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ORIGINAL PAPER

Effects of polygamy on the activity/rest rhythm of male fruit flies *Drosophila melanogaster*

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Abstract Although polygamy is common in insects, its extent varies enormously among natural populations. Mating systems influence the evolution of reproductive traits and the difference in extent of polygamy between males and females may be a key factor in determining traits which come under the influence of sexual selection. Fruit flies *Drosophila melanogaster* are promiscuous as both males and females mate with multiple partners. Mating has severe consequences on the physiology and behaviour of flies, and it affects their activity/rest rhythm in a sex-specific manner. In this study, we attempted to discern the effects of mating with multiple partners as opposed to a single partner, or of remaining unmated, on the activity/rest rhythm of flies under cyclic semi-natural (SN) and constant dark (DD) conditions. The results revealed that while evening activity of mated flies was significantly reduced compared to virgins, polygamous males showed a more severe reduction compared to monogamous males. In contrast, though mated females showed reduction in evening activity compared to virgins, activity levels were not different between polygamous and monogamous females. Although there was no detectable effect of mating on clock period, power of the activity/rest rhythm was significantly reduced in mated females with no difference seen between polygamous and monogamous individuals. These results suggest that courtship motivation, represented by evening activity, is successively reduced in males due to mating with one or more partners, while in females, it does not depend on the number of mating partners. Based on these results we conclude that polygamy affects the activity/rest rhythm of fruit flies *D. melanogaster* in a sex-dependent manner.

Keywords *Drosophila melanogaster* · Polygamy · Natural conditions · Circadian · Activity

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Introduction

Individuals in a population show differential reproductive success due to variability in traits such as development time, survival, mating, and fecundity (Stearns 1976). Since mating success is a key determinant of future genetic composition of any population, viable strategies for optimal mating are likely to be selected for (Thornhill and Alcock 1983). Mating systems are typically described and classified based on courtship rituals, number of matings acquired, and parental care (Emlen and Oring 1977; Markow 1996). In most animals, females usually exercise mate choice and require mating only once or twice to reach their maximal fertility compared to males in which reproductive success increases linearly as a function of number of matings (Bateman 1948; Trivers 1972). Moreover, due to resource limitations, there is a trade-off in resource allocation between survival and reproduction (Williams 1966; Gadgil and Bossert 1970). Mating can be a costly affair in terms of time and energy expenditure, predation risk, and exposure to sexually transmitted diseases (Wing 1988; Arnqvist 1989; Hurst et al. 1995). Therefore, it is expected that only males would seek multiple matings since cost of mating in males is likely to be offset by the gain in reproductive fitness, while females would prefer to mate less

often since they would not gain in proportion to the number of matings acquired (Bateman 1948). Contrary to the expectations from parental investment theory (Trivers 1972), females of most species mate with multiple males, suggesting that females may gain benefits of mating, which would induce them to mate with multiple males despite the associated costs (Arnqvist and Nilsson 2000). For instance, mating and presence of large number of viable sperms stimulate egg production resulting in increased fertility (Opp and Prokopy 1986; Tregenza and Wedell 1998). Hence, it would be interesting to see if males and females show similar effects of exposure to multiple partners since the costs and benefits of polygamy are different across the two sexes.

Although evolutionarily stable strategies in populations with sexual conflict *usually* result in females exhibiting rejection behaviour and males showing persistence of courtship (Smith 1984), the extent of polygamy seen in natural populations of insects varies vastly across species. While females of some species such as the Egyptian cotton moth *Earias insulana* Bois show low levels of repeated mating, others such as the green-veined white butterfly *Pieris napi* show high levels of remating (Safonkin 2011). Even closely related species such as the ermine moths, *Yponomeuta padellus* and *Yponomeuta cagnagellus*, show different levels of remating (Bakker et al. 2008). In insects, polyandry is known to increase sperm competition and affect the evolution of accompanying traits such as ejaculate volume and genital modifications (Kvarnemo and Simmons 2013). Hence, difference in the extent of polygamy between the two sexes is a key factor in determining various traits that are under the influence of sexual selection.

Mating, like several other behaviours, is known to show daily rhythm in fruit flies *Drosophila melanogaster* (Sakai and Ishida 2001). It has been suggested that mating rhythm may play a critical role in creating reproductive isolation between species (Hardeland 1972; Sakai and Ishida 2001). It has been shown that courtship rhythm requires presence of functional clocks in males (Fujii et al. 2007), whereas rhythmic mating (measured in terms of mating frequency at different times of the day) requires presence of functional clocks in females (Sakai and Ishida 2001). Some key aspects of the molecular and neuronal regulation of mating rhythm in *D. melanogaster* have also been elucidated in a few recent studies (Hamasaka et al. 2010; Fujii and Amrein 2010; Hanafusa et al. 2013; Krupp et al. 2013). For instance, it has been shown that recognition of sex as well as species is aided by cuticular hydrocarbons produced by specialised cells located on the abdominal cuticle called oenocytes (Billeter et al. 2009). These oenocytes host peripheral clocks, which accumulate pheromones in a rhythmic manner via mechanisms involving the clock-controlled gene *desat* (Krupp et al. 2008). Mating-related chemical signals are further influenced by the social environment as well as ambient light conditions (Kent et al. 2008). Given the pervasive control of circadian rhythms on mating, it would be interesting to examine if mating in turn affects circadian rhythms.

In a recent study, it was shown that oscillation of clock proteins in the central clock neurons [*pigment dispersing factor* (PDF)-expressing lateral neurons] is necessary for the persistence of courtship rhythm in *D. melanogaster*, while a subset of the dorsal neurons (DN1s) (part of the evening oscillator) is required for synchronising its phase (Fujii and Amrein 2010). This role of the evening oscillator has been confirmed in other studies, which showed that dorsal neurons (DN1s and dLNs) are necessary for the persistence of close proximity (surrogate of courtship) rhythm in *Drosophila*, while the morning clock neurons (PDF-neurons) are dispensable (Hamasaka et al. 2010; Lone and Sharma 2012). Moreover, there is evidence to suggest that presence of females affects clock protein cycling in the DN1 neurons of males (Hanafusa et al. 2013) and that prior exposure to sociosexual interactions results in decreased evening activity, and in lengthening of clock period in males (Lone and Sharma 2011), providing further evidence of the role of evening oscillators in mating. Thus, circadian clocks are necessary for courtship-related evening activity, which in turn is affected by mating. Hence, it would be interesting to compare the effects of mating with multiple partners on the activity/rest rhythm of male and female flies considering evening activity as proxy for courtship motivation. Such experiments may provide insights into if and how mating affects courtship motivation in the two sexes.

Since it is believed that mating with more than one partner confers costs and benefits differentially across the two sexes, we hypothesised that mating with additional partners would affect the activity/rest rhythm of male and female *D. melanogaster* flies differently. Although it is known that mating behaviour and responses to mating differ between males and females, we asked if such differences would be reflected in their post-mating activity/rest rhythm, since patterns of activity are believed to be functionally related to foraging and mating-related behaviours (De et al. 2013). In a previous study, Lone and Sharma (2011) have shown that post-mating activity/rest rhythm of flies housed in mixed-sex groups was different from those living in same-sex groups; however, this study did not examine the effects of mating with single versus multiple partners. In the present study, we examined the effects of polygamy versus monogamy on the post-mating activity/rest rhythm of fruit flies *D. melanogaster*. Since activity/rest rhythm of flies living in mixed-sex groups have been shown to be affected under laboratory light/dark cycles, we asked if mating affects the activity/rest rhythm of flies maintained under semi-natural conditions (SN), wherein the rhythm of virgin males is known to be remarkably different from that seen under standard laboratory conditions (Vanin et al. 2012; De et al. 2013).

Material and methods

Fly populations

All experiments described here were conducted on a large outbred population of fruit flies *D. melanogaster* maintained for over 200 generations under laboratory 12:12 h light/dark cycles at constant temperature (25 °C) and humidity (~80 %) on banana-jaggery food medium (Sheeba et al. 2001). These populations were kept on a 21-day discrete generation cycle with eggs collected in each generation on the 12th day after emergence. All flies used in our experiments were collected from vials seeded with low-to-moderate density of eggs (~60 eggs/vial). Virgin males and females were collected within 5–6 h of emergence following anesthetisation with carbon dioxide and loaded into locomotor activity tubes (7×65 mm). In all the three treatments, locomotor activity tubes with fresh food were provided to flies every day. Flies exposed to all the three treatments were maintained and handled in a similar manner, and their locomotor activity was recorded simultaneously. The only difference in terms of treatments was that virgin flies were maintained solitarily, monogamous flies were maintained in fixed pairs, and individuals exposed to polygamous treatment were rotated sequentially.

Experimental design to study effect of polygamy

Four- to six-day-old virgin flies were used in all our experiments. We studied effects of polygamy on the activity/rest rhythm of both males and females assigned to three treatments—virgin, monogamous, and polygamous. A total of 100 males and 100 females were used in each of the treatments. Each treatment was continued for 8 days by maintaining flies in locomotor activity tubes (7×65 mm) containing corn medium. As controls, virgin males (n=100 individuals) and females (n=100 individuals) were maintained solitarily for a period of 8 days in locomotor activity tubes, which were changed every day for uniformity in handling across treatments. For the monogamy treatment, 100 male–female pairs were maintained in locomotor activity tubes for 8 days, and the tubes were changed every day. For the polygamy treatment, males and females (100 pairs) were initially paired, and subsequently, on each successive day, males were separated from their partners without anesthesia and paired with a different female such that each fly in the treatment was paired every day with a new individual of the opposite sex (Fig. 1). For example, on the first day, male 1 was paired with female 1, male 2 with female 2 and so on till male 10 was paired with female 10. On the second day, male 1 was separated from female 1 and paired with female 2; similarly, male 2 was paired with female 3 and so on till male 9 was paired with female 10, and male 10 with female 1. On the third day, male 1 was separated from female 2 and paired with female 3; male 2 was paired with female 4 and so on till male 9 was paired with female 1 and male 10 with female 2. This process of rotating males across females was continued till 8 days such that each fly encountered eight flies of the opposite sex at 24-h intervals. This was carried out on ten such sets of flies (Fig. 1).

Recording of locomotor activity behaviour

Following 8 days of treatment, flies were isolated and introduced individually into locomotor activity tubes (7×65 mm) with corn food at one end and cotton plug at the other, and their locomotor activity behaviour was monitored for about 10 days under SN or constant darkness (DD, 25 °C) of the laboratory in BOD incubators (Percival, USA) using Drosophila Activity Monitors (Trikinetics, Waltham, USA). Food in all recording activity tubes was changed every third day to avoid eggs/larvae from interfering with the activity recording and to avoid physiological effects of presence of eggs and larvae. Locomotor activity recording under SN was done simultaneously for flies across all the three treatments in the month of July, 2012 inside an enclosure (De et al. 2013) constructed under a leafy canopy within the Jawaharlal Nehru Centre for Advanced Scientific Research campus, Bangalore (12°59′ N, 77°35′ E). Unlike previous studies (Vanin et al. 2012; De et al. 2013), the afternoon peak under SN in this assay was seen during 0900–0915 hours (hence pre-noon peak) due to sudden increase in light and temperature around this time on most days of recording. This can be distinguished from the morning peak, which occurred close to dawn (0600 hours) with anticipation prior to dawn and due to quality of the pre-noon peak, which was abrupt and occurred for a short duration.

Statistical analyses

To compare average activity profiles of males (n=32 virgins, n=31 monogamous and n=30 polygamous) and females (n=31 virgins, n=30 monogamous and n=31 polygamous), activity data collected for over 10 days under SN were plotted separately. To compare cumulative activity of flies during different parts of the day, 24 h of the day was divided into light (day) and dark (night) phases. Since under SN, dawn (time in the morning when light intensity exceeded 0 lx) occurred at 0600 hours and dusk (time in the evening when light intensity dropped to 0 lx) at 1900 hours, we took these two times as reference points to divide activity into four intervals−early day (ED: 0600–1230 hours), late day (LD: 1230–1900 hours), early night (EN: 1900–0030 hours) and late night (LN: 0030–0600 hours). The offset of evening activity was estimated by taking the first time-point after dusk with activity lower than 10 counts/15 min in the average activity profile of individual flies. This cutoff was chosen since the peaks of activity were >10 counts/15 min, while

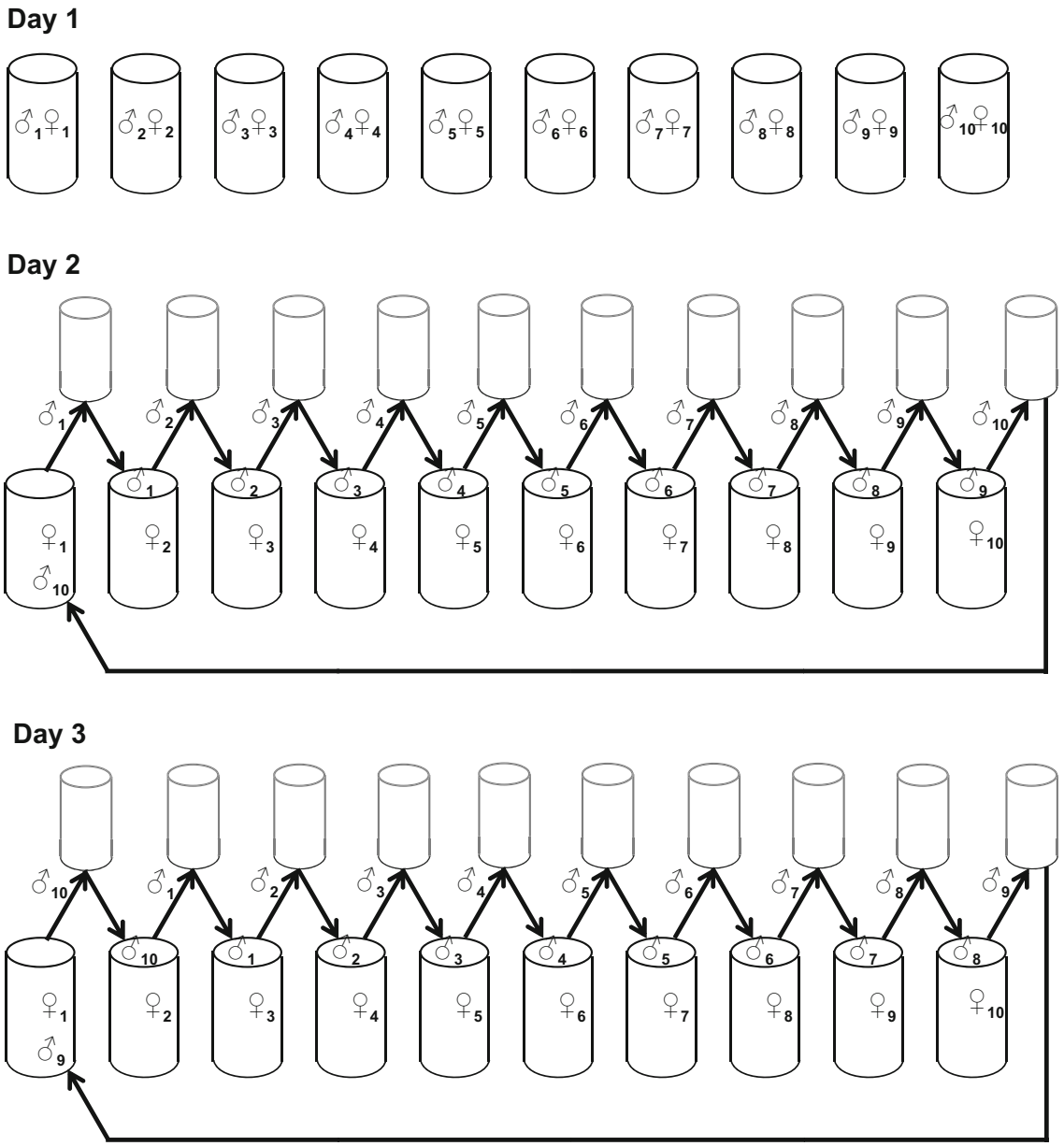

Fig. 1 Schematic representation of the protocol used for subjecting fruit flies *Drosophila melanogaster* to polygamy treatment. For the polygamy treatment, males and females (100 pairs) were initially paired, and subsequently, on each successive day, males were separated from their partners without anesthesia and paired with a different female such that each fly in the treatment was paired every day with a new individual of the opposite sex. On day 1, hundred males were paired with hundred females in ten sets of ten pairs each. On day 2, male 1 was separated from female 1 by shifting into an empty tube (light gray tube) and then paired with female 2. Before male 1 was paired with female 2, male 2 was separated from female 2 and introduced into an empty tube where from it was then paired with female 3 and so on, till male 9 was paired with female 10 and male 10 was paired with female 1. Similarly, on day 3, male 1 was separated from female 2 by shifting into an empty tube (light gray tube) and then paired with female 3. Before male 1 was paired with female 3, male 2 was separated from female 3 and introduced into an empty tube wherefrom it was then paired with female 4 and so on, till male 9 was paired with female 1 and male 10 was paired with female 2. This process was continued for 8 days

baseline activity rarely exceeded the same. The raw activity data of flies maintained under DD were plotted as actograms, and analysed for period and power of activity/rest rhythm using Lomb Scargle (LS) Periodogram in CLOCKLAB (Actimetrics, USA). Activity data of flies that died early (within 5 days of recording) were not considered for analysis.

Activity data collected under SN were analysed using analysis of variance (ANOVA) to test for statistically significant differences in activity at different times of the day, using time of the day and treatment as fixed factors. Repeated measures ANOVA was also carried out on the cumulative activity data from the four intervals considering treatment as a fixed factor and interval as repeated measures. The activity offset, free-

running period and power of the activity/rest rhythm (amplitude of the LS periodogram) was analysed separately considering treatment as a fixed factor. Post hoc multiple comparisons were carried out using Fisher's LSD test. ANOVA was executed on Statistica for Windows, Release 5.0B (Statsoft 1995).

Results

Polygamy affects evening activity of males most severely

While evening activity peak of mated males under SN was reduced compared to that of virgins, evening peak of polygamous males was most severely affected (Fig. 2a, b). ANOVA on the activity profile data of males revealed statistically significant effects of treatment ($F_{2,8928}$=7.06, p<0.01), time of the day ($F_{95,8928}$=25.36, p<0.01) and treatment×time of the day interaction ($F_{190,8928}$=2.53, p<0.01). Post hoc multiple comparisons showed statistically significant reduction in the evening activity of polygamous males compared to monogamous males, which in turn showed significant reduction compared to virgin males during 1930 to 2045 hours (p<0.05; Fig. 2b). The evening activity of both polygamous and monogamous males was additionally reduced compared to virgins during the interval between 2045 and 2145 hours (p<0.05). The pre-noon peak, on the other hand, was significantly higher in monogamous males compared to virgin and polygamous males with post hoc multiple comparisons showing significantly greater activity at 0915 hours (p<0.01). Although at 0915 hours, polygamous males were as active as virgin males (Fig. 2b), they were significantly more active than virgin males at 0900 hours (p<0.05). The offset of evening activity in males was significantly different across the three treatments (Fig. 2b). ANOVA on the offset data revealed a statistically significant effect of treatment ($F_{2,90}$=6.79, p<0.01) with polygamous males showing significantly advanced offsets compared to monogamous and virgin males (p<0.05), while activity offsets of monogamous and virgin males did not differ statistically (p>0.05).

To compare activity during different parts of the 24-h cycle, we computed cumulative activity in four intervals. Repeated measures ANOVA on the cumulative activity data of males revealed statistically significant effects of interval ($F_{3,270}$= 18.52; p<0.01), and interval×treatment interaction ($F_{6,270}$= 10.26, p<0.01). Post hoc multiple comparisons showed that early day (ED) activity of polygamous and monogamous males was significantly greater than virgin males (p<0.05), while ED activity of monogamous and polygamous males did not differ statistically from each other (Fig. 2c). There was no difference in activity between the treatments during late day (LD). Post hoc comparisons of activity during early night (EN) revealed that polygamous males were less active compared to monogamous males (p<0.01), which in turn were less active than virgin males (p<0.01; Fig. 2c). During late night (LN), polygamous males showed significantly greater activity than virgin males (p<0.01). These results suggest that, for most parts of the day, activity of mated males differ from virgins; however, evening activity was further reduced in polygamous males relative to monogamous males, indicating that effect of mating is more severe in males which mate with multiple females than in males which mate with a single female.

Polygamy has no additional effect on activity of females

Mated females were less active than virgins, while activity of polygamous and monogamous females did not differ (Fig. 3). ANOVA on the activity data of females revealed statistically significant effects of treatment ($F_{2,8544}$=249.91, p<0.01), time of the day ($F_{95,8544}$=42.22, p<0.01) and treatment×time of the day interaction ($F_{190,8544}$=4.69, p<0.01). Post hoc multiple comparisons revealed that activity during a large part of the evening (between 1645 and 2345 hours) was significantly lower in mated females compared to virgin females (p<0.05; Fig. 3b). However, in comparison to virgins, the evening activity peaks of both polygamous and monogamous females were reduced by a similar extent. The pre-noon peak of females coincided with maximum light and temperature conditions around 0900 hours (Fig. 3a, b). The virgin females showed smaller pre-noon peak compared to polygamous and monogamous females, which showed significantly higher activity at 0915 hours (p<0.01). However, during 0830 to 0845 hours, polygamous females were significantly more active compared to both monogamous and virgin females (p<0.01). The offset of evening activity in females was significantly different between mated and virgin males (Fig. 3b). ANOVA on the offset of activity data revealed a statistically significant effect of treatment ($F_{2,89}$=30.14, p<0.01) with both polygamous and monogamous females having significantly advanced offsets compared to virgin females (p<0.01).

Repeated measures ANOVA on the cumulative activity data across the four intervals revealed statistically significant effects of interval ($F_{3,267}$=149.56, p<0.01), and interval× treatment interaction ($F_{6,267}$=12.74, p<0.01). Post hoc multiple comparisons on activity during early day (ED) showed that mated females were significantly more active than virgin females (p<0.01; Fig. 3c). Post hoc multiple comparisons on activity during early night (EN) showed that mated females were less active compared to virgin females (p<0.01; Fig. 3c). However, there was no difference between the activity of polygamous and monogamous females during ED or EN (Fig. 3c). There was also no difference in activity between all the three treatments during late day (LD) and late night

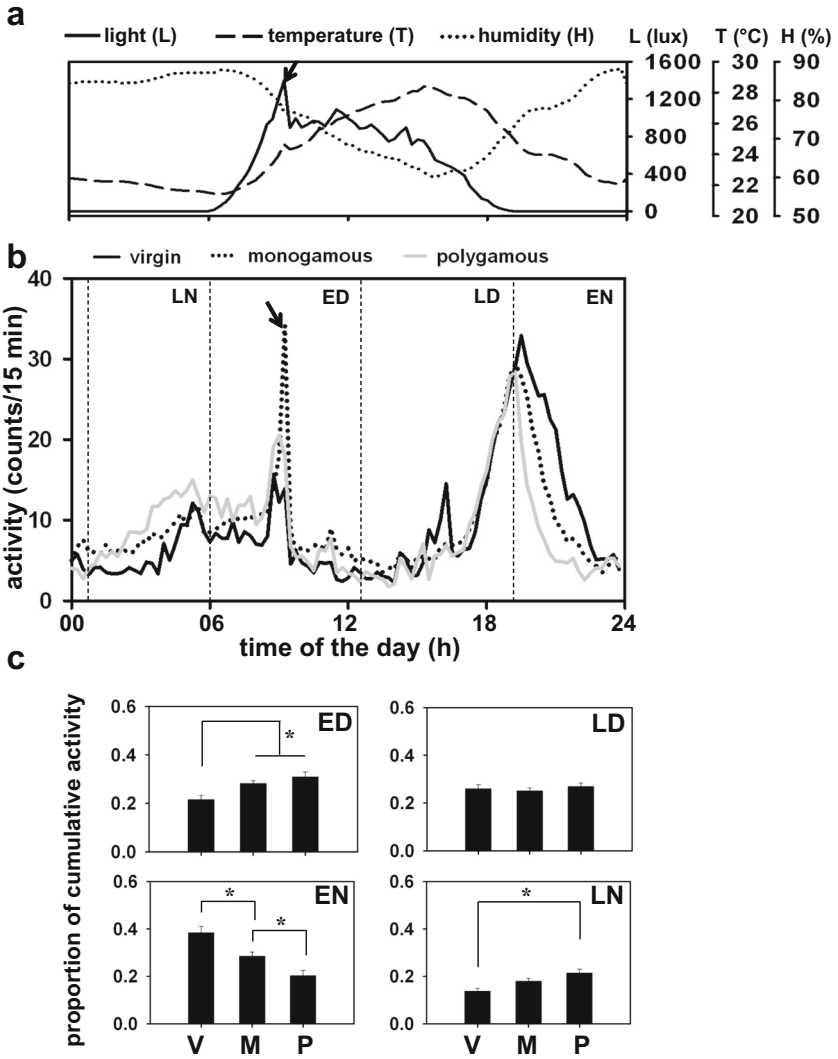

Fig. 2 Activity profiles of virgin, monogamous, and polygamous males under semi-natural (SN) conditions. **a** Average profiles of light (*L*), temperature (*T*), and humidity (*H*) over 10 days of recording. *Arrow* in **a** indicates sudden increase in light and temperature at 0915 hours. **b** Average activity profiles of virgin (*V*), monogamous (*M*), and polygamous (*P*) *Drosophila melanogaster* males over 10 days of recording. Virgin males showed highest evening activity followed by monogamous males, and then polygamous males that showed least amount of evening activity. *Arrow* in **b** indicates the pre-noon peak. Monogamous males showed highest pre-noon activity peak followed by polygamous males, and then virgin males. **c** Proportion of cumulative activity in the four 6-h intervals of early day (ED: 0600–1230 hours), late day (LD: 1230–1900 hours), early night (EN: 1900–0030 hours), and late night (LN: 0030–0600 hours) compared across the three treatments. Virgin males showed significantly lower early day (*ED*) activity compared to both monogamous and polygamous males. During the early night (*EN*), virgin males showed maximum activity followed by monogamous males, which showed significantly higher activity than polygamous males. During the late night (*LN*), polygamous males showed significantly greater activity than virgins, while other comparisons were not statistically significant. Error bars represent SEM. *Asterisk* denotes statistically significant differences of $p<0.05$ from post hoc multiple comparisons

(LN) in females. These results suggest that, though females show a clear effect of mating, unlike males, there is no additional effect of mating with more than one partner. However, the pre-noon peak of polygamous females did show enhanced activity compared to their monogamous counterparts. These results suggest that evening activity of mated females is significantly reduced compared to virgins with no additional effect of mating with multiple partners.

Mating slows down clocks without incurring any effect of polygamy

Since socio-sexual interactions are known to lengthen clock period in males and reduce amplitude of the activity/rest rhythm in females, we asked if polygamy has any effect on the free-running rhythm. In males, neither period nor power of the rhythm was affected by the treatments (Fig. 4a, b). However, monogamous males showed a trend towards longer

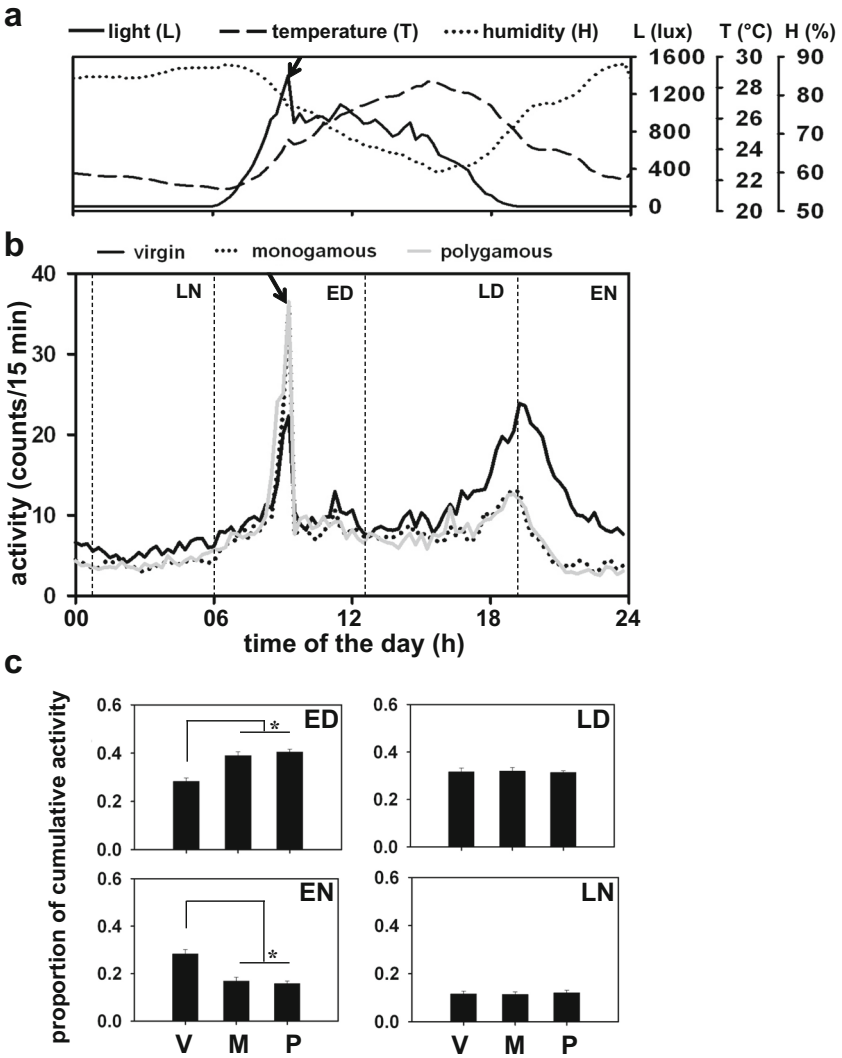

Fig. 3 Activity profiles of virgin, monogamous, and polygamous females under semi-natural conditions. **a** Average profiles of light (*L*), temperature (*T*), and humidity (*H*) over 10 days of recording. **b** Average activity profiles of virgin (*V*), monogamous (*M*), and polygamous (*P*) *Drosophila melanogaster* females over 10 days of recording. Virgin females showed significantly greater evening activity than mated females with no difference observed between monogamous and polygamous females. Mated females showed greater pre-noon peak compared to the virgins. **c** Proportion of cumulative activity in the four 6-h intervals of early day (ED: 0600–1230 hours), late day (LD: 1230–1900 hours), early night (EN: 1900–0030 hours), and late night (LN: 0030–0600 hours) compared across the three treatments. During the early day (*ED*), activity of mated females was significantly higher than that of virgins while early night (*EN*) activity of mated females was significantly reduced compared to virgins. At both times, activity of polygamous and monogamous females was not different from each other. Remaining details same as in Fig. 2

period and lower power of rhythm compared to both polygamous and virgin males (Fig. 4). Although period of mated females (both monogamous and polygamous) was slightly longer than that of virgins, this difference was not statistically significant (p=0.55; Fig. 4). ANOVA on power of the rhythm in females showed a statistically significant effect of treatment ($F_{2,85}$=4.83, p<0.05) with both polygamous and monogamous females showing reduced power compared to virgins (p<0.05). However, there was no difference in power of the rhythm between monogamous and polygamous females (Fig. 4), suggesting no additional effect of mating with more than one partner on the free-running rhythm. These results suggest that mating affects the free-running rhythm of fruit flies in a sex-specific manner, with mated females showing reduced power of activity/rest rhythm.

Discussion

In *Drosophila*, a large part of its daily activity, particularly in the evening is believed to be due to courtship/mating-related behaviours, which is significantly reduced after mating (Fujii et al. 2007; Lone and Sharma 2011). In the present study, we

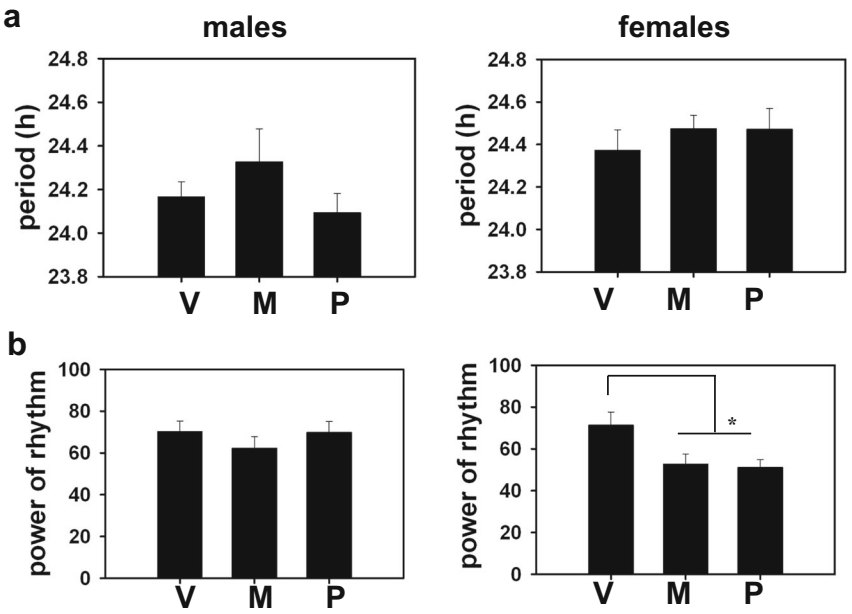

Fig. 4 Circadian period and power of activity/rest rhythm under constant dark conditions compared across different treatments. **a** Free-running period of male (*left panel*) and female (*right panel*) fruit flies *Drosophila melanogaster* under constant dark (*DD*) conditions compared across virgin (*V*), monogamous (*M*), and polygamous (*P*) treatments. **b** Power of the free-running rhythm in males and females under DD. Only mated females (both polygamous and monogamous) showed significantly lower power of rhythm compared to virgin females. Monogamous males showed longer period and lower power of rhythm though these differences were not statistically significant. Remaining details same as in Fig. 2

observed that evening activity was significantly reduced in mated males and females relative to their virgin counterparts; however, this reduction was more severe in polygamous males compared to monogamous males (Figs. 2 and 3). On the other hand, the evening activity of both polygamous and monogamous females was reduced by a similar extent, without females incurring any additional cost of mating with multiple partners (Fig. 3). Thus, effects of mating with multiple versus single partners on the activity of females seem to be not as severe as it is for males, suggesting that behavioural response to polygamy is different across the two sexes. Such differential effects of mating on male and female activity/rest rhythms are consistent with the observations of sexual dimorphism in the fly brain (Belgacem and Martin 2002) as well as in their mating behaviour (Billeter et al. 2006; Dickson 2008). Apart from mating behaviour, sexual dimorphism is widely seen in traits such as size, shape, colouration, mortality rates, brain circuits, and parental behaviours (Trivers 1972; Shine 1994; Owens and Hartley 1998; Simerly 2002). Sexual dimorphism is often attributed to intra-sexual competition for mates (Darwin 1871) and, to some extent, to niche separation between the two sexes (Shine 1989). Therefore, differences in the effects of mating on evening activity between males and females, and hence in the level of courtship motivation, may represent sex-specific adaptations to maximise reproductive success while minimising adverse effects of repeated mating.

Males usually indiscriminately court females, while mated females often reject sexual advances since females require only a single mating to attain close to maximum fertility, and with each subsequent mating, females bear more cost than males (Sturtevant 1915; Bateman 1948; Bastock and Manning 1955; Markow 2002). Hence, males experience higher levels of intra-sexual selection due to greater competition for mates and higher variance in reproductive success compared to females (Bateman 1948; Trivers 1972). This pattern is thought to be due to the fundamental differences in energy investment in the production of gametes (or anisogamy), whereby males are capable of producing large numbers of sex cells with relatively less investment compared to females (Bateman 1948). Hence, females (which produce large gametes) become a limiting resource for whom males have to compete (Bateman 1948). Although males also incur costs of reproduction in terms of reduced survival and future reproduction (Partridge and Farquhar 1981; Dewsbury 1982; Cordts and Partridge 1996; Kotiaho and Simmons 2003), such costs are offset by the increase in fertility that they gain with every additional partner they mate with. Our results on the activity/rest rhythm of flies revealed that polygamous males are significantly less active in the evening than monogamous males, which in turn are less active than virgin males (Fig. 2). These results can be taken to suggest that mating with a single female probably does not reduce motivation for courtship in males as much as mating with multiple females does. Since courtship is necessary for the realisation of reproductive fitness, reduced sex drive (as indicated by reduction in evening activity) would imply a cost of mating. This cost appears to be greater in

polygamous males compared to monogamous males, while it does not differ between polygamous and monogamous females. This difference in the effects of mating with multiple partners may represent sex-specific differences in the cost of mating and gain in reproductive success with each successive mating. Since males gain fertility while suffering minimal costs with each mating, the reduction in their motivation for courtship after mating with a single partner is lower than the effects of mating with multiple partners. In contrast, females show as much reduction in activity after mating with a single partner as with multiple partners probably because they do not receive proportionate gains in reproductive success with subsequent matings.

Besides *Drosophila*, differences in the number of matings between males and females are seen in insects such as the spiny bollworm, *E. insulana* (Kehat and Gordon 1977), where males show a tendency to mate more often than females. Similarly, higher costs and lower benefits of remating in females are also observed in insect species such as *Gerris buenoi* and *Grapholita molesta* (Rowe 1994; de Morais et al. 2011). In such cases, polygamy may be favoured in males but not females in a manner similar to *D. melanogaster*, and mating with a single partner may lower the sex drive of females much more than that of males. In contrast, insects that are predominantly polyandrous such as *Callosobruchus maculatus* and *Kawanaphila nartee* often show increased benefits of multiple matings in females due to nuptial gifts present in the ejaculate (Arnqvist et al. 2005; Kvarnemo and Simmons 2013). For such insects, sex-specific differences in effects of mating seen in *Drosophila* may not be relevant. Therefore, although *D. melanogaster* females are not strictly monogamous, the optimal number of matings for females is likely to be low, in contrast to highly polyandrous insect species, which gain benefits from multiple mating and may not be inclined to reduce their courtship motivation after a single mating.

The male sex drive or courtship motivation rhythm in *Drosophila* was shown to require the evening oscillator neurons (DN1s and LNds) for its persistence (Fujii and Amrein 2010; Hamasaka et al. 2010). Molecular oscillation of clock proteins in the DN1 neurons was found to be affected due to socio-sexual interactions (Hanafusa et al. 2013). Taken together with the above findings, our results suggest that mating-related reduction in the evening activity of *Drosophila* is regulated by the evening clock neurons. However, in females, after-effects of mating on the activity/rest rhythm are likely to be clock-independent because even clock manipulated flies show effects similar to wild-type flies (Lone and Sharma 2011).

In contrast to males, monogamous and polygamous females show very little difference in activity, though both show lower activity levels compared to virgin females (Fig. 3). While reduction in activity of females is restricted mainly around the evening peak, there also seems to be an overall decrease in activity through most of the day (barring the pre-noon peak) in females, which is not seen in males (Fig. 3). This is complemented by a reduction in the power of free-running rhythm in mated females (Fig. 4). These results suggest that mating has an overall effect on the activity levels of females irrespective of the number of partners they mate with.

Although we tried to minimise the effects of eggs and larvae in the food medium of mated females by providing flies with fresh food every third day, it is not possible to completely rule out the possibility that part of the effects seen in the mated females is due to eggs and larvae being present in the food for the intervening 2 days. Nevertheless, the results of our study show that, in females, there is no additional effect of mating with multiple partners, in contrast to males. Furthermore, virgins in our treatment were maintained solitarily, which may confound the effects of mating with the effects of social interaction. However, the main result of our study focuses on the effect of polygamy in comparison to monogamy, and a previous study (Lone and Sharma 2012) has shown that the effect of pairing two males or two females on activity/rest rhythm was negligible.

The activity profiles in the present study were assayed under SN as opposed to standard laboratory conditions under which previous experiments were conducted (Fujii et al. 2007; Lone and Sharma 2011). The consistency in the reduction of evening peak in mated males suggests that this peak under both the conditions is controlled by processes similarly affected by mating. However, the pre-noon peak, which appears to be a response to stressful environmental conditions, is enhanced in mated flies compared to virgins, in contrast to the evening peak, which is reduced in mated flies (Figs. 2 and 3). Although polygamous and monogamous females show no difference in their pre-noon peak, polygamous males exhibit a lower peak than monogamous males. This result is counter-intuitive since mating with multiple partners would be expected to cause a greater effect than that with a single partner, as seen for the evening peak. Hence, we conclude that pre-noon and evening activity peaks in males are differently affected by mating as well as by the number of mating partners.

The free-running period of mated and virgin males or females do not differ in our present study (Fig. 4) contrary to what was observed previously in mated males (Lone and Sharma 2011). A possible reason for this disparity could be the protocols used in the two studies. While Lone and Sharma (2011) placed flies in mixed-sex groups of 30 flies per vial (25×90 mm) with equal number of males and females, in the present study, we exposed individual males to individual females in small glass tubes (7×65 mm), and rotated the partners in case of the polygamous treatment. Therefore, the differences seen are likely to be due to the effects of exposure to multiple partners successively versus simultaneously.

In conclusion, the evening activity of males, representing male sex drive, is further reduced upon mating with multiple partners relative to a single partner, which suggests that males retain some of their courtship motivation even after mating with a single partner. However, post-mating, females show a general reduction in activity with a clear suppression in power of the rhythm. This reduction is of comparable magnitude in both polygamous and monogamous females, suggesting that the effect of mating with single or multiple partners is similar in females. In summary, *D. melanogaster* males show differential effects of mating with single and multiple partners, whereas females show only a general effect of mating.

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