1/24	1/22	1/22	1/25	1/25	
	Р	0.250	0.076	0.063	0.006	0.004	0.009	0.023	0.005	
LM	F	1.329	2.536	0.579	2.577	4.030	3.295	9.126	9.282	
	Df	1/20	1/20	1/24	1/24	1/22	1/22	1/25	1/25	
	Р	0.263	0.127	0.454	0.121	0.057	0.083	0.006	0.005	
MD	F	0.189	1.460	3.390	6.092	0.336	0.051	30.399	49.613	
	Df	1/20	1/20	1/24	1/24	1/22	1/22	1/25	1/25	
	Р	0.668	0.241	0.078	0.021	0.568	0.824	<0.001	<0.001	
EE	F	0.130	1.063	2.168	5.043	2.624	0.170	6.719	15.830	
	Df	1/20	1/20	1/24	1/24	1/22	1/22	1/25	1/25	
	Р	0.722	0.315	0.154	0.034	0.119	0.684	0.016	0.001	
LM	F	0.735	0.638	1.579	3.758	6.830	1.075	1.713	0.418	
	Df	1/20	1/20	1/24	1/24	1/22	1/22	1/25	1/25	
	Р	0.401	0.434	0.221	0.064	0.016	0.311	0.202	0.524	
MN	F	0.112	0.899	37.029	32.116	18.304	20.160	3.561	8.939	
	Df	1/20	1/20	1/24	1/24	1/22	1/22	1/25	1/25	
	Р	0.742	0.354	<0.001	<0.001	<0.001	<0.001	0.071	0.006	

Notes: The independent factor "Temperature" (20°C and 29°C). Data on each of four stains in 2 long day experiments (20°C vs 29°C) were analyzed. Activity Measure: Locomotor Activity per 30 min; Sleep Measure: Sleep duration per 30 min. F, Df, P: F-ratio, degree of freedom, and level of significance for the main effects. U28, the strain #28265 maintained in the Bloomington Drosophila Stock Center and collected in Raleigh, NC, USA; F30, the strain from P. Haddrill collected in Montpellier; G10 and G15, two strains from P. Haddrill collected in Ghana.

Abbreviations: DD, constant darkness; LD20:4, light-dark cycle consisting of 20 h of light and 4 h of darkness; EM, early morning (3:00–7:00); LM, late morning (7:00–11:00); MD, middle of the day (11:00–15:00); EE, early evening (15:00–19:00); LE, late evening (19:00–23:00); MN, middle of the night (23:00–3:00).

strain-specific differences between their sleep–wake patterns in winter season (Figure 4, upper part). Again, two-way rANOVAs revealed a significant interaction term (Table 1).

Chronotypes under long day and its combination with heat in Experiments 3 and 4

We also found that the strains remained to be clearly distinguished on their sleep–wake cycle and its response to heat in the two other experiments (Figure 4, lower part; Figure 2, right graphs; and Figure 3).

For instance, as is shown in Table 1, the two-way rANO-VAs with repeated measure "Time" and independent factor "Strain" always yielded a highly significant main effect of factor "Strain," as well as a highly significant main effect of interaction between this factor and "Time" (eg, $P \le 0.001$ for sleep measure in any of these rANOVAs). Moreover, the results on the two-way rANOVAs with repeated measure "Time" and independent factor "Temperature" reported in Table 2 and illustrated in Figure 3 statistically supported the differences between the strains in response of their sleep– wake patterns to high temperature.

Response of strain G10 to experimental conditions

In more details, the following specific responses to experimental conditions were found in four selected strains. The strain G10, representing "woodcocks" (late morning and early evening activity peaks) responded by dramatic reduction of the circadian variation under a long photoperiod (Figure 4, lower part, and Figure 3). However, the expected deleterious impact of heat on night sleep was not observed in this strain, as indicated by nonsignificant main effect of factor "Temperature" and nonsignificant interaction of this factor with repeated measure "Time" (Table 2). Moreover, the results of MANOVAs shown in Table 3 suggested that, in fact, only in this strain sleep–wake behavior was not additionally affected by heat on any of six intervals of the 24-h cycle (see also Figure 3, left).

Response of strain U28 to experimental conditions

In contrast, in the strain U28 representing "swifts" (early morning and late evening activity peaks), the night sleep episode remained undisturbed only at 20°C, whereas the circadian pattern observed at 29°C was characterized by dramatic reduction of night sleep at the expense of extended "siesta" (Figure 4, lower part, and Figure 3, left). Such a disturbance of sleep–wake pattern is statistically proved by significant "Temperature" by "Time" interaction (P<0.001) and a significant increase of levels of locomotor activity and wakefulness during a 4-h interval of darkness at midnight (Table 3). Moreover, only this strain demonstrated a significant increase of mean level of locomotor activity at 29°C, as indicated by a significant main effect of factor "Temperature" (Table 2).

Response of strain G15 to experimental conditions

Such a significant increase of locomotor activity (P < 0.01) in U28 contrasted with a significant decrease of sleep (P < 0.05) in G15 (Table 2), the strain representing "larks" (early morning and evening activity peaks). Unlike G10 and similar to U28 (Figure 3), G15 responded to the combination of a long photoperiod with heat by the disturbance of night sleep episode at the expense of deepened "siesta" (Figure 3). As a result, the amount of sleep at the midday interval significantly increased, while the amount of sleep at the midnight interval significantly decreased (Table 3). Due to the early time of the evening peak these flies were already very sleepy prior to the transition from light to darkness, as sleepy as during the darkness interval (Figure 3). However, at 20°C, the sleep-wake pattern of this strain was least affected by a long photoperiod (Figures 3 and 4). The flies of this strain, unlike flies of other strains, succeeded in adjustment of their morning peak of activity to the very early time of transition from darkness to light, but only at 20°C (Figures 3 and 4). At 29°C, the amount of wakefulness during the 4-h early morning interval was significantly reduced in this strain, as well as in U28 and F30 (Table 3).

Response of strain F30 to experimental conditions

The strain F30, representing "owls" (late morning and evening activity peaks), was different from African strains (G10 and G15) but similar to U28 in the ability to keep a rather high level of activity in the evening hours at 29°C (Figure 3). However, the evening peak time at 20°C did not demonstrate any delay under a long photoperiod compared with this peak time in African strains (eg, Figure 3). On the other hand, the preceding episode of "siesta" in F30 was very deep compared with night sleep that contrasted with the pattern shown by the two African strains (Table 3). Finally, only this strain demonstrated a significant increase of total sleep duration at 29°C (Table 2), by sleeping more on three 4-h intervals in a row, from early morning to midday (Table 3).

Discussion

Given the complexity of the human sleep timing system and numerous limitations imposed on human studies, D. melanogaster might serve as an excellent model for addressing such kinds of research questions as to whether a heritable individual variability contributes to the response of the sleepwake cycle to such external sleep-disturbing factors as short dark night interval and high air temperature. The following results were obtained by testing four hypotheses. We were able to identify four distinct chronotypes among the strains originating from wild populations ("larks," characterized by early morning and evening peaks; "owls," with late morning and evening peaks; "swifts," with early morning and late evening peaks; and "woodcocks," characterized by late morning and early evening peaks). We also found that such differences between chronotypes persisted, despite adaptive seasonal changes in the sleep-wake patterns. Finally, we demonstrated that these four chronotypes differentially responded to extreme lengthening of day length, and additional differences were identified when such lengthening of the photoperiod was combined with heat stress.

Notably, despite such profound differences between selected strains, the results of the present study also supported the previously reported findings on the general pattern of response of the fly's sleep–wake cycle to either a long photoperiod^{2–8} or heat.^{9–12} As was already noted in these previous reports, a general way by which this cycle might be reorganized by ambient light and high temperature seems to be very similar to that shown by the human sleep–wake cycle.

Normally, the circadian pattern of locomotor activity in the fruit fly is bimodal, and this bimodality seems to be an additional attractive feature of this animal model for research on heritable differences in four rather than two extreme chronotypes. The differences between these types and the differences in their response to environmental stressors cannot be purely attributed to the fundamental difference in circadian phase position. Most likely, these differences can be underlined by a complex timing system consisting of circadian, homeostatic, and allostatic regulators. Human studies provided a rationale for distinguishing between, at least, two components of morning–evening preference associated with morning and evening-early night sleep–wake behavior (see Putilov¹³ for more details).

For instance, factor analysis of the structure of a multidimensional questionnaire for self-assessment of

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sleep-wake pattern yielded two morningness-eveningness dimensions,^{19,20} and replicability of this division into two dimensions has been confirmed, at least twice, by applying conventional psychometric procedures for selection of items for new chronotypological questionnaires.^{21,22} It was shown that the 24-h time course of alertness-sleepiness level significantly differs in the groups of early-early (larks), late-late (owls), early-late (swifts), and late-early (woodcocks) participants of the sleep deprivation experiment.¹⁸ Importantly, the two components of morningness-eveningness demonstrated differential relationships with health,^{17,23} somatic dysfunction,^{17,24} affective state,²⁵⁻²⁸ seasonality,^{24,29,30} etc. Therefore, further research on four chronotypes in fruit flies might be, in particular, aimed on testing whether this chronotypology is related with lifespan, reproductive success, sleep disruption, circadian misalignment, etc, and, if yes, what might be the particular mechanisms underlying such relations.

On the other hand, fundamental research might be aimed at deepening our understanding of the mechanisms underlying the variation in phase relationships between timing of morning and evening peaks of activity under different environmental conditions. Although the existence of two, morning and evening, circadian oscillators was proposed and experimentally supported, their genetic and neuronal underpinnings require further elaboration (see Kistenpfennig et al,³, Peschel and Helfrich-Förster,⁵ Helfrich-Förster,³¹ and Yoshii et al³² for more details).

The major limitation of the present exploratory study is the inclusion in our experiments of strains originating from populations evolved on three different continents. Our preliminary testing of several other strains (>8) from each of these three wild populations showed that much more efforts and time would be required to find all four chronotypes in each of these populations. Also, it is likely that the directional selection of the strains' breeds might be necessary to obtain the representatives of four distinct chronotypes in each of the populations. Further research can be aimed at identification of the genetic background of the within-population variation in the sleep–wake cycle, and its maladaptive response to an extremely long day and heat.

Conclusion

An experimental search for heritable chronotypes in the fruit fly can facilitate the study of the genetic underpinnings of individual variation in vulnerability to maladaptive sleep– wake behavior, circadian misalignment, and sleep disorders.

Author contributions

LPZ and DVP participated in discussion of the study design and in analysis of the collected data sets, and they also contributed to the writing this paper. AAP discussed the design of the study, participated in statistical analysis of the experimental datasets, and made the major contribution to the writing this paper.

Disclosure

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