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Animal behaviour

Sexual harassment induces a temporary fitness cost but does not constrain the acquisition of environmental information in fruit flies

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Across animals, sexual harassment induces fitness costs for females and males. However, little is known about the cognitive costs involved, i.e. whether it constrains learning processes, which could ultimately affect an individual's fitness. Here we evaluate the acquisition of environmental information in groups of fruit flies challenged with various levels of male sexual harassment. We show that, although high sexual harassment induces a temporary fitness cost for females, all fly groups of both sexes exhibit similar levels of learning. This suggests that, in fruit flies, the fitness benefits of acquiring environmental information are not affected by the fitness costs of sexual harassment, and that selection may favour cognition even in unfavourable social contexts. Our study provides novel insights into the relationship between sexual conflicts and cognition and the evolution of female counterstrategies against male sexual harassment.

1. Introduction

In sexually reproducing animals, the different reproductive interests of males and females may result in sexual conflicts [1]. In species where male reproductive investment is low, maximizing the number of sexual partners is often advantageous; conversely, when reproductive investment is high, females are usually selected for mating just once or a few times. Owing to this difference, in many species, males attempt to coerce females into mating more frequently than they would, i.e. they sexually harass them [2]. This results in costs for females, which include physical damage, increased exposure to predators, reduction of feeding time, excessive energetic investment in male avoidance [2], and limited access to the most favourable resources [3,4]. Although sexual harassment has received much attention from behavioural ecologists, little is known about its effects on cognitive tasks such as the acquisition of environmental information. According to the 'neural constraints' hypothesis, limitations exist on the amount of information animals can process, as well as the number and accuracy of decisions that can be made in a given period of time [5,6]. For example, sexually harassed females invest a considerable amount of time avoiding coercive copulations, and therefore may focus more on avoiding males rather than learning about environmental cues. This might prevent individuals from being adequately aware of the surrounding environment, which could lead to disadvantageous decisions and ultimately reduce fitness. Similarly, males that focus exclusively on copulation attempts might pay less attention to the environment and incur an analogous fitness cost. In the fruit fly, *Drosophila melanogaster*, males and females are in conflict over reproduction. Female fitness depends strongly on oviposition site choice [7], which requires the ability to readily acquire environmental information, whereas males benefit from maximizing their sexual partners, which results in

sexual harassment. In order to study the impact of sexual harassment on cognition, we created groups which experienced different intensities of male sexual harassment and placed them in artificial environments with two food sources, one of which contained a toxic substance. Afterwards, we tested the food preferences of males and females with the aim of understanding whether the intensity of sexual harassment constrained the acquisition of environmental cues.

2. Material and methods

(a) Experimental protocol

We based our procedure on a two-phase protocol testing how flies associate odours with aversive gustatory stimuli [8]. In the first phase, we placed groups of 30 flies in transparent boxes containing two Petri dishes with different flavoured (banana or strawberry) agar media, one of which contained a gustatory aversive cue, quinine (electronic supplementary material, figure S1). In the second phase, six male or female flies were transferred and kept overnight in boxes where they were exposed to a choice between two traps, one scented with banana and the other with strawberry (electronic supplementary material, figure S1). The proportion of flies found in the traps was used to quantify food preference. The details of our protocol can be found in the electronic supplementary material.

(b) Experiments

To detect any cognitive differences between sexes or with respect to the reproductive status of females (virgin or mated), we tested males, virgin females and mated females in single-sex groups of 30 individuals. Then, to investigate the actual effects of sexual harassment, we tested two treatment groups with different sex ratios, i.e. a high-harassment treatment including 24 males and six virgin females and a low-harassment treatment including six males and 24 virgin females. Because *D. melanogaster* males learn not to attempt copulation with already mated females under certain conditions [9], we suspected that males bred in mixed-sex environments (sexually experienced) would exhibit lower motivation to copulate, ultimately decreasing sexual harassment. In order to control for this effect, we replicated the experiment by using sexually naive (virtually virgin) males collected shortly after eclosion.

The concentration of quinine and the length of the conditioning phase may have also affected the learning performance of flies, because acquiring environmental information is in principle easier when informative stimuli are conspicuous and the exposure to stimuli is long. Consequently, to take into account the impact of these factors we repeated the experiment three times: we first conditioned flies for 4 h with agar medium containing 3 g l⁻¹ quinine, then for 4 h with 0.3 g l⁻¹ quinine medium and finally for 1 h with 0.3 g l⁻¹ quinine medium.

In order to quantify the actual occurrence of sexual harassment in our experimental settings, and its temporary fitness costs, we conducted two different tests, which reproduced the 4 h conditioning phase of our learning assay. In the first experiment, we visually scanned experimental boxes containing high- or low-harassment fly groups at 10-min intervals, noting the occurrence of sexual harassment and counting the female flies observed on the medium lacking quinine. In this assay, sexual harassment was defined as every time male courtship behaviour resulted in females rejecting copulation or fleeing. In the second experiment, we counted the number of eggs non-virgin female flies laid in high- and low-harassment conditions and divided it by the number of females in each box. We used this measure as a proxy for fitness during the time frame of the assay.

Table 1. The statistics used throughout the study.

comparison	statistic	R function	response variable	fixed factors	random factors	error	link
test the food preference of each fly group against chance	χ^2 -test for proportions	prop.test	fly proportion in each trap	—	—	—	—
compare the learning performance of fly groups	GLMM	glmer	fly proportion in each trap	group	day	binomial	logit
compare the occurrence of sexual harassment	GLMM	glmer	proportion of boxes in which sexual harassment was observed	treatment	progressive scan number	binomial	logit
compare the proportion of female flies observed on the correct medium	GLMM	glmer	proportion of female flies on the correct medium	treatment	progressive scan number, experimental box	binomial	logit
compare the number of eggs at different intensities of sexual harassment	GLM	glm	number of eggs	treatment	—	quasi-Poisson	log

Table 2. Learning performance of fly groups.

group	sexual harassment	quinine concentration (g l ⁻¹)	male sexual experience	conditioning phase duration (h)	n	preference for correct food (mean ± s.d.)	d.f.	χ ²	p-value
30 virgin females	—	3	—	4	100	0.59 ± 0.34	99	282.48	<0.001
30 mated females	—	3	—	4	100	0.65 ± 0.29	99	230.22	<0.001
30 males	—	3	experienced	4	100	0.64 ± 0.29	99	229.76	<0.001
six females	high	3	experienced	4	80	0.64 ± 0.32	79	250.76	<0.001
24 males	high	3	experienced	4	78	0.59 ± 0.30	77	183.20	<0.001
24 females	low	3	experienced	4	78	0.59 ± 0.36	77	204.47	<0.001
six males	low	3	experienced	4	78	0.63 ± 0.29	77	165.66	<0.001
six females	high	3	naive	4	60	0.6 ± 0.31	59	180.85	<0.001
24 males	high	3	naive	4	57	0.62 ± 0.3	56	128.93	<0.001
24 females	low	3	naive	4	60	0.66 ± 0.33	59	143.03	<0.001
six males	low	3	naive	4	59	0.61 ± 0.31	58	146.63	<0.001
six females	high	0.3	naive	4	60	0.61 ± 0.33	59	164.11	<0.001
24 males	high	0.3	naive	4	59	0.56 ± 0.34	58	171.57	<0.001
24 females	low	0.3	naive	4	59	0.56 ± 0.38	58	207.62	<0.001
six males	low	0.3	naive	4	60	0.55 ± 0.38	59	211.91	<0.001
six females	high	0.3	naive	1	98	0.54 ± 0.36	97	312.26	<0.001
24 males	high	0.3	naive	1	100	0.56 ± 0.33	99	265.29	<0.001
24 females	low	0.3	naive	1	99	0.51 ± 0.38	98	341.90	<0.001
six males	low	0.3	naive	1	100	0.51 ± 0.36	99	321.35	<0.001

Figure 1. Comparisons of performance (preference for the correct food) among fly groups. (a) Single-sex groups. (b) Female groups which spent the conditioning phase with experienced males. (c) Female groups which spent the conditioning phase with naive males. (d) Experienced male groups. (e) Naive male groups. (f) Female and male groups at various quinine concentration and duration of conditioning phases; for the treatment at 3 g l⁻¹ quinine during 4 h, we pooled the groups including experienced and naive males. Bars represent mean $\pm$ s.e.m.; the dotted bar represents the absence of response to environmental cues; n.s. = non-significant differences; *** $p < 0.001$.

(c) Statistics

Data were analysed in the software R [10], in which we implemented generalized linear mixed models (GLMMs) by using the package lme4 [11]; within GLMMs, if the residual deviance exceeded the residual degrees of freedom, we corrected for overdispersion by setting observations (each experimental replicate) as an additional random factor. Details on the statistics we used are given in table 1.

3. Results

With 3 g l⁻¹ quinine agar medium and 4 h of conditioning, groups of 30 males, virgin females or mated females exhibited a significant preference for the flavour that did not contain quinine during the first phase (table 2 and figure 1a). No difference was observed between the three groups ($n = 100$ for each

Figure 2. (a) Proportion of boxes in which we observed coercive copulation attempts during each scan. (b) Proportion of females observed on the medium lacking quinine during each scan. (c) Number of eggs laid in different harassment conditions. Bars represent mean $\pm$ s.e.m.; *** $p < 0.001$.

treatment group, $\chi^2 = 0.46$, d.f. = 2, $p = 0.292$; figure 1a), suggesting that sex and females' reproductive status did not influence the response to the environmental conditions experienced in the first phase. We found no significant difference between groups of females who interacted with males at male:female ratios of 24:6, 6:24 and 0:30 (with experienced males: $78 \leq n \leq 100$ for each treatment group, $\chi^2 = 0.37$, d.f. = 2, $p = 0.50$, figure 1b; with naive males: $60 \leq n \leq 100$ for each treatment group, $\chi^2 = 0.56$, d.f. = 2, $p = 0.45$, figure 1c). All female groups significantly preferred the flavour that did not contain quinine during the first phase (table 2 and figure 1b,c). Similarly, groups of experienced or naive males showed no significant difference in performance (experienced males: $78 \leq n \leq 100$ for each treatment group, $\chi^2 = 0.64$, d.f. = 2, $p = 0.43$, figure 1d; naive males: $57 \leq n \leq 100$ for each treatment group, $\chi^2 = 0.18$, d.f. = 2, $p = 0.55$, figure 1e), with all groups preferring the flavour not containing quinine (table 2 and figure 1d,e).

Decreasing the quinine concentration by a factor of 10 during conditioning and shortening the conditioning resulted in overall lower learning performance, but all fly groups learned to avoid the quinine-associated scent (table 2 and figure 1f). Once again, we observed no significant differences among the group performances (0.3 g l^{-1} quinine and 4 h conditioning: $59 \leq n \leq 60$ for each treatment group, $\chi^2 = 0.76$, d.f. = 3, $p = 0.85$; 0.3 g l^{-1} quinine and 1 h conditioning $98 \leq n \leq 100$ for each treatment group, $\chi^2 = 0.37$, d.f. = 3, $p = 0.71$; figure 1f).

Sexual harassment was observed significantly more often in the high-harassment treatment than in the low-harassment treatment ($n = 20$ for each treatment, $\chi^2 = 0.32$, d.f. = 1, $p < 0.001$; figure 2a). Throughout the experiment, we observed proportionally fewer female flies on the medium lacking quinine in the high-harassment treatment compared with the low-harassment treatment ($n = 10$ for each treatment, $\chi^2 = 8.37$, d.f. = 1, $p < 0.001$; figure 2b). Finally, high-harassment fly groups laid proportionally fewer eggs (on average five times less, with the number of eggs corrected for the number of flies) than low-harassment groups ($n = 20$ for each treatment, $\chi^2 = 6.38$, d.f. = 1, $p < 0.001$; figure 2c).

4. Discussion

In our study, we investigated whether sexual harassment constrains the acquisition of environmental information in *D. melanogaster*. We first found that coercive copulation attempts were more frequent in boxes including males and females at 24:6 ratio than in boxes with a 6:24 male:female ratio. Then, we showed that, during the conditioning phase, female flies in high-harassment conditions avoided the oviposition media more than females in low-harassment conditions; this indicates that the presence of many males can prevent females from approaching favourable food sources and egg-laying sites. We finally showed that females in high-harassment conditions laid proportionally fewer eggs than females in low-harassment conditions. This suggests that male sexual harassment limits the female egg-laying rate by preventing females from approaching favourable egg-laying sites, therefore imposing a direct fitness cost, at least during the time frame of the assay. However, most interestingly, whereas male sexual harassment produced a significant fitness decrease for female flies, it did not affect their learning performance. In fact, all fly groups avoided the odours previously paired with the aversive stimuli, regardless of sexual harassment intensity, sex, sexual experience, reproductive status, intensity of the aversive conditioning stimulus and duration of the conditioning phase.

In our experiments, there was no effect of the intensity of sexual harassment on females' acquisition of environmental information; learning quickly and efficiently about the features of feeding substrates probably leads to significant fitness benefits for both female and male flies. Fruit flies mate and lay eggs on patchily distributed food sources, and choosing high-quality patches results in producing high-quality offspring [12]. However, these patches may host many flies, which could also increase the risk of being sexually harassed. In nature, a high frequency of sexual coercion in *D. melanogaster* could lead females to maximize the efficiency of their learning processes, minimize the disturbance from males and make advantageous reproductive decisions. Similarly, we found that sexual harassment did not affect the learning performance of males, perhaps because they had time to experience both fruit-flavoured media, either

between matings at high female-biased sex ratios or when they could not approach females owing to competition at high male-biased sex ratios.

In our study, we expected to find that the conflicting reproductive interests between sexes in *D. melanogaster* would result in detrimental effects on information acquisition. Despite the impact of male copulation attempts on female behaviour, and their negative effects on female fitness, we found that sexual conflict had no influence on the ability to learn about food patch quality in males and females. However, it is difficult to conclude that the efficient female cognitive performance observed in our study results from selection via male sexual harassment over evolutionary time. Indeed, the ability to acquire environmental information within unfavourable social contexts may be a by-product of the strong selection pressure for food patch choice in *D. melanogaster* [13].

Our results provide novel insights into the evolution of individual counterstrategies against the cognitive constraints

imposed by male sexual coercion. Future research should examine whether sexual harassment impairs cognitive performance in those species where it seems to be more costly, as well as focus on the effects of sexual harassment on differentially complex cognitive challenges in various ecological contexts.

Ethics. The research performed in this study on the fruit fly, *Drosophila melanogaster*, did not require approval by an ethics committee.

Data accessibility. The data underlying this study are available from Dryad: <http://dx.doi.org/10.5061/dryad.sg7k2>.

Authors' contributions. S.T. and F.M. designed the experiments, analysed the data and wrote the manuscript; S.T., C.M. and L.V. conducted the experiments. All authors gave final approval for publication.

Competing interests. Authors declare no competing interests.

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