The Effect of Social Isolation on Locomotor Activity in the Houseflies (*Musca Domestica*)

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Abstract Social behavior is broadly defined as the interaction between members of the same species that changes their subsequent behavior. Isolation has been shown to affect behavioral traits such as courtship, mating aggression, foraging, learning and memory. This study investigated the effect of isolation (8 days) on adult *Musca domestica* locomotion. We found that isolation significantly affected male locomotor activity and that the effect of isolation was reduced over time giving a significant interaction of isolation and time. Females' locomotion was not affected by isolation. These results suggest differences in response to social deprivation between sexes and add to the understanding of immediate consequences of behavioral interactions between houseflies.

Keywords Isolation · sexual dimorphism · locomotor activity · social deprivation · fitness

Introduction

Social behavior can be defined broadly as the interaction between individuals of the same species that changes their subsequent behavior and can affect traits such as

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courtship, mating, aggression, parenting, foraging, learning, and memory as well as non-behavioral phenotypes such as development and mortality (Sokolowski 2010). Many effects of social isolation have been shown in studies of invertebrates. In *Drosophila* social isolation leads to reduced lifespan (Ruan and Wu 2008), increased aggression (Hoffmann 1987, 1990; Zhou et al. 2008), reduced need for sleep (Ganguly-Fitzgerald et al. 2006; Donlea et al. 2009) and a decrease in the fiber number of the mushroom bodies (Technau 2007). In spiderlings, maternal and sibling social isolation appears to hinder the spiderling's capability in exploring new environments, learning and hunting skills (Punzo and Alvarez 2002). These behavioral effects were associated with retarded development of the central nervous system (Punzo and Ludwig 2002), which has also been found in *Caenorhabditis elegans* (Rose et al. 2005) and *Apis mellifera* (Maleszka et al. 2009). Work on gregarious cockroaches has shown that isolation has a significant effect on their physiology, for instance by reducing developmental rates and egg production (Lihoreau and Rivault 2008; Lihoreau et al. 2012). In *Blattella germanica*, individuals kept in isolation show reduced willingness to interact with others, reduced ability to asses mating partner quality and reduced foraging activity collectively described as "isolation syndromes" (Lihoreau et al. 2009). It is suggested that all domiciliary species suffer from the effects of isolation which have significant impacts on their rate of maturity and reproductive capability (Lihoreau et al. 2012).

The housefly, *Musca domestica*, is associated with humans, particularly waste and animal husbandry (Malik et al. 2007) and can reach population increase rates of 1.25-2.82 per day (Imai 1984). Given the high densities that populations can reach, social interactions may have strong consequences on the expression of individual behavior. Male houseflies show preferences in females during courtship (Shin et al. 2003), depending on past experience of female reciprocation. They show learning within courtship behavior (Hagenbuch 2005) and differences are intricate enough that divergence in courtship behavior and premating isolation are observed in bottleneck lines (Meffert and Bryant 1991). Mating also influences female behavior considerably because of the quantitative effect of the male accessory seminal fluid transferred during copulation which stimulates oviposition and reduce females receptiveness to further mating. This is enforced by a nutritional effect which enhances female fitness (Riemann and Thorson 1969; Arnqvist and Andrés 2006). Thus, the housefly has evolved a complex courtship repertoire (Meffert and Hagenbuch 2005) which selection acts upon and makes it suitable for studies on communication, social interactions, and learning.

Here we explored the effect of isolation on locomotor activity of houseflies as locomotion is an integral part of many behaviors and high basal locomotor activity has been shown to be correlated with fitness (Patterson 1957; Murvosh et al. 1964; Partridge et al. 1987; Long and Rice 2007). We hypothesized that socially deprived houseflies show reduced locomotor activity as has been found for exploratory and foraging behavior in other species (Lihoreau et al. 2009). Females are known to be more sedentary than males (Patterson 1957; Murvosh et al. 1964) and have been shown to have lower basal levels of locomotor activity (Bahrndorff et al. 2012) so the observational effect of isolation may be less pronounced in females than males.

Material and Methods

Rearing

The houseflies used in the present study were from a Spanish field population collected in 2011 at Centre de Recerca en Sanitat Animal, Barcelona (41.30°N, 2.05°E) (Kjærsgaard et al. 2013). The population was kept in rearing cages (30×30×30 cm) (MegaView Science Co., Ltd., Taiwan) at a population size of approximately 1000 individuals for 10 generations and maintained at 25 °C before being used. Larvae were kept in larval medium consisting of wheat bran (24.6 %), alfalfa (12.3 %), yeast (0.6 %), malted sugar (0.9 %) and tap water (61.6 %) until 75 % were puparia. Puparia used in the experiment were separated from the medium into 250 ml glass flasks with foam stoppers, with one individual per vial to ensure that all flies used were virgins. Emerging adults were fed on 8 % sugar (sucrose) solution in 2 ml Eppendorf tubes sealed with cotton. Flies experienced a photoperiod of 16:8 h light:dark (L:D) and were kept in separate vials before being sexed and assorted into treatment groups.

Experimental Design

The effect of behavioral isolation of males and females were carried out in two independent experiments. In both experiments the virgin flies were sorted into two groups 24 h after eclosion – 16 vials with either one male or female fly (socially isolated), and another 16 vials with either two male or two female flies together (socially maintained). As mating could have a confounding effect on locomotor activity there were no mixed sex treatments. During the treatment all the flies were fed ad libitum to an 8 % sugar solution and were kept at 25 °C and a photoperiod of 16:8 h (L:D). The treatment lasted for 8 days.

After terminating the isolation treatment locomotor activity, comprising primarily walking but also jumping and brief flight in the vial, was measured in a locomotor activity monitor (LAM, TriKinetics). The activity was estimated as the number of times in a 5 s interval that a photocell in the middle of the vial was crossed, thereby monitoring the frequency with which a fly crossed the sensors in the vial (see Bahrndorff et al. 2012 for details). One fly was placed in each vial – 16 vials with a male or female from the isolation treatment and 16 vials with a single male or female of the socialized treatment. The flies were anaesthetized using brief carbon dioxide exposure twice during the experiment – for dividing flies into vials for social mainte-nance treatments and for placing flies in vials prior to the experiment in the LAM. Tests on *Drosophila melanogaster* have shown that there is little effect of CO_2 anesthesia on locomotor activity specifically (Van Dijken et al. 1977) and on performance in general (MacAlpine et al. 2011).

Vials were placed horizontally into the LAM by random assignment to one of the 32 slots. Food in the form of the sugar solution described above was placed at both ends of the vial in lidless 1.5 mL Eppendorf tubes placed inside foam stoppers. A piece of cotton prevented the solution from spilling and provided a moist substrate where flies could feed. Flies spent 3 days in the machine. The first 24 h of recording were not included in the analysis due to the potential effect of handling the flies. Two full days and nights of recordings were collected and used for analysis. The light program

followed a photoperiod of 16:8 h (L:D) (16 h of light from 07.00 a.m. to 11.00 p.m. and 8 h of complete darkness (0 lux) from 11.00 p.m. to 07.00 a.m.).

Data Analysis

The activity registered in each vial was divided into activity recorded during two light and dark periods. Prior to analysis the activity was summed into 1 h bins. R statistical software version 2.14.1 (R Development Core Team 2011) was used for all analyses. Data were tested for normality (Shapiro-Wilk's test) and equal variances (variance ratio F tests). Day time activity data were normally distributed with equal variances so differences were tested with generalized linear mixed models (lmer, lme4 library). Wald χ^2 tests (type II) were applied on the restricted maximum likelihood (REML) estimates and errors testing for differences in locomotor activity as an effect of treatment (isolated or socially maintained), time of day (hourly intervals) and day (day 1 or day 2). REML is an alternative to maximum likelihood offering less biased standard deviations by averaging the random effect parameters over the fixed effect parameters (Bolker et al. 2009). Treatment, time of day and day were entered as fixed effects including their first order interactions and vial nested in time of day as a random effect to account for the repeated hourly activity measurements (Everitt 2010). Sexes were tested in separate models because they were not tested simultaneously. Since visual inspection of the data indicated that a difference between treatments in the males would be confined to day 1, we also ran a model testing only day 1. Night time activity was low and did not meet assumptions for a parametric test so we summed the total activity and $\log_{10}$ transformed the data before analysis. Because of the nature of the data with usually only high activity in the first hour interval of the night period this approach seems appropriate in determining if there are differences in activity between treatments. Similar to the daytime models we included treatment (isolated or socially maintained) and night (1 or 2) as fixed effects and vial as a random effect to account for the repeated measure on consecutive nights. Graphs depicting the locomotor activity over time were produced by smoothing the raw data for graphical presentation, taking the average activity of all replicates across hourly intervals.

Results

The daytime activity of socially maintained male flies was significantly higher than that of isolated male flies only on day 1 when analyzed separately (isolated vs. socially housed males, day 1: Wald $\chi^2_1=2.88$, $P=0.045$, one-tailed test; Fig. 1, Table 1). The difference declined on the second day giving a significant effect of day (Wald $\chi^2_1=41.42$, $P<0.001$) and treatment by day interaction (Wald $\chi^2_1=14.62$, $P<0.01$; Table 1). Daytime activity of the female flies showed a different pattern. The effect of isolation did not significantly reduce activity compared to socially maintained females (Wald $\chi^2_1=0.28$, $P=0.59$) (Fig. 1). There was a significant effect of day (Wald $\chi^2_1=122.36$, $P<0.001$) and time of day (Wald $\chi^2_1=6.31$, $P=0.012$) but no significant interactions (Table 1). Night time activity did not differ between treatments for both sexes (Table 1) although there was a significant effect of night on activity of the females which were less active on the second night (Wald $\chi^2_1=3.42$, $P=0.007$; Table 1). This was mainly
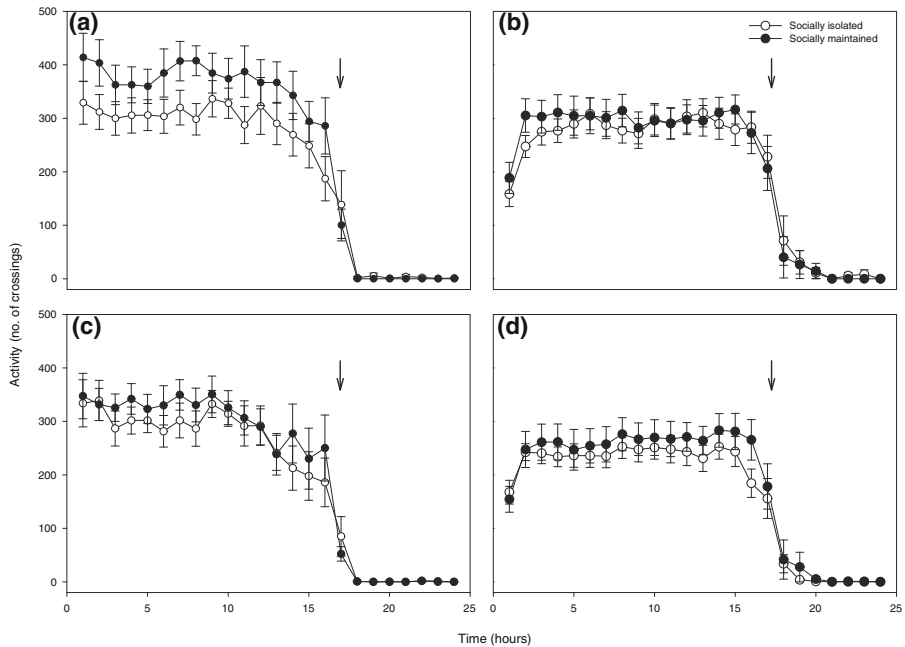
Fig. 1 The influence of isolation on locomotor activity in *Musca domestica*. Hourly locomotor activity (mean ±SE) during daytime and nighttime of (**a**) male flies (day1), (**b**) female flies (day 1), (**c**) male flies (day2) and (**d**) female flies (day 2), previously isolated or socially maintained. N=16 replicates per treatment. Arrow indicates change from light to dark

due to lower activity of the isolation treatment females giving a marginally non-significant interaction (Wald χ^2_1=7.32, P=0.06).

Discussion

Male houseflies that were kept isolated for 8 days without the stimulation of other conspecifics showed reduced locomotor activity throughout daytime on day 1, but not at night time (Fig. 1a). The effect of isolation was most apparent in the first day where the isolated flies were less active than the socially maintained flies. With time, activity of the control decreased towards the level of the isolation treatment males (Fig. 1c). Females on the other hand did not show any effect of isolation on locomotor activity on day 1 or 2 (Fig. 1b and d) and were noticeably less active in the first 2 h of the first day. Similar results have been obtained on mice, where males were more easily affected by isolation (Guo et al. 2004). In *Drosophila* studies have shown gender-selective patterns of aggressive behaviour (Nilsen et al. 2004) and that isolation can affect aggression (Ueda and Kidokoro 2002).

Male flies of *M. domestica* differ substantially from female flies with respect to behavior and show both higher activity and different circadian rhythm patterns (Buchan and Sohal 1981; Bahrndorff et al. 2012). This sexual dimorphism in behavioral activity patterns could explain why we only observed an effect of isolation on male flies. Male houseflies optimize fitness by fertilizing as many eggs as possible (Hosken et al. 2009),

Table 1 Wald χ^2 tests (type II) on the REML estimates testing for differences in locomotor activity as an effect of treatment (isolated or socially maintained) and day (day 1 or day 2)

Model	Effect	χ^2	D.F.	$P > \chi^2$
Males light	Treatment	1.60 (2.88)	1	0.103 (**0.045***)
	Day	41.42	1	**<0.001*****
	Time of day	11.79 (7.76)	1	< **0.001***** (**0.003****)
	Treatment×Day	14.62	1	< **0.001*****
	Treatment×Time of day	0.008 (0.01)	1	0.464 (0.459)
	Day × Time of day	3.23	1	0.072
Females light	Treatment	0.28	1	0.59
	Day	122.36	1	< **0.001*****
	Time of day	6.31	1	**0.012***
	Treatment × Day	1.39	1	0.239
	Treatment × Time of day	0.03	1	0.860
	Day × Time of day	0.58	1	0.445
Males dark	Treatment	0.01	1	0.917
	Night	1.66	1	0.198
	Treatment × Night	0.76	1	0.384
Females dark	Treatment	0.009	1	0.924
	Night	7.32	1	**0.007****
	Treatment × Night	3.42	1	0.064

See text for difference in model structure between light and dark regimes. D.F.: degrees of freedom. $P > \chi^2$: probability of obtaining a larger χ^2 statistic then by random sampling of the data. Numbers in parentheses give the significance for day 1 separately

*$P < 0.05$

**$P < 0.01$

***$P < 0.001$

and in the presence of another male will still attempt mating strikes (Murvosh et al. 1964). Thus, other than light and food, mating can be considered a main motivator for male activity and stimuli such as conspecifics that might trigger mating strikes would consequently result in higher activity. Females are monogamous mating usually only once (Riemann et al. 1967) and have not been observed initiating mating (Murvosh et al. 1964). High activity may attract unwanted male attention which could explain the lack of effect of isolation on female locomotor activity. Females also invest much energy in egg production, which could result in a trade-off with activity levels to save energy. However, in this experiment the flies had unlimited access to food so the observed sex differences in activity probably do not reflect physiological needs or requirements, which is in agreement with earlier studies (Schou et al. 2013). Behavioral differences may instead be genetically determined. The genes of the neurons controlling sexually dimorphic behaviour have recently been mapped and it was shown that males expressing a dominant feminizing transgene in a small cluster of neurons in the *pars intercerebralis* had a female-like pattern of locomotor activity (Gatti et al. 2000).

At night time there was no difference between isolated or socially maintained flies of either sex. In *Drosophila* isolation leads to reduced need for sleep (Ganguly-Fitzgerald et al. 2006; Donlea et al. 2009). However, in houseflies isolation does not seem to affect night time activity, as the activity levels of the treatments largely overlap for both sexes. Male houseflies appear to anticipate the darkness as there is a clear decline in activity in the hours leading up to the night interval and a further rapid drop in activity the first 2 h of darkness (Fig. 1a and c). These changes are not as distinct in females (Fig. 1b and d).

It is not clear whether the behavioral differences seen between treatments in males in this experiment were caused by early ontogenetic neurodevelopment caused by lack of social stimuli or if it is due to behavioral plasticity. The lack of responsiveness or reactivity of the isolated male flies to the test environment (i.e., lower activity on day 1 compared to the controls) suggests that it could be irreversibly fixed developmentally. On the other hand, the decline of the social male activity towards the lower activity of the isolated males on the second day, suggests that it could be reversible behavioral plasticity showing a beginning effect of isolation or acclimation to the test settings of the previously socially maintained flies. In order to disentangle these possibilities a second test could be conducted on the same flies reversing the treatments although it would require marking the flies of the social treatment that had initially been maintained in isolation. In *Apis mellifera*, the mushroom body sizes of adult bees has been shown to be highly plastic in response to social experience (Maleszka et al. 2009) suggesting that effects of isolation can be countered by subsequent socialization.

Locomotor activity is widely used for studying behavior in invertebrates (Zordan et al. 2007; Schou et al. 2013). In such studies the importance of social stimuli should be taken into consideration. As we have shown here, individuals kept in isolation may not reflect natural conditions, which could be problematic depending on the context of the experiment. The observed effect of isolation on housefly behavior indicates that this organism could be a good candidate for further studies investigating the effects of isolation.

References

Arnqvist G, Andrés JA (2006) The effects of experimentally induced polyandry on female reproduction in a monandrous mating system. Ethology 112:748–756

Bahrndorff S, Kjaersgaard A, Pertoldi C, Loeschcke V, Schou T, Skovgard H, Hald B (2012) The effects of sex-ratio and density on locomotor activity in the housefly (*Musca domestica*). J Insect Sci 12:71

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135

Buchan PB, Sohal RS (1981) Effect of temperature and different sex ratios on physical activity and life span in the adult housefly, *Musca domestica*. Exp Gerontol 16:223–228

Development Core Team R (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Donlea JM, Ramanan N, Shaw PJ (2009) Use-dependent plasticity in clock neurons regulates sleep need in *Drosophila*. Science 324:105–108

Everitt BS (2010) A handbook of statistical analyses using R. Chapman & Hall/CRC, Boca Raton

Ganguly-Fitzgerald I, Donlea J, Shaw PJ (2006) Waking experience affects sleep need in *Drosophila*. Science 313:1775–1781

Gatti S, Ferveur JF, Martin JR (2000) Genetic identification of neurons controlling a sexually dimorphic behaviour. Curr Biol 10:667–670

Guo M, Wu CF, Liu W, Yang JY, Chen D (2004) Sex difference in psychological behavior changes induced by long-term social isolation in mice. Prog Neuro Psychoph 28:115–121

Hagenbuch KL (2005) Experience-dependent courtship modification in the housefly, *Musca domestica*. Dissertation, Rice University

Hoffmann AA (1987) A laboratory study of male territoriality in the sibling species *Drosophila-melanogaster* and *Drosophila simulans*. Anim Behav 35:807–818

Hoffmann AA (1990) The influence of age and experience with conspecifics on territorial behavior in *Drosophila melanogaster*. J Insect Behav 3:1–12

Hosken DJ, Stockley P, Tregenza T, Wedell N (2009) Monogamy and the battle of the sexes. Annu Rev Entomol 54:361–378

Imai C (1984) Population dynamics of houseflies, *Musca domestica*, on experimentally accumulated refuse. Res Popul Ecol 26:353–362

Kjærsgaard A, Blanckenhorn WU, Pertoldi C, Loeschcke V, Bahrndorff S (2013) Plasticity in behavioural responses and resistance to temperature stress in *Musca domestica*. 99:123–131

Lihoreau M, Rivault C (2008) Tactile stimuli trigger group effect in cockroach aggregations. Anim Behav 75:1965–1972

Lihoreau M, Brepson L, Rivault C (2009) The weight of the clan: even in insects, social isolation can induce a behavioural syndrome. Behav Process 82:81–84

Lihoreau M, Costa JT, Rivault C (2012) The social biology of domiciliary cockroaches: colony structure, kin recognition and collective decisions. Insect Soc 59:445–452

Long TA, Rice WR (2007) Adult locomotory activity mediates intralocus sexual conflict in a laboratory-adapted population of *Drosophila melanogaster*. Proc R Soc B 274:3105–3112

MacAlpine JLP, Marshall KE, Sinclair BJ (2011) The effects of CO2 and chronic cold exposure on fecundity of female *Drosophila melanogaster*. J Insect Physiol 57:35–37

Maleszka J, Barron AB, Helliwell PG, Maleszka R (2009) Effect of age, behaviour and social environment on honey bee brain plasticity. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 195:733–740

Malik A, Singh N, Satya S (2007) Housefly (*Musca domestica*): A review of control strategies for a challenging pest. J Environ Sci Health, Part B 42:453–469

Meffert LM, Bryant EH (1991) Mating propensity and courtship behavior in serially bottlenecked lines of the housefly. Evolution 45:293–306

Meffert LM, Hagenbuch KL (2005) The genetic architecture of housefly mating behavior. Curr Top Dev Biol 66:189–213

Murvosh CM, Fye RL, Labrecque GC (1964) Studies on the mating behavior of the housefly, *Musca domestica* L. Ohio J Sci 64:264–271

Nilsen SP, Chan YB, Huber R, Kravitz EA (2004) Gender-selective patterns of aggressive behavior in *Drosophila melanogaster*. Proc Natl Acad Sci U S A 101:12342–12347

Partridge L, Ewing A, Chandler A (1987) Male size and mating success in *Drosophila melanogaster* - the roles of male and female behavior. Anim Behav 35:555–562

Patterson RS (1957) On the causes of broken wings of the housefly. J Econ Entomol 50:104–105

Punzo F, Alvarez J (2002) Effects of early contact with maternal parent on locomotor activity and exploratory behavior in spiderlings of *Hogna carolinensis* (Araneae : Lycosidae). J Insect Behav 15:455–465

Punzo F, Ludwig L (2002) Contact with maternal parent and siblings affects hunting behavior, learning, and central nervous system development in spiderlings of *Hogna carolinensis* (Araeneae: Lycosidae). Anim Cogn 5:63–70

Riemann JG, Thorson BJ (1969) Effect of male accessory material on oviposition and mating by female houseflies. Ann Entomol Soc Am 62:828–834

Riemann JG, Moen DJ, Thorson BJ (1967) Female monogamy and its control in houseflies. J Insect Physiol 13:407–418

Rose JK, Sangha S, Rai S, Norman KR, Rankin CH (2005) Decreased sensory stimulation reduces behavioral responding, retards development, and alters neuronal connectivity in *Caenorhabditis elegans*. J Neurosci 25:7159–7168

Ruan HY, Wu CF (2008) Social interaction-mediated lifespan extension of *Drosophila* Cu/Zn superoxide dismutase mutants. Proc Natl Acad Sci U S A 105:7506–7510

Schou T, Faurby S, Kjaersgaard A, Pertoldi C, Loeschcke V, Hald B, Bahrndorff S (2013) Temperature and population density effects on locomotor activity of the housefly, *Musca domestica*. Environ Entomol 42:1322–1328

Shin JG, Seo MJ, Shin HJ, Youn YN (2003) Mating preferences and theoretical discussion on courtship in the male housefly, *Musca domestica*. J Asia Pac Entomol 6:21–27

Sokolowski MB (2010) Social interactions in "simple" model systems. Neuron 65:780–794

Technau GM (2007) Fiber number in the mushroom bodies of adult *Drosophila melanogaster* depends on age, sex and experience. J Neurogenet 21:183–196

Ueda A, Kidokoro Y (2002) Aggressive behaviours of female *Drosophila melanogaster* are influenced by their social experience and food resources. Physiol Entomol 27:21–28

van Dijken FR, van Sambeck MJPW, Scharloo W (1977) Influence of anaesthesia by carbon dioxide and ether on locomotor activity in *Drosophila melanogaster*. Experientia 33:1360–1361

Zhou C, Rao Y, Rao Y (2008) A subset of octopaminergic neurons are important for *Drosophila* aggression. Nat Neurosci 11:1059–1067

Zordan MA, Benna C, Mazzotta G (2007) Monitoring and analyzing *Drosophila* circadian locomotor activity. Methods Mol Biol 362:67–81