

Adaptive variation among *Drosophila* species in their circadian rhythms

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ABSTRACT

Observation: In cool, high latitude regions, *Drosophila* are often active throughout the day, whereas in lower latitudes, they are most active early and late during the daylight hours.

Question: Is this difference in activity due to variation in the conditions experienced by flies in the wild or their perceptual sensitivity to these conditions, or their genetically encoded circadian rhythms.

Methods: The activity patterns of 11 species of *Drosophila* were recorded continuously in activity monitors under identical 12 h light/12 h dark conditions.

Results: There was a significant positive correlation between the midday activity of flies and the latitudinal midpoint of the species range, showing that behavioural differences in the wild are at least in part genetically based. An independent contrasts test, correcting for phylogeny, confirmed this correlation. Among species from the same latitudinal region, those that inhabit swampy areas and breed on skunk cabbage have significantly greater midday activity than do mycophagous species. Both the latitudinal and breeding site effects suggest that potential desiccation stress has influenced the evolution of their daily activity patterns.

Keywords: desiccation stress, independent contrasts, latitudinal variation, mycophagous insects.

INTRODUCTION

Southwood (1977) proposed that an organism's ecological strategies – growth, dispersal, reproduction, and so on – can be viewed as having been selected to fit the spatial and temporal template of favourableness characteristic of its habitat. While Southwood's ideas are most frequently applied to life-history theory, they are also applicable to the daily cycles of an organism's activities. Thus, the circadian rhythms exhibited by organisms can be viewed as a means by which periods of activity are timed to coincide with periods of environmental favourableness. However, as for any trait, one must beware of attributing adaptive significance to any particular circadian rhythm in the absence of supporting evidence, such as comparative analysis of organisms inhabiting different environments (Sharma, 2003; Johnson, 2005).

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Drosophila have long been model organisms for the study of circadian rhythms, because of the ease with which their behaviour can be studied under controlled laboratory conditions and the existence of various mutants affecting clock phenotypes (Pittendrigh, 1993; Klarsfeld *et al.*, 2003; Hall, 2005). *Drosophila* is also an ecologically and biogeographically diverse genus, with various species occupying such habitats as tropical rainforests, deserts, and cold boreal forests. Thus, one might expect *Drosophila* to vary adaptively in their circadian rhythms in a habitat-specific fashion. Previous studies have, in fact, documented intra-specific geographical variation in the daily rhythms of oviposition in *D. melanogaster* (Allemand and David, 1976), eclosion in *D. littoralis* (Lankinen, 1986), and locomotor activity in *D. ananassae* (Joshi, 1999; Khare *et al.*, 2005) and the *Drosophila*-parasitic wasp *Leptopilina heterotoma* (Fleury *et al.*, 1995). At the molecular level, the clock gene *period* of *D. melanogaster* exhibits latitudinal clines in the frequencies of different variants, suggestive of adaptive variation (Costa *et al.*, 1992).

Whether this geographical variation is the outcome of natural selection remains to be determined. However, it is noteworthy that the proportion of eggs laid during the midday hours by *D. melanogaster* and the midday activity of *D. ananassae* both increase with latitude (Allemand and David, 1976; Joshi, 1999). Such evolutionarily independent, but parallel patterns are reminiscent of the best early evidence for the role of selection governing the F/S polymorphism of *Adh* in *D. melanogaster* – that is, similar latitudinal clines in allele frequencies on different continents (Oakeshott *et al.*, 1982). Here we ask whether the geographical patterns found within *D. melanogaster* and *D. ananassae* can be extrapolated up to the level of macroevolutionary variation among *Drosophila* species that occur at different latitudes, and thus whether *Drosophila* species broadly are subject to similar selective regimes affecting circadian phenotypes.

Relatively few *Drosophila* are active during the midday hours in the southern United States, whereas in the northern United States and Canada flies may be active throughout the day (personal observations). Does this difference simply reflect the fact that midday periods are hotter in southern regions and that flies respond opportunistically to the environmental conditions they experience? Or are there genetically encoded differences among *Drosophila* species in their circadian rhythms, with southern species programmed for midday inactivity, while their northern relatives are not? In the present study, we examined the patterns of daily activity in 11 species of *Drosophila* with different latitudinal ranges. Our findings indicate that the differences among flies in the wild are at least in part genetically based, and an independent contrasts test suggests that the differences are adaptive. The latitudinal variation we find among species is very similar to the pattern of variation seen within *D. melanogaster* and *D. ananassae*, suggesting that selection acts on the circadian phenotypes of different *Drosophila* species in the same general manner.

MATERIALS AND METHODS

Drosophila species

The species examined in this study and the sites from which the experimental strains were collected are shown in Table 1. Their phylogenetic relationships are presented in Fig. 1 (from Perlman *et al.*, 2003). These species were chosen for study because none is cosmopolitan or a human commensal, unlike *D. melanogaster*. Therefore, their activity patterns are likely to reflect adaptation to the natural conditions in which they are currently found.

Table 1. The *Drosophila* species studied, their breeding sites, the locations (with dates in parentheses) from which the experimental strains were collected, the northern and southern latitudinal limits of their known ranges, and the midpoint of the latitudinal range

Species	Breeding site	Collection localities	Latitude		
			Southern limit	Northern limit	Midpoint
<i>D. falleni</i>	Mushrooms	Ithaca, NY (2002)	30.3	53.5	41.9
<i>D. recens</i>	Mushrooms	Munising, MI (2002)	36	54	45
<i>D. subquinaria</i>	Mushrooms	Edmonton, AB (2002)	40	61	50.5
		Bethlehem, NH (2002)			
		Vancouver, BC (2000)			
<i>D. innubila</i>	Mushrooms	Portal, AZ (2000)	19.5	34.7	27.1
<i>D. pictiventris</i>	Mushrooms	Don Pedro I., FL (2003)	25.5	27.6	26.55
<i>D. acutilabella</i>	Mushrooms	Ft. Lauderdale, FL (1999)	26.5	27.6	27.05
<i>D. suboccidentalis</i>	Mushrooms	Coeur d'Alene, ID (2001)	28	53	40.5
<i>D. cardini</i>	Mushrooms	Don Pedro I., FL (2003)	26.5	29.7	28.1
<i>D. palustris</i>	Skunk cabbage	Rochester, NY (2002)	37.5	47.5	42.5
<i>D. quinaria</i>	Skunk cabbage	Rochester, NY (2002)	34.7	45.5	40.1
<i>D. deflecta</i>	Skunk cabbage	Princeton, NJ (1998)	29	40.2	34.6

Note: The range data are for the contiguous ranges encompassing the sites where the flies were collected. Authorities for species names are available at Bächli (2005).

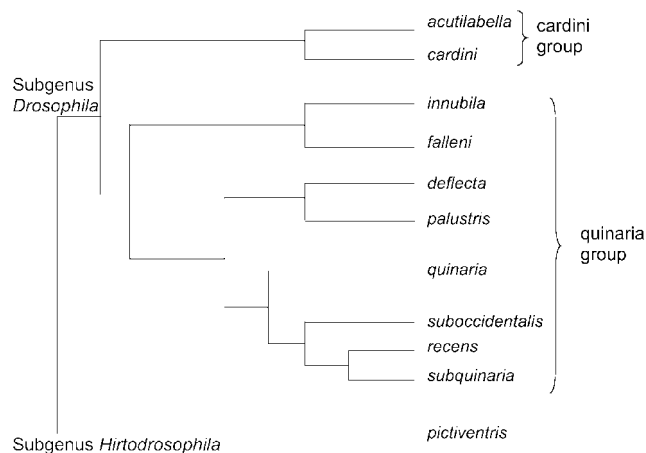


Fig. 1. Phylogenetic relationships of the study species (from Perlman *et al.*, 2003). As a member of the sub-genus *Hirtodrosophila*, the position of *D. pictiventris* is assumed to be comparable to that of *D. histrioides* in Perlman *et al.* (2003). Branch lengths are not to scale.

The strains examined were derived from multiple (generally 5–10) wild-caught females and males, and were thus genetically representative of the populations from which they were obtained. Most of the species examined breed on mushrooms in forests and woodlands, but

our sample also included three species – *D. deflecta*, *D. palustris*, and *D. quinaria* – that inhabit swampy areas and breed in decaying skunk cabbages (*Symplocarpus foetidus* (L.) Nutt.). The fly cultures were maintained in the laboratory on instant *Drosophila* medium supplemented with commercial *Agaricus bisporus* (Lange) mushroom. The cultures were maintained at 22°C, ~70% relative humidity, and a 12 h light/12 h dark cycle.

The latitudinal ranges of these species are presented in Table 1. These ranges were determined on the basis of published records (Spencer, 1942; Patterson, 1943; Patterson and Wagner, 1943; Stalker, 1953; Wheeler, 1960; Throckmorton, 1962; Bächli, 2005), our personal collection records, and records from the extensive *Drosophila* collection at the American Museum of Natural History (kindly provided by Dr David Grimaldi). Because disjunct populations are often genetically divergent (and may even belong to different biological species), for analysis purposes we considered the contiguous range of the population that included the site from which a species was collected. For most of the species listed in Table 1, this was simply the entire known range of a species. However, *D. cardini*, *D. acutilabella*, and *D. pictiventris* are known not only from mainland North America, where we collected them, but also from more southern regions, including Caribbean islands such as Cuba and Jamaica. For these three species, we computed the latitudinal midpoint of their mainland North American ranges.

Activity monitoring

For the experimental runs, male flies 3–5 days post-eclosion were placed in groups of 15 in vials containing sugar–agar medium plus a small piece of banana peel. The experimental flies were then placed in chambers with a 12 h light/12 h dark cycle, with lights on and off at the same time as during their developmental period.

Fly activity was quantified using *Drosophila* Population Monitors linked to a *Drosophila* Activity Monitor IV (Trikinetics, Inc., Waltham, MA). The monitors include a series of ring detectors to detect fly movement; each time a fly passes an infrared beam, a count was recorded. The counts from the three sensors within a unit were summed to determine the total activity of the flies for each 30-min period throughout the duration of the experiment. To allow flies to habituate to their new environments, activity was recorded on days 3–5 after the flies had been introduced to the monitors. Because *Drosophila* species differ in their overall activity, the number of counts per 30-min period was normalized by dividing the actual count by the number of counts during the 30-min period of maximum activity during the 3 days over which counts were made. The numbers of 5-day experimental runs for each species were as follows: *D. acutilabella* (1), *D. cardini* (2), *D. deflecta* (1), *D. falleni* (2), *D. innubila* (2), *D. palustris* (1), *D. pictiventris* (1), *D. quinaria* (2), *D. recens* (1 for each of three strains), *D. suboccidentalis* (2), and *D. subquinaria* (1 for each of three strains). The time-specific levels of activity for each species were computed as the mean \pm standard deviation of the run-normalized activity, including data from all experimental runs.

Statistical analysis

We devised a simple statistic to quantify relative midday activity. For each day during which activity was quantified, we determined the total activity during the first 3 h of the daylight period (early: 08.00–11.00 h), the middle 6 h of the day (midday: 11.00–17.00 h), and the last 3 h of the daylight hours (late: 17.00–20.00 h). We then summed the counts for the early

Table 2. Analysis of covariance of midday activity ($Y = \log(\text{midday}/(\text{early} + \text{late}))$) as a function of a species' breeding site and the latitudinal midpoint of its range

Source of variation	d.f.	SS	<i>F</i>	<i>P</i>
Breeding site	1	0.2137	7.9574	0.0025
Latitude	1	0.3565	13.2769	0.0066
Error	8	0.2148		

and late periods and contrasted this with the midday count, and defined midday activity as $Y = \log(\text{midday}/(\text{early} + \text{late}))$. We calculated Y for each 24-h period during which activity was measured for a species and determined its overall mean across days (Table 2).

This study was designed to determine whether species from higher latitudes exhibit greater midday activity than species from lower latitudes. For our initial analysis, we simply examined the correlation between Y (relative midday activity) and the latitudinal midpoint of a species' range. The initial analysis revealed an additional pattern that we had not considered *a priori*. Specifically, it appeared that species that breed in skunk cabbage differed from mycophagous species in their midday activity, even for species from the same latitudinal region. Therefore, we carried out an analysis of covariance to examine Y as a function of breeding site (mushroom vs. skunk cabbage) and latitudinal midpoint of a species range.

Such straightforward statistical analyses consider each species as an independent sample. From an evolutionary perspective, such analyses assume that species can instantaneously adapt to the current environmental conditions – that is, that there is no effect of phylogeny. To correct for possible effects of phylogeny, we conducted an independent contrasts test of the correlation between latitudinal range and midday activity (Felsenstein, 1985). Because the null hypothesis tested in this analysis assumes a Brownian motion model of evolutionary change, the expected variance in character change is proportional to time, or lineage branch length (Felsenstein, 1985). We estimated the relative branch lengths using the molecular phylogeny of our study species presented in Perlman *et al.* (2003). This phylogeny is based on mitochondrial COI, COII, and COIII sequences, which in total were 1517 bp long.

RESULTS

Mean normalized activity (\pm standard error) for each species as a function of time during a 24-h period are shown in Fig. 2. The qualitative impression from Fig. 2 is that the southern species exhibit pronounced peaks of activity at dawn and dusk, whereas the northern species are more active throughout the daylight hours. There is, in fact, a significant positive correlation between midday activity and the latitudinal midpoint of a species range, using both parametric ($r^2 = 0.53$, $P = 0.011$) and non-parametric measures of association ($\rho = 0.64$, $P = 0.035$; Fig. 3). On average, the relative midday activity (not log-transformed) of the four northern mycophagous species was three times greater than that of the four southern species. The correlation between latitudinal range and midday activity was strongly supported by an independent contrasts test, with both parametric ($r^2 = 0.58$, $P < 0.01$) and non-parametric tests ($\rho = 0.79$, $P < 0.01$; see Fig. 4). Thus, the correlation between latitudinal range and midday activity is not due to phylogenetic pseudoreplication.

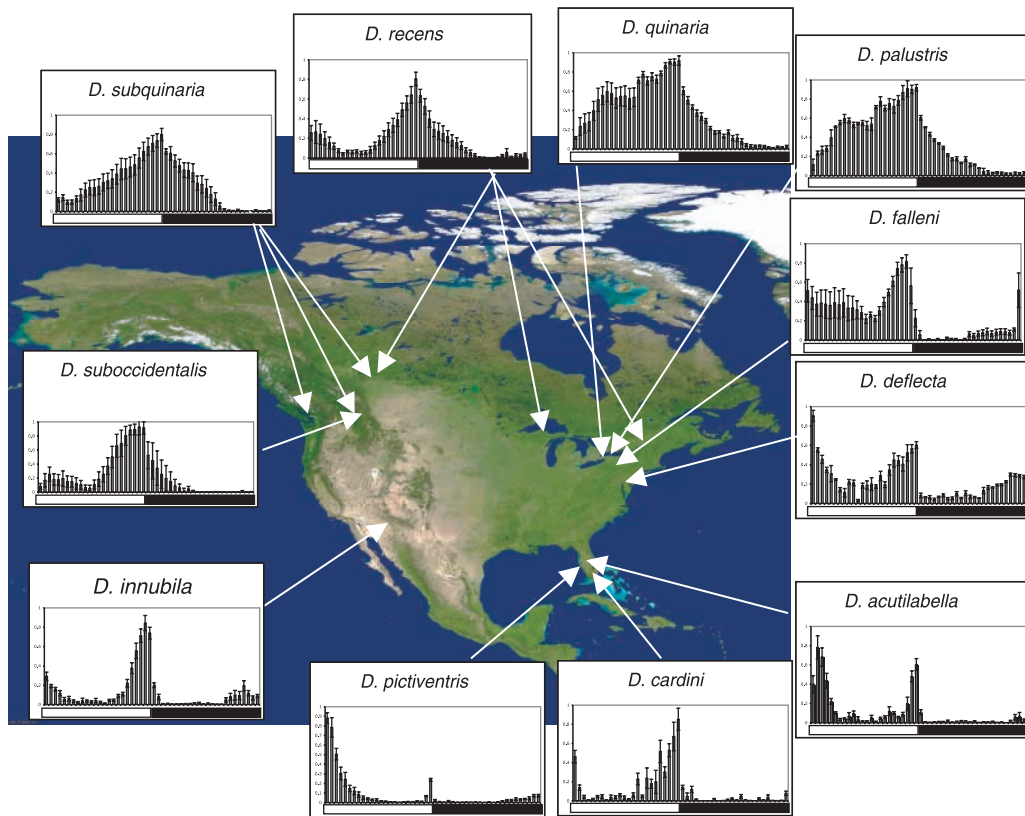


Fig. 2. Activity patterns (normalized mean \pm standard error) of *Drosophila* species. Light and dark bars at the bottom of each graph denote periods of light and dark during the experimental runs. Arrows indicate sites from which these species were collected.

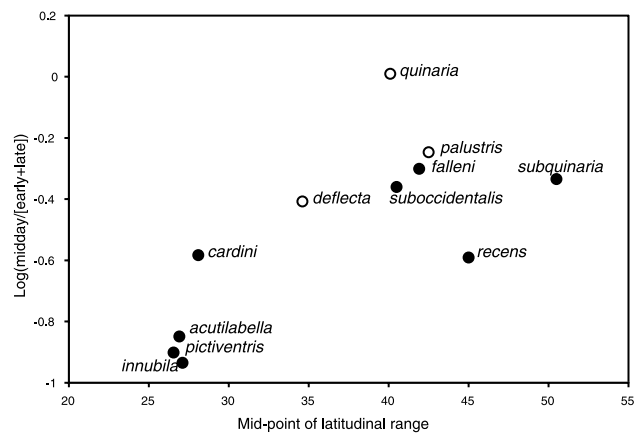


Fig. 3. Relative midday activity ($Y = \log(\text{midday}/(\text{early} + \text{late}))$) plotted as a function of the latitudinal midpoint of a species range. Solid and open points represent mycophagous and skunk cabbage breeding species, respectively.

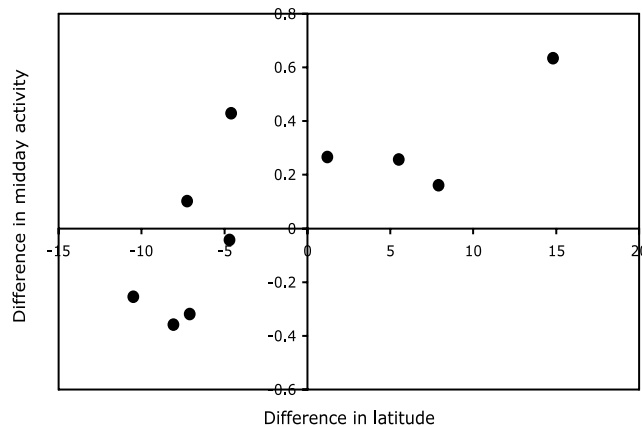


Fig. 4. Phylogenetic independent contrasts of the difference in relative midday activity ($\log(\text{midday}/(\text{early} + \text{late}))$) as a function of the difference in latitudinal midpoint of ranges ($r = 0.76$, $P < 0.01$).

An analysis of covariance indicates that the skunk cabbage-breeding species exhibit greater midday activity than do the mycophagous species ($P = 0.0025$), and confirms the finding that midday activity increases significantly with the latitudinal midpoint of a species' range ($P = 0.0066$; Table 1). Among the northern species, those that breed on skunk cabbage had relative levels of midday activity about 35% greater than the mycophagous species.

The species we examined also exhibit other interesting differences in their activity patterns, although we have not analysed these statistically. First, some northern species (*D. recens*, *D. subquinaria*, *D. quinaria*, and *D. palustris*) have prolonged periods of activity during the early part of the night, whereas other, primarily southern species (*D. falleni*, *D. innubila*, *D. cardini*, and *D. acutilabella*) exhibit a pronounced decline in activity immediately after 'sunset'.

Second, the species with a noticeable lull in midday activity differ considerably in the relative magnitudes of the early and late peaks. For instance, *D. innubila* and *D. cardini* exhibit greater activity late in the day, whereas *D. pictiventris* is much more active early in the day.

Finally, these species vary in the extent to which they anticipate sunrise, as indicated by their activity prior to lights on (the right-hand side of the black bars in Fig. 2). For instance, *D. pictiventris*, *D. deflecta*, and *D. innubila* exhibit a noticeable anticipation of sunrise, whereas *D. suboccidentalis*, *D. subquinaria*, *D. cardini*, and *D. acutilabella* show virtually none at all.

DISCUSSION

Although the present study included only 11 species of *Drosophila*, there is clearly substantial variation among them in their daily activity patterns. One difference that stands out is that species from lower latitudes exhibit a much greater lull in midday activity than do species from higher latitudes. Thus, the observed differences in *Drosophila* midday activity in different regions of the world are probably due in part to variation in their genetically programmed circadian rhythms. The independent contrasts test also revealed a significant

correlation between latitudinal range and midday activity, indicating that there is little phylogenetic constraint on *Drosophila* activity patterns.

The latitudinal variation in midday activity exhibited among species of *Drosophila* is very similar to the intraspecific patterns previously described in *D. melanogaster* and *D. ananassae* (Allemand and David, 1976; Joshi, 1999). The parallel between these intra- and inter-specific correlations suggests that the circadian rhythms of flies are subject to similar selective regimes across *Drosophila* species.

We also found that species that inhabit swampy areas and breed in skunk cabbages – *D. quinaria*, *D. palustris*, and *D. deflecta* – exhibit greater midday activity than do woodland mycophagous species from the same latitudinal region. Both the latitudinal and breeding site correlates of activity patterns may be related to the desiccation stresses experienced by flies in the wild. Desiccation rates depend on temperature and humidity, which are critical limiting factors in the physical environment of *Drosophila* (Parsons, 1978). The potential for desiccation during the midday hours should be greater at lower latitudes, because such regions are hotter, and because the saturation deficit (the difference in water vapour pressure between the respiratory system of a fly and the external environment) increases much faster than linearly as a function of temperature (Barbour *et al.*, 1980). Moreover, the midday sun is more directly overhead at lower latitudes, resulting in deeper sunlight penetration through the forest canopy (Terborgh, 1985), which in turn decreases the availability of shady microhabitats in which flies might remain active. Thus, flies from high latitudes can remain active throughout the day, whereas flies from lower latitudes are generally restricted to the less stressful hours early and late in the day.

The skunk cabbage-breeding species, which exhibit high midday activity, may experience particularly low desiccation stress for two reasons. They inhabit swampy areas where the relative humidity is higher than in the woodlands inhabited by mycophagous species. In addition, the skunk cabbage *Symplocarpus foetidus* has very large leaves that provide a shady, humid microhabitat for flies utilizing this plant. Thus, in a physically permissive environment, selection may favour those individuals that continue their activities – mating, egg laying, and so on – throughout the day.

In summary, the *Drosophila* species examined in the present study exhibit substantial variation in their circadian patterns of activity. Given the great phylogenetic and ecological diversity of the genus, their standing as a model system for the study of the genetics and molecular biology of circadian rhythms, and their continuing emergence as models of speciation and adaptive divergence, *Drosophila* may also turn out to be ideal for exploring the adaptive evolution and diversification of circadian activity patterns.

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