Daily behaviour can differ between colour morphs of the same species: A study on circadian activity behaviour of grey and pied zebra finches

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To investigate if the plumage colour mutation relates to circadian activity behaviour in the zebra finch, Taeniopygia guttata, wild type grey and pied mutant males were sequentially subjected for three weeks each to 12 h light:12 h darkness (12L:12D) and constant dim light (LL$_{dim}$) condition. During the first 3 h of the 12 h day, pied finches were significantly greater active than grey finches. Also, as compared to grey, pied finches had longer activity duration in the day, with early activity onsets and late activity offsets. This was changed under free-running condition (LL$_{dim}$), when the activity later in the subjective day (clock hour 9 and 11) was significantly greater in grey than in pied finches. Two colour morphs differed in daily activity profile, but not in the total daily activity or circadian rhythm period. Results suggest that grey zebra finches represent late chronotype, and could perhaps be better adapted to a seemingly stressful environment, such as low intensity LL$_{dim}$ in the present study.

Keywords: Chronotype, Circadian behaviour, Colour morphs, Zebra finch

Organisms keep track of time and anticipate changes in the daily and seasonal environment which they inhabit. This they achieve through in-built circadian (German: circa = about; dian = day) clocks. In the natural environment, there is a synchrony between endogenous clock and external cyclic environment such that behavioural and physiological processes occur at the optimal time of the day and the year. Whereas multiple environmental zeitgebers (German: zeit = time; gegers = givers) have been experimentally shown to have synchronizing influence on circadian system, the light-dark (LD) cycle remains the primary zeitgeber$^{1,2}$. Circadian rhythms are ubiquitous. Among vertebrates, circadian rhythms are involved in the regulation of a wide array of behaviour and physiology, including activity–rest pattern, sleep-wake cycle, cognitive performance, hormonal secretions, metabolism, etc.$^{3-7}$. A circadian behaviour is such phased that diurnal birds are active during the day and nocturnal sare active at night. In fact, birds may begin their activity and rest phase in anticipation of the approaching day and night, respectively$^{8,9}$. In the absence of day-night periodic environment (i.e. under constant conditions), the time taken to complete a full cycle of events is defined as circadian period (tau, $\tau = \approx 24$ h)$^{10}$, which can differ at individual and species levels$^{10,11}$, and it is perhaps one of the key factors that can influence the chronotype in many species. Individuals with a shorter $\tau$ will become active early in the morning (early chronotype), whilst individuals with relatively longer $\tau$ will become active later in the morning (late chronotype)$^{12,14}$. Such a linkage between $\tau$ and chronotype may not hold good, however, in some species$^{10,14,15}$. Instead, the phase angle difference between activity and day light hours, as measured by relationship between the onsets, midpoints or ends of activity and light hours, defines the chronotype$^{16}$. As a measure of the adaptation to day-night environment, circadian activity behaviour has been widely studied among animals including several birds$^{17,18}$. Although intraspecific colour polymorphism has been extensively studied in birds, particularly in the context of habitat and mate selection advantages and reproductive fitness$^{19}$, it is unclear whether colour morphs of a species will exhibit differences in their circadian behaviour. This study addresses on this, by comparing the daily and circadian activity behaviours of zebra finches.
(Taeniopygia guttata) that were sequentially exposed to an equinox daylight and constant dim light environment, respectively.

**Materials and Methods**

This study was done on adult male zebra finches (Taeniopygia guttata) that were bred and raised in captivity. Both grey and pied zebra finches were born out of the same colony birds and maintained together in the aviary receiving same day-night environment until recruited for the experiment. They always had food and water ad libitum. Birds (grey, n = 8; pied, n = 7) were moved indoors and individually housed in the activity recording cages (size 38 × 36 × 46 cm) that were placed inside light-tight wooden boxes, providing 12 h light : 12 h darkness (12L : 12D, L = 250±10 lux; D = ~ 0.2 lux) by compact fluorescent lamps (Phillips CFL lamp, 5W,220-240 V). Mueller automatic timer (Mueller, Bedienungsanleitung SC 88) controlled the timing of L:D periods. After three weeks of 12L:12D, birds were released into constant dim light (LL_{dim}, ~0.2 Lux, light intensity of dark phase of the preceding LD) for another three weeks.

Each activity cage was furnished with two perches and mounted with a passive infrared motion sensor (DSC, LC100 PI Digital PIR detector, Canada). The activity behaviour of each bird was continuously monitored, as previously described by Malik et al. Briefly, an infrared sensor mounted on the cage detected the general movement of bird in its cage and transmitted it to a designated channel of the computerized data acquisition system. The collection, graphics, and analysis of activity behaviour were done by “The Chronobiology Kit” software program of Stanford Software Systems, Stanford, USA. A double-plotted activity record of each individual, called actogram, was obtained for the whole duration of the experiment.

In LD and LL_{dim}, daily activity over 10- or 11-day period was used to calculate total activity per day, activity duration, phase angle difference and circadian period (τ) both in the LD and LL_{dim} conditions. Activity counts over 2 min bin was pooled for five consecutive bins (10 min) to measure the precise activity onsets and offsets. A count of 10 or more in activity during the first minute of the pooled 10 min bin during the hour before and after lights on was taken as the activity onset. Similar activity counts during the last minute of the 10 min bin during the hour before and after lights off were taken as the activity offset. Activity counts of <10 in the ten min bin was considered as noise. Phase relationship between daily activity rhythm and LD cycle was analysed with reference to the activity onset (compared with times of light on), activity mid-point (compared with the mid, hour 6, of light) and activity end (compared with times of light off), and used it to designate the chronotype of the two colourmorphs. A few days data were unusable for technical reasons, therefore 67-day data from seven pied and 76-day data from eight grey finches were used to assess for the chronotype. Data were statistically analysed by two-way analysis of variance (two-way ANOVA), followed by the post hoc Bonferroni test, if the ANOVA indicated the significant difference. Student’s t-test was used to compare two groups at one time point. Significance was considered at P<0.05 level. All statistics were done using GraphPad Prism (version 5.01) software program.

**Results**

**Activity behaviour under 12L:12D**—Both colour morphs were active during the day time with activity abruptly ending around the time of lights off (Fig. 1A and B). Under 12L : 12D, pied finches had significantly higher activity bouts per day, as compared to grey finches (P<0.05; Student t-test; Fig. 2A). There was a significant effect of the colour morph (F_{1,23} = 50.59, P<0.0061), time of day (F_{23,23} = 28.90, P<0.0001) as well as interaction between these two (F_{23,23} = 2.364; P<0.0005 ) on activity profile measured every hour over 24 h day. Pied finches had significantly higher activity than the grey finches during the first half of the day, particularly in the hour ending at ZT1, 2, 3 and 6 (Bonferroni post test, P<0.05; Fig. 1C). They also had significantly longer activity duration (P < 0.05; Student t-test, Fig. 2C). In relation to lights on, activity onsets was significantly phase advanced (occurred earlier) in pied than in the grey finches (P<0.05; Student t-test). In actual terms, pied finches began activity 14.47±2.0 min prior to lights on, whilst in the grey finches activity onset coincided with lights on (Fig. 3). In relation to lights off, activity end was significantly phase delayed (occurred later) in pied than in grey finches (P<0.05, Student t-test). However, both colour morphs showed a positive phase relationship of offset of activity with time of lights off. Pied finches ended their activity 26.05±2.0 min prior to lights off, whilst grey finches ended their activity 35±4.0 min before lights off. There was also a positive phase relationship between midpoints, without a significant
difference between two colour morphs. On average, as compared to grey finches, pied finches had early activity onsets and later activity offsets, and showed higher activity and of longer duration.

Activity behaviour under LL\textsubscript{dim}—Birds free ran with a circadian period but with no significant difference between two colour morphs (Fig. 2D). In comparison to 12L:12D, total activity per day under LL\textsubscript{dim}, was reduced for both colour morphs, with no significant difference between them. However, distribution of activity over subjective 24 h, corresponding to preceding LD cycle, showed an inversed activity pattern as compared to birds under 12L:12D. That is, grey finches had higher activity than pied finches (Fig. 1D and 2B). Two way ANOVA further validated significant difference in the 24 h activity profile between two colour morphs. There was a significant effect of the plumage colour...
Fig. 2—Mean (± SE) daily activity profile of grey (n=8) and pied (n = 7) finches under 12L: 12D (A) and LL (B) condition. Asterisk in ‘A’ indicates a significantly higher activity level (P<0.05; Student’s t-test) in pied than in the grey finches. (C) Mean (± SE) daily activity duration (alpha, α) under 12L: 12D over 10 days (four days activity of one grey finch, and three days activity of pied finch was excluded from analysis; hence for 76 days for grey finch and for 67 days for pied finch). Asterisk indicates significantly longer activity duration in pied as compared to that in the grey finch (P<0.05, Student t-test). (D) Circadian Period (τ) under LL_dimm.

Fig. 3—Mean (± SE) timing of activity of grey and pied colour morphs of zebra finches in relation to day onset (activity beginning, A), mid-day (midpoint of activity, B) and day end (activity end, C). Horizontal lines crossing symbols indicate standard error. Data are plotted for number of days (76 – grey finch; 67 – pied finch). Asterisk(*) indicates significantly early activity onsets in pied finches as compared to that in the grey finches (P<0.05, Student t test).

Discussion

Pied colour morph is the result of a recessive mutation, where white markings are seen on the head flight and tail feathers. These results show a difference in daily and circadian behaviours between two colour morphs. This may possibly be taken to indicate their chronotype. An early chronotype, such as those shown by pied finches by phase, duration and period of circadian behavior could be advantageous in terms of sexual selection. For example, male birds that begin singing earlier in the day could have better mating chances, since females consider morning singing for better male quality. This interpretation is based on studies in humans, when individuals with shorter and longer τ are defined as early and late chronotypes, respectively. However, many studies also provide evidence for the absence of a direct influence of period on chronotype.

Results (cf. Figs. 1-3) show that pied finches had significantly greater activity, early activity on set, late activity off set and longer activity duration. A 24 h activity profile also revealed that pied finches were significantly more active in the first three hours (ZT1 - ZT3) of morning. It should be noted however, that the two colour morphs did not have significantly different circadian period under LL_dimm, which is a key factor in defining the chronotype of an individual. Perhaps, a larger sample study may have given better resolution of the result on differences between two colour morphs. Nonetheless, differences in the activity behaviour between two colour morphs under 12L:12D do indicate pied finches to be of an early chronotype. As compared to the daily activity profile under 12L:12D in which pied finches had a higher amplitude of activity, 24 h activity profile was inversely related to external light condition. In other words, wild type grey finches have greater ability to cope with a stressful continuous lighting environment at 0.2 lux than the pied mutant colour morphs.

To sum up, the present results show a colour morph dependent variability in the activity behaviour of zebra finches. It may be of much interest to look into
colour morph dependent variations in other behavioural and physiological markers across several other bird species, to understand whether such mutations have implications on habitat and sexual selections, and reproductive fitness.

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