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RESEARCH ARTICLE

Light conditions affect sexual performance in a lekking tephritid fruit fly

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SUMMARY

Sensory systems are very susceptible to early environment experience. Mating success depends on the transmission of information from the signaller to the receiver, which means that sensory biases caused by developmental environment are likely to affect sexual selection. We investigated the impact of the developmental visual environment (light spectrum) on male copulation behaviour and female preference in the lekking tephritid *Anastrepha ludens*. We reared flies in four different light spectrum conditions – red light, blue light, shaded light and darkness – during their first 16 days after emerging from pupae. We found that the light environment experienced during early adulthood affected mating frequency and, in some cases, the latency to copulate, but not copulation duration. Males exposed to any of the three light treatments (red, blue or shaded light) were more frequently chosen as mating partners than dark-reared males. Flies reared under dark conditions exhibited the lowest mating performance out of any of the rearing environments. Under field cage conditions, a slight assortative mating between blue- and red-light-reared flies was detected. Additionally, females reared in blue light and darkness mated less compared with females reared in red and shaded light. Our data demonstrate that male mating behaviour is flexible in response to light environment. The findings suggest that light spectrum only weakly affects the direction of sexual selection by female choice; however, dark rearing environments deeply affect mating success.

Key words: courtship display, early experience, light spectrum, copulation behaviour, phenotypic plasticity, Diptera

INTRODUCTION

Environmental effects on the sensory system play an important role in the maintenance of genetic variation (Ryan, 1990; Ryan, 2007; Endler, 1993a). Signal traits and receptor organs are selected depending on environmental conditions, not only maximizing signal attractiveness but also minimizing negative effects of factors such as predation (Gamble et al., 2003; Fuller, 2002; Bailey and Zuk, 2008). Light is a highly variable factor, because two important characteristics – spectrum and intensity (Endler, 1993b) – exhibit both spatial (among microenvironments) and temporal (diurnal and seasonal) variation. Thus, variation in visual communication through differences in light environment can favour directional selection. For example, in the bluefin killifish, *Lucania goodei*, the relative abundance of male colour morphs varies with lighting environment (Fuller, 2002). One explanation is that the development of the signal reception organ is affected by light conditions during rearing. For instance, the expression of the visual protein opsin in the bluefin killifish varies with rearing light spectrum (Fuller et al., 2005). In *Drosophila melanogaster*, the development of the optic lobes and mushroom body depends on the light environment; optic lobe size in flies reared in dark conditions is smaller than that of flies reared under full light (Barth et al., 1997b). Additionally, when *D. melanogaster* are reared in light environments, photoreceptor voltage responses to light contrast changes are amplified, favouring signalling performance (Wolfram and Juusola, 2004).

It has been demonstrated that light environment during early adulthood favours assortative mating and profoundly affects mating success in *D. melanogaster* (Hirsh and Tomkins, 1994; Hirsh et al., 1995; Barth et al., 1997a). Also, acoustic experience at an early age can modulate female choice and shape alternative mating tactics in field crickets *Teleogryllus oceanicus* (Bailey and Zuk, 2008; Bailey et al., 2010). Thus, rearing environment could be crucial in modelling mating behaviour, especially in species that exhibit elaborated courtship displays. In species that exhibit lek mating systems, males display complex signals during courtship in areas where signals could be amplified by microhabitat conditions, and males that monopolize those territories gain mating advantages (Emlen and Oring, 1977; Andersson, 1994; Endler and Théry, 1996).

Lekking fruit flies offer an excellent opportunity to explore the ontogenetic effect of light regimes on sexual selection because lekking takes place under specific environmental conditions that favour male signalling. In tephritid flies, lekking takes place on trees that offer both visual and olfactory stimuli (Hendrichs et al., 1991; Kaspi and Yuval, 1999a). Plant spectral quality (particularly hue and intensity) appears to be the principal stimulus that elicits landing on living plants (Prokopy and Owens, 1983). Tephritid males select lekking sites based on tree size and architecture (Shelly and Whittier, 1994; Field et al., 2002), and inside the lekking tree, leaves, which represent male territories, are chosen based on light and microclimatic conditions (Kaspi and Yuval, 1999a; Kaspi and Yuval, 1999b). From these territories, males display complex visual and acoustic signals combined with the release of pheromones (Prokopy and Hendrichs, 1979; Field et al., 2002). Furthermore, the transparent areas of tephritids' wings exhibit striking and stable structural colour patterns, called wing interference patterns, that could play an important role in the sexual selection process because there is sexual dimorphism (Shevtsova et al., 2011). Additionally, sexual

dimorphism in UV reflectance has been observed in some tephritid species (Sivinski et al., 2004).

Here, we studied the ontogenetic effect of light spectrum on copulation behaviour of the lekking tephritid Mexican fruit fly, *Anastrepha ludens* Loew 1873. These flies can be found from southern Texas to Central America in tropical, temperate and semiarid environments (Aluja et al., 2000). They are synovigenic, polyphagous and multivoltinous and exhibit circadian mating rhythm (Flitters, 1964). Mating occurs at dusk, and females are highly choosy (Robacker et al., 1991; Aluja et al., 2000; Díaz-Fleischer and Aluja, 2003). However, calling and mating behaviour schedules vary among populations within Mexico. For instance, flies from Nayarit start courtship 1.5 h earlier than flies from Chiapas and Sinaloa (Orozco-Dávila et al., 2007). Males have free sperm available 5 days after emergence whereas females have mature eggs when they reach 15 days of age (Dickens et al., 1982; Servin-Villegas and Jimenez-Jimenez, 1995). Given its pest status, *A. ludens* is mass-reared, irradiated and released as part of control efforts involving the sterile insect technique (SIT). SIT effectiveness is based on sterile male sexual competitiveness in mating with wild females (Knipling, 1955). However, long-term rearing conditions deeply modify mating schedules and behaviour of flies, thus reducing inter-breeding between wild and laboratory flies (Robacker and Hart, 1985; Cayol, 2000; Briceño and Eberhard, 2002; Meza-Hernández and Díaz-Fleischer, 2006; Weldon et al., 2010). Additionally, after emergence and before release, mass reared flies are held for 5–7 days either in paper bags or in plastic aerial release containers. These containers are kept in a dark room in order to allow flies to rest and avoid wasting energy (Enkerlin, 2007). Keeping flies under darkness could have important implications for SIT if, as in *D. melanogaster*, assortative mating occurs.

So far, studies reporting effects of light regimes on insect mating behaviour have been performed under laboratory conditions and, basically, contrast the effect of light and dark conditions. As such, little is known about the effects of light spectrum on insect mating success. Specifically, there is a scarcity of information concerning the relationship between the light spectrum environment of the sexually immature adults and their mating success in nature. Here, we test experimentally the possibility that variation in ambient light spectrum is responsible for variation in male and female mating success and sexual selection. We were interested in determining: (1) whether there is a relationship between the environmental conditions of the rearing experience and the mating success under those particular conditions (e.g. males reared under red light may have higher mating success in a red light environment) and (2) whether light environmental experience, especially during the period of sexual development, affects mating success under seminatural conditions.

MATERIALS AND METHODS

Study insects

Mexican fruit flies originated from pupae produced at the Moscafrut mass-rearing facility in Chiapas, Mexico, for approximately 150 generations. Pupae, 250 per cage, were placed inside eight emergence cages of 50×50×50 cm, made of wood and cotton mesh screen. Emergence cages, two per treatment, were placed under one of the following light conditions: blue light, red light, shaded light and total darkness. Adult flies received water and a standard diet (3:1 mixture of sugar and hydrolyzed yeast protein; ICN Biochemicals, Aurora, OH, USA) *ad libitum*. The flies were maintained at 25±1°C and 60±10% relative humidity under a 12 h:12 h light:dark cycle with lights on at 07:00 h.

Light rearing environment treatments

Light was provided through fluorescent tubes with a colour rendering index of more than 90% (Vita-Lite 75 W_T12, Duro de México, S.A., Tultitlán, State of Mexico, Mexico). Coloured cellophane Roscolux filters [Rosco, Stamford, CT, USA; number 26 (Light Red) for the red light treatment and number 75 (Twilight Blue) for the blue light treatment] were used to obtain red and blue light spectra. Red and blue light spectra were chosen to obtain contrasting light wavelength environmental conditions. As a control, we used a shaded light treatment by covering a fluorescent tube with a shade cloth. Flies were also kept in total darkness by covering cages with black cardboard. We kept the light intensity relatively low and minimized differences in light intensity between the treatments by varying the numbers of layers of filter or shade net. The mean ± s.d. light colour and light intensity were measured at three standard positions inside the cages for each treatment (Table 1). Insects emerged and were kept under these light conditions until they were fully sexually mature (14±2 days after emergence) (Orozco et al., 2001).

Forty-eight hours after adult emergence, flies were separated by sex in cages of 30×30×30 cm to ensure their unmated state. To separate flies, all lights in the laboratory were turned off except the light of the respective treatment. Forty flies of one sex were placed inside each cage. Five cages of each sex/treatment were prepared. Cages were isolated from the effect of normal laboratory illumination by covering them with black cardboard. Flies submitted to the darkness treatment were separated using a digital camera (Sony Cyber-shot model DSC-V1) equipped with infrared light. The colour temperature (T_{colour}; K) was measured using an Eye-One® Gretag Macbeth spectroradiometer (X-Rite, Grand Rapids, MI, USA). Colour temperature is a measurement in that indicates the hue of a specific type of light source (Kitsinelis, 2010). To transform colour temperature data to wavelength (λ; nm), we used the relationship between colour temperature and the peak wavelength in its spectrum using Wien's law, where $\lambda=3{,}000{,}000/T_{colour}$ (Mazenko, 2000). Thus, at 4500 K, the peak wavelength is 666 nm (red); at 6000 K, the peak wavelength is 500 nm (bluish green); and at 7500 K, the peak wavelength is 400 nm (deep blue).

No-choice experiment

Cages with 12 males and 12 females from the same treatment were placed under one of the four light treatments: red light, blue light, shaded light or darkness. Number of mating pairs, latency to mate and copulation duration were registered. Observations took place from 14:00 to 18:00 h. Observations in the darkness treatment were carried out using a digital camera. In this case, the observer used a black cloth to cover both the cage and the observer. This experiment was replicated 15 times.

Table 1. Colour and light intensity (mean ± s.e.m.) used in the rearing cages

Treatment	Light colour temperature (K)	Wavelength (nm)	Light intensity (lux)*
Shade cloth	4745±25.4	610±10.5	135.4±5.67
Red filter	4313±68.69	672.4±3.3	137.2±13.3
Blue filter	6483±369.5	453.1±27.9	128.6±24.6

*ANOVA for light intensity: $F_{2,14}=0.18$, $P=0.83$.

Choice experiment

We placed three pairs of flies from each of the four treatments inside the cages (12 pairs per cage). Two hours before the test, flies were marked on the thorax with a small spot of vinyl paint (Vinci de México, S.A. de C.V., Mexico City, Mexico) to distinguish individuals during the experiment. Colours were assigned at random and rotated among treatments. Additionally, previous studies indicated that this type of mark does not interfere with fly sexual activity (Meza et al., 2005). Cages were placed under one of the four light treatments. Observations were carried out as in the no-choice experiments. Fifteen replicates were carried out.

Field cage experiment

Field tests were run in cages made of Lumite® light brown polypropylene, 20×20 mesh per square inch (cat. no. 7250P; BioQuip Products, Gardena, CA) (Chambers et al., 1983; Calkins and Webb, 1983). Ten potted citrus trees and 10 potted mango trees were distributed in an alternating sequence around the perimeter (16 trees) and the centre (four trees) of each cage.

Ten males from each light rearing treatment were released 1 h prior to testing to allow them to establish territories. Ten females of each treatment were released 1 h later (17:00 h). As in the choice experiment (above), prior to field cage observations, males and females were marked on the thorax with a small spot of vinyl paint to distinguish individuals during the test. One observer carefully inspected all branches by standing on a stool to search the higher branches. During evening observations, the tree was surveyed between 17:00 and 17:30 h to determine the locations of flies. Prior to this time the flies were relatively inactive. Beginning at 17:00 h, locations where fly activity was high were observed closely throughout the remainder of the evening, although frequent scans of the tree were also conducted. The number of mating pairs, latency of the copulations and copulation duration were registered. The experiment was replicated 20 times.

Temperature and relative humidity were registered every 15 min by means of a hygrothermometer data logger (Extech Instruments model 42275, Waltham, MA, USA). The light colour and light intensity were registered every 5 min by means of the spectroradiometer and a photometer (Lutron model YK-10LX, Lutron Electronic Enterprise Co., Ltd, Taipei, Taiwan).

Data analysis

Small integer counts recorded for one of the response variables (i.e. number of copulations) were analysed using a generalised linear model (GLM) (JMP v.7, SAS Institute, Cary, NC, USA) with Poisson errors, a log-link function and type III significance tests (Crawley, 1993; Agresti, 1996). Contrasts were used to test for differences in levels within a variable. For the continuous response variables, such as latency until copulation and copulation duration, a two-way ANOVA was used. The effect of cage was included as a random factor, and least-square means Tukey's honestly significant difference tests were used to compare means (JMP v.7).

For the field cage experiment, the pair sexual selection (*PSS*), pair sexual isolation (PSI) and pair sexual isolation index (I_{PSI}) coefficients and their bootstrap significances were obtained using the software JMATING (Carvajal-Rodríguez and Rolán-Alvarez, 2006). *PSI* and *PSS* statistics measure sexual isolation and sexual selection effects independently. *PSS* is the statistic obtained when the expected pair types from mates is divided by the expected pair types from total numbers. Because both expected pair types were calculated assuming full random mating, *PSS* only measures the sexual selective differences between copulating and noncopulating samples for every pair type, that is, the effect of sexual selection. *PSI* is defined for every pair combination as the number of observed pair types divided by the number of expected pair types from mates. This statistic compares the observed pairs with the expected pairs from mating individuals (assuming random mating), so it is a measure of sexual isolation effects. *PSI* is independent of any sexual selection effect, because mating frequencies are used to obtain the expected pair types (Rolán-Alvarez and Caballero, 2000). For example:

$$PSI_{aa} = \frac{(aa)t}{(aa+ab)(aa+ba)} \text{ and } PSS_{aa} = \frac{(aa+ab)(aa+ba)S}{(AA')t^2}, \quad (1)$$

where A and B are the two types studied, with A and B being the number of males and A' and B' the corresponding number of females. The number of copulating pairs observed is t, and aa, ab, ba and bb are the observed number of copulating pairs for every male and female combination. S is the total number of expected pairs from population frequencies.

The *PSI* coefficients reflect the sexual isolation effects of each pair. The *PSS* coefficients represent the fitness of each pair and are an additive decomposition of the cross product estimator (W) (Rolán-Alvarez and Caballero, 2000). Values of *PSI* above/below one indicate excess/deficit of observed pairs relative to expected pairs under random mating using marginal frequencies. Values of *PSS* above/below one indicate excess/deficit of expected pairs from marginal frequencies relative to expected pairs using total population frequencies. The best alternative for estimating sexual isolation caused by mating preferences under biologically realistic sample sizes is the I_{PSI} statistic (Pérez-Figueroa et al., 2005). Differing from other indexes such as Yule V, Yule Q, YA, joint I and Cayol's index of compatibility, I_{PSI} uses *PSI* coefficients for the respective mating pair combinations instead of observed numbers of matings (see Rolán-Alvarez and Caballero, 2000):

$$I_{PSI} = \frac{(PSI_{aa} + PSI_{bb}) - (PSI_{ab} + PSI_{ba})}{(PSI_{aa} + PSI_{ab} + PSI_{ba} + PSI_{bb})}. \quad (2)$$

I_{PSI} varies from –1 to 1, with –1 representing maximum disassortative mating, 0 representing random mating and 1 representing the maximum possible degree of assortative mating (complete sexual isolation). The observed mating pairs were resampled 10,000 times, and the statistics were calculated for every resampling. In addition, we calculated the mating propensity (W) for each mate type, in males and females separately, relative to the type with the highest fitness (Carvajal-Rodríguez and Rolán-Alvarez, 2006).

RESULTS

No-choice experiment

A total of 1709 copulations (out of a possible 2880) were observed during the experiment. Fewer copulations were observed in arenas without any light (χ^2=58.2, d.f.=3, P<0.0001; Fig. 1A). Additionally, those flies that were reared in darkness copulated less frequently than flies that were reared in the other three treatments (χ^2=680.4, d.f.=3, P<0.0001). The interaction between test light condition and light rearing experience was significant, indicating that mating success (i.e. number of copulations) of flies of at least one of the groups was affected differently by the test environment (χ^2=130.8, d.f.=9, P<0.0001). Thus, number of copulations of shade-reared flies was not significantly different from red- and blue-reared flies under blue, shaded or red light environments; however, under darkness,

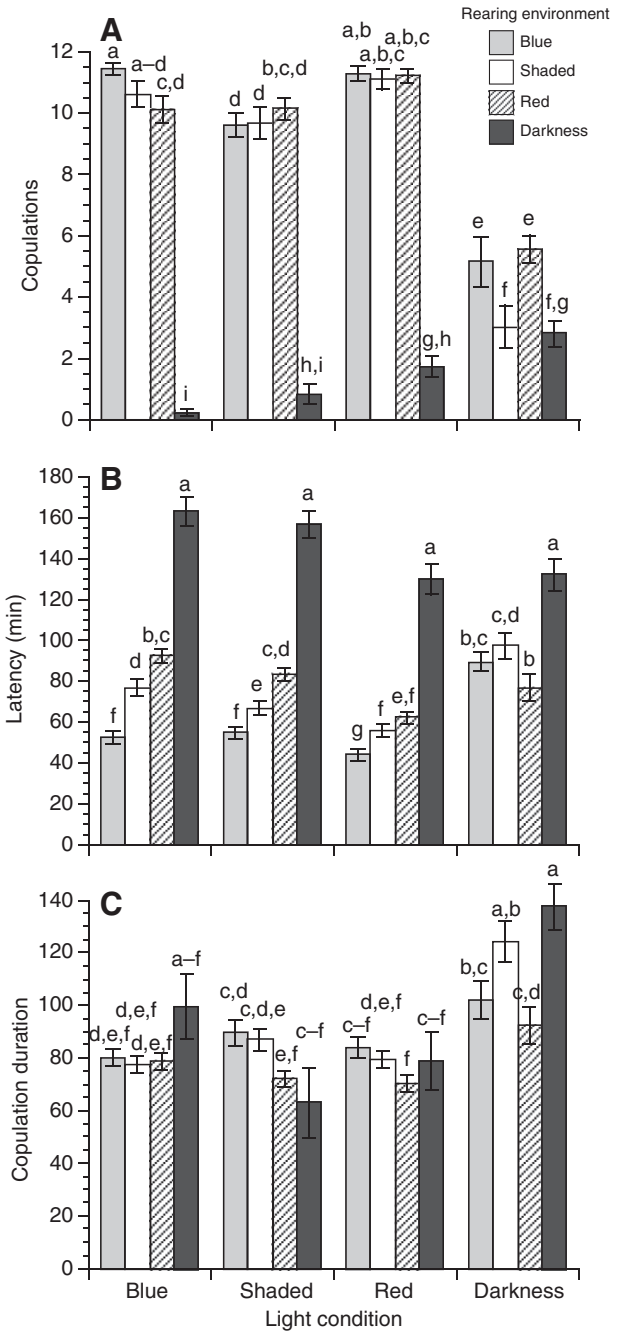

Fig. 1. No-choice experiment. Copulation behaviour in four light conditions of flies reared in different light environments. For a given test lighting, the two sexes had the same light rearing experience. (A) Number of copulations obtained, (B) latency to copulation and (C) copulation duration. Data are means ± s.e.m. Contrasts and least-square means *t*-tests were run among treatments according to the type of analysis (GLM or ANOVA). Bars with the same letters are not significantly different ($P>0.05$).

shade-reared flies obtained fewer copulations than red- or blue-reared flies.

Significant differences in latency to copulation were observed depending on light conditions during testing; latency to copulate was longer in darkness ($F_{3,1637}=21.5$, $P<0.0001$) (Fig. 1B). Also, the type of light flies experienced during early adulthood significantly affected the latency to copulation. In the case of flies exposed to light treatments, blue-reared flies started copulation faster than flies of reared under the red light treatment. Flies exposed to darkness exhibited the longest latency to mate ($F_{3,1637}=55.4$, $P<0.0001$). The interaction between testing and rearing light conditions was significant, indicating that light environment affected flies differently according to their light rearing experience. Thus, blue-reared flies copulated faster than other flies under red, blue and shaded light conditions but not flies in darkness ($F_{14,1637}=4.2$, $P<0.0001$).

Copulations lasted longer in darkness during testing ($F_{3,1628}=12.7$, $P<0.0001$; Fig. 1C). Also, longer copulations were observed in those flies that experienced darkness during rearing ($F_{3,1628}=3.5$, $P<0.014$). The interaction between light conditions during testing and rearing was not significant for copulation duration ($F_{9,1628}=1.5$, $P<0.125$).

Choice experiment

Significant differences in the number of copulations were recorded depending on light conditions. Fewer copulations were observed in darkness ($\chi^2=44.5$, d.f.=3, $P<0.0001$; Fig. 2A). Also, the number of copulations varied with light rearing experience. Males that were reared in darkness obtained fewer copulations than other males ($\chi^2=57.4$, d.f.=3, $P<0.0001$). The interaction between the test light conditions and light rearing experience was significant; blue-reared males copulated less than the other males under darkness, whereas darkness-reared flies copulated less in blue, red and shaded light arenas ($\chi^2=44.0$, d.f.=9, $P<0.0001$). Similar to males, fewer females copulated in darkness ($\chi^2=33.5$, d.f.=3, $P<0.0001$). Again, rearing conditions had a significant effect on the number of copulations females obtained, because females reared in darkness mated fewer times ($\chi^2=119.0$, d.f.=3, $P<0.0001$). However, differing from males, there was no significant interaction between light conditions during testing and rearing, indicating that female flies, independent of rearing experience, responded in a similar way under any of the four light environments ($\chi^2=8.5$, d.f.=9, $P=0.48$).

The light conditions during testing significantly affected latency to mate. Longer latencies were observed in darkness for both genders (males: $F_{3,290}=12.2$, $P<0.0001$; females: $F_{3,290}=4.0$, $P<0.008$; Fig. 2B). Also, light conditions during rearing significantly affected latency to mate. Again, insects reared in darkness exhibited longer latencies independently of sex (males: $F_{3,290}=15.7$, $P<0.0001$; females: $F_{3,290}=4.0$, $P<0.007$). However, the interaction was significant for males ($F_{9,290}=3.0$, $P<0.002$) but not for females ($F_{9,290}=1.0$, $P<0.41$). Red-reared males took less time to copulate under shaded light and darkness, whereas in the other two light environments no significant differences were observed among all flies.

Copulation duration was not significantly different among the four arenas for both genders (males: $F_{3,290}=1.5$, $P=0.21$; females: $F_{3,290}=0.5$, $P=0.661$) (Fig. 2C). Light conditions during rearing did not affect copulation duration for either sex (males: $F_{3,290}=2.1$, $P<0.09$; females: $F_{3,290}=0.99$, $P=0.4$). The interaction between light conditions during testing and rearing was not significant for either sex (males: $F_{9,290}=0.6$, $P=0.80$; females: $F_{9,290}=0.2$, $P=0.99$).

Field cage experiment

A reduction in both light intensity and wavelength was observed along the observation period (Fig. 3). A total of 246 copulations (out of a possible 800) were observed. As in the previous experiment under laboratory conditions, both females and males that were reared in darkness copulated fewer times than flies that were reared under any of the light spectra (females: $\chi^2=79.43$, d.f.=3, $P<0.0001$; males: $\chi^2=101.2$, d.f.=3, $P<0.0001$; Fig. 4A).

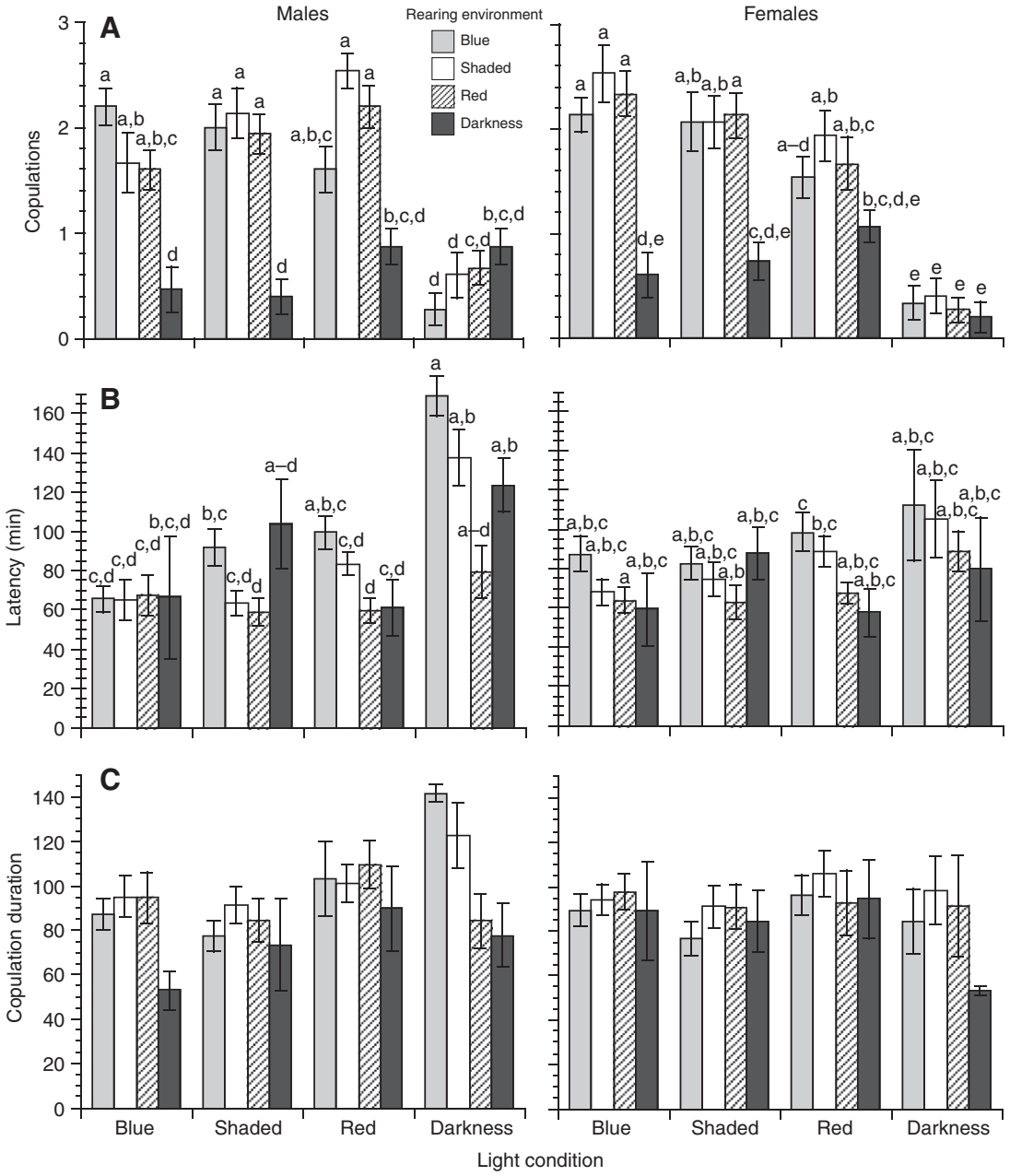

Fig. 2. Choice experiment. Copulation behaviour in four light conditions of flies reared in different light environments. Three pairs of flies from each of the four light rearing conditions were placed in a given test lighting. (A) Number of copulations obtained, (B) latency to copulation and (C) copulation duration. Data are means ± s.e.m. Contrasts and least-square means *t*-tests were run among treatments according to the type of analysis (GLM or ANOVA). Bars with the same letters are not significantly different (P>0.05).

Latency to copulation was not significantly affected by light conditions during rearing for either sex (males: $F_{3,221}$=1.3, P=0.28; females: $F_{3,221}$=1.3, P=0.27; Fig. 4B). Copulation duration was not significantly different among rearing treatments in either sex (males: $F_{3,218}$=0.07, P=0.97; females: $F_{3,218}$=0.5, P=0.71; Fig. 4C).

Values of *PSI* were not significantly higher or lower than one for any pair type (Table 2). However, *PSI* values were higher than one for homotypic blue–blue and red–red pairs, whereas they were lower than one for heterotypic blue–red and red–blue pairs. Values of I_{PSI} were slightly but significantly higher than zero only for flies reared in red and blue light, but not in the other light treatments, indicating that there was subtle sexual isolation between these fly types (Table 2).

The *PSS* estimates for (male–female) red–red, red–blue, red–shaded, shaded–blue, shaded–red and shaded–shaded were close to each other and significantly greater than one, whereas values for blue–blue, blue–red and blue–shaded were also close to each other but were not statistically greater than one (Table 2). These results indicate that, in general, red- and shaded-reared flies participated in more mating pairs (both homotypic and heterotypic; 160 and 176, respectively) than blue-reared flies (133 pairs). Conversely, *PSS* estimates for any combination with darkness-exposed flies were very close to each other and significantly lower than one. This indicates that females reared in darkness exhibited lower propensity to copulate than females of any of the other light treatments. For males of the same darkness treatment, this value indicates that they obtained fewer matings (Table 2). Females exposed to shaded light had more matings compared with those exposed to blue light and darkness, but not compared with those exposed to red light (blue light, W=0.6776, s.d.=0.1139, P=0.0081; darkness, W=0.1256, s.d.=0.0404, P<0.0001; red light, W=1.0107, s.d.=0.1529, P=0.5167). For males, only those exposed to darkness exhibited a significantly lower mating performance than those exposed to shaded light (blue light, W=0.86, s.d.=0.1377, P=0.1618; red light,

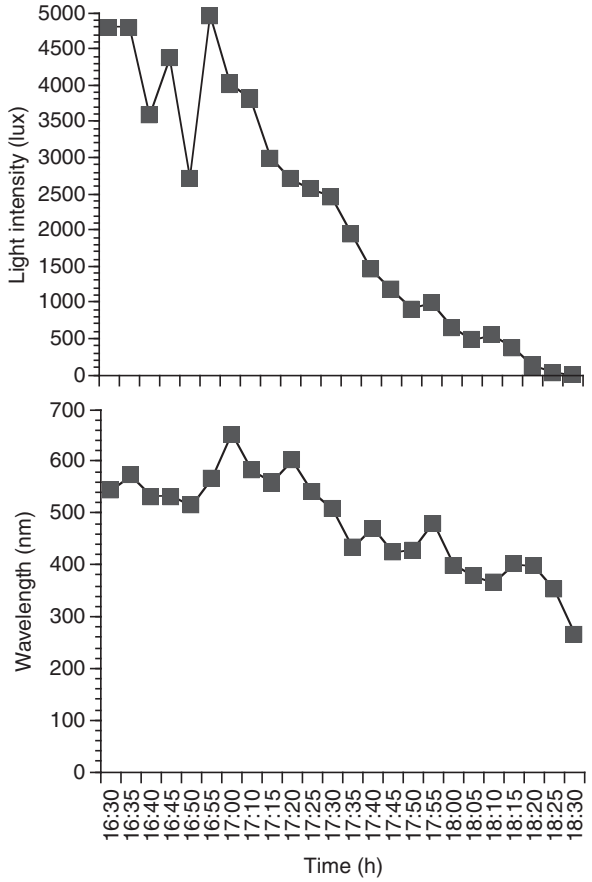

Fig. 3. Mean light intensity and light wavelength variation under field cage conditions.

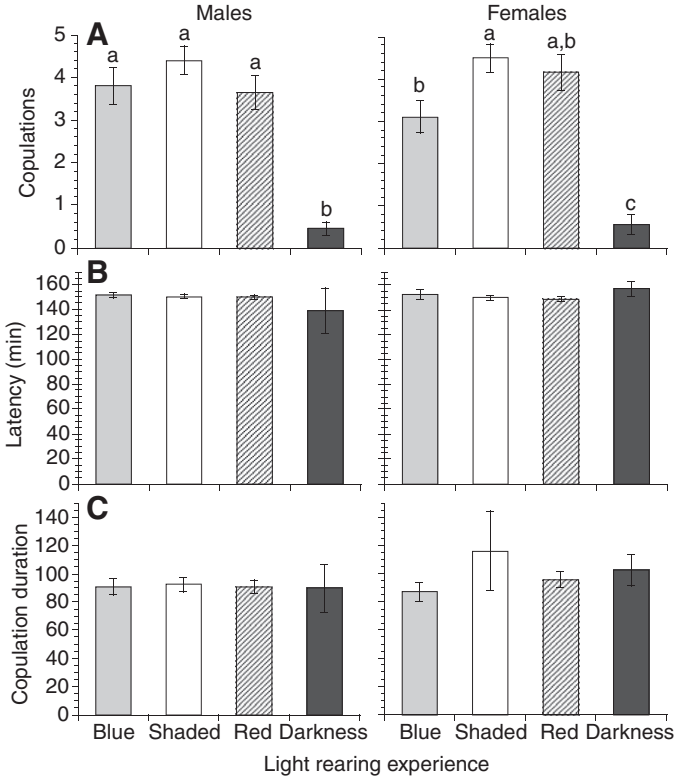

Fig. 4. Field cage experiment. Copulation behaviour in field cage conditions of flies reared under different light environments. Ten pairs from each light rearing treatment were released inside each cage. (A) Number of copulations obtained, (B) latency to copulation and (C) copulation duration. Data are means ± s.e.m. Contrasts and least-square means *t*-tests were run among treatments according to the type of analysis (GLM or ANOVA). Bars with the same letters are not significantly different ($P>0.05$).

W=0.8829, s.d.=0.1418, P=0.2019; darkness, W=0.1051, s.d.=0.0364, $P<0.0001$).

DISCUSSION

In this study, we provide empirical evidence that light conditions during rearing affect mating success of *A. ludens* flies. Flies that were reared in darkness exhibited the lowest mating success. Contrary to our hypothesis, there was not a relationship between the environmental conditions of the rearing experience and the mating success under those particular conditions. Under field cage conditions, males that were exposed to any of the three light environments mated more often than males exposed to darkness. In field cage conditions, a subtle assortative mating was detected between blue-light- and red-light-exposed flies. This pattern was the result of a low number of mating pairs of the blue-light-exposed females with the red-light-exposed males.

As observed in *D. melanogaster* (Hirsh et al., 1995), the experience of being held in darkness during early adult life affected the mating success of *A. ludens*. However, differing from *D. melanogaster*, no assortative mating was observed in this treatment (Barth et al., 1997a). This difference could be explained by differences in the mating behaviour of these two species. Although the mating activity of *D. melanogaster* is light independent, *A. ludens* seems to be a facultative-dark-mating species, as some individuals copulated under dark conditions (Grossfield, 1971). *Drosophila melanogaster* uses visual, auditory and chemosensory stimuli in its courtship, and the exclusion of any single sensory input does not block mating (Spieth and Ringo, 1983). In contrast, sexual activity and the beginning of mating activities, including the release of pheromones as long distance signals, is dependent on light intensity during dusk in *A. ludens*, and it occurs during a relatively inflexible schedule (Robacker et al., 1991; Meza-Hernández and Díaz-Fleischer, 2006). Thus, *D. melanogaster* individuals that were exposed to darkness may use other signals to elicit female response, whereas flies under illuminated conditions have the complete courtship repertoire and so light-reared females respond preferentially to them. In the case of *A. ludens*, darkness-exposed flies obtained fewer copulations and, in general, exhibited longer mating latencies. Highly dependent on visual stimulus, males and females reared in darkness resulted in less attractive males and less responsive females. For instance, it has been reported that *Strumeta* (currently *Bactrocera*) *tryoni* female flies exposed to darkness laid no eggs and exhibited a reduction in the amount of feeding (Barton-Browne, 1957). Because food quality has a dramatic impact on fly activity (Shelly and Kennelly, 2002), flies that reduce food intake could exhibit impaired sexual activity. However, circadian rhythms could be altered in flies reared in darkness. The ontogenetic development of the circadian system can be modified or imprinted by changing environmental conditions (Weinert, 2005). For example, it has been reported in *Drosophila* that dark rearing may produce a high frequency of locomotor arrhythmia and increase copulation latencies (Dowse and Ringo, 1989; Hirsh and Tomkins,

Table 2. Analysis of sexual isolation and sexual selection using the pair sexual isolation index (I_{PSI}), pair sexual selection (PSS) and pair sexual isolation (*PSI*) coefficients and bootstrapping statistical tests on matings among *Anastrepha ludens* flies exposed to four different light spectra

	Male			
Female	Blue	Shaded	Red	Darkness
Blue				
Count	21	23	11	4
PSI	1.2558±0.4277, *P*=0.527	1.0182±0.2814, *P*=0.9976	0.9388±0.2649, *P*=0.7664	1.1747±1.0936, *P*=0.9482
PSS	1.1529±0.2631, *P*=0.5956	1.7223±0.3128, ***P*=0.0164**	1.724±0.3191, ***P*=0.0172**	0.215±0.1172, ***P*=0.0001**
I_{PSI}			0.2456±0.1085, ***P*=0.03**	
Shaded				
Count	26	33	27	2
PSI	1.1594±0.354, *P*=0.658	1.0887±0.2642, *P*=0.7688	0.9286±0.2345, *P*=0.7246	1.0059±0.9686, *P*=0.882
PSS	1.3564±0.2831, *P*=0.2154	2.0288±0.338, ***P*=0.0016**	2.0227±0.3365, ***P*=0.0006**	0.2529±0.1274, ***P*=0.0001**
I_{PSI}	0.0379±0.1018, *P*=0.7126		0.0989±0.0922, *P*=0.2934	
Red				
Count	24	28	34	2
PSI	0.6381±0.2564, *P*=0.198	1.034±0.2782, *P*=0.9296	1.2888±0.3247, *P*=0.3182	1.5483±1.3715, *P*=0.684
PSS	1.1841±0.2695, *P*=0.5358	1.7602±0.3208, ***P*=0.0146**	1.7728±0.3191, ***P*=0.011**	0.2207±0.118, ***P*=0.0001**
Darkness				
Count	3	4	3	1
PSI	2.1874±1.7125, *P*=0.3318	0.8076±0.8341, *P*=0.7572	0.8094±0.8607, *P*=0.7602	0.9215±0.968, *P*=0.2902
PSS	0.1405±0.0957, ***P*=0.0001**	0.2114±0.1146, ***P*=0.0001**	0.2092±0.1169, ***P*=0.0001**	0.026±0.041, ***P*=0.0001**
I_{PSI}	0.06±0.391, *P*=0.8764	0.2683±0.4743, *P*=0.7292	0.2195±0.451, *P*=0.7388	

Count, number of pairs of each treatment and sex combination.
Data are means ± s.d. pooled over all replicates, calculated by resampling the observed values 10,000 times.
N=200 individuals for all treatments and sexes.

1994). These differences could represent disadvantages when competing for mates.

During the field cage test, we observed that the light environment that was experienced in early adulthood had a slight but significant effect on fly sexual selection. Males reared under any of the three light treatments were more preferred as mates or were more competitive than males reared under dark conditions. Interestingly, those females exposed to blue light copulated fewer times with red-light-reared males than with males reared in other light conditions. Apparently, the effect of divergent wavelengths provoked differences in the sensory system that reduced random mating in these two fly types. In polymorphic fishes, assortative mating take place according to light spectrum, some morphs are successful under specific light conditions (Gamble et al., 2003; Fuller et al., 2005). However, this is not the case in our study. The flies used in the present study have been reared in a laboratory environment for many years, a condition that has standardized many individual traits (Cayol, 2000). So, it could be inferred that light spectrum must have impacted male signalling displays, whereas in females the effect could have distorted visual signalling reception. In Diptera and Lepidoptera, there are differences in the shape and amount of visual neurons between males and females (Strausfeld, 1980; Arikawa et al., 2005). It has been suggested that vision development responds to different arrangement of stimuli. Thus, the effect of light rearing environment could reduce the perception of visual cues that help females locate males (Endler and Théry, 1996; Juusola and Hardie, 2001). It is possible that these females are not capable of detecting the UV reflectance of males (Sivinski et al., 2004) or, as observed in *D. melanogaster*, females have different visual capabilities in contrasting semi-natural light environments (Wolfram and Juusola, 2004).

Our findings suggest that light spectrum only weakly affects the direction of sexual selection by female choice; however, dark rearing environments deeply affect mating success. Male fruit flies reared in red, blue and shaded light environments had a strong mating advantage over males from dark environments. Flies reared in darkness exhibited the lowest mating performance. We suggest that the effect of light rearing environment could reduce the perception of visual cues that help females locate males. Additionally, an important question arises with respect to light condition effects on fly vision. In the case of *D. melanogaster*, changes in the development of the optic lobe of flies exposed to darkness at early age are not reversible (Barth et al., 1997b), whether this effect can be modified in *A. ludens* remains to be determined. As advice for those programs that use SIT as a control tool, our results also suggest that darkness conditions in the holding room where adults emerge could dramatically reduce mass-reared insect mating performance.

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