Drosophila melanogaster females change mating behaviour and offspring production based on social context

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In Drosophila melanogaster, biological rhythms, aggression and mating are modulated by group size and composition. However, the fitness significance of this group effect is unknown. By varying the composition of groups of males and females, we show that social context affects reproductive behaviour and offspring genetic diversity. Firstly, females mating with males from the same strain in the presence of males from a different strain are infecund, analogous to the Bruce effect in rodents, suggesting a social context-dependent inbreeding avoidance mechanism. Secondly, females mate more frequently in groups composed of males from more than one strain; this mitigates last male sperm precedence and increases offspring genetic diversity. However, smell-impaired Orco mutant females do not increase mating frequency according to group composition; this indicates that social context-dependent changes in reproductive behaviour depend on female olfaction, rather than direct male–male interactions. Further, variation in mating frequency in wild-type strains depends on females and not males. The data show that group composition can affect variance in the reproductive success of its members, and that females play a central role in this process. Social environment can thus influence the evolutionary process.

Keywords: Drosophila melanogaster; social behaviour; reproduction; mate choice; sperm competition; Bruce effect

1. INTRODUCTION

The adaptive value of group life has been illustrated in many species by field and laboratory studies [1,2]. Group membership can facilitate biological functions including reproduction, foraging and protection against predators [1]. Such traits are controlled by the genotype of an individual as well as by social partners, a phenomenon termed indirect genetic effects (IGEs) [3,4]. IGEs are predicted to affect the evolutionary process when interactions with social partners affect variance in the individual’s fitness, known as social selection [3–6].

Drosophila melanogaster swarm on food substrates, where they feed and breed [7,8]. Members of these groups derive benefits from this social environment. For instance, females lay eggs communally [9], which increases larval survival [10]. Group composition is an important parameter influencing Drosophila social life. Studies on wild populations and laboratory lines suggest that the genotype of a minority can influence behaviour in the whole group [11–13]. For example, mixing arrhythmic period mutants with wild-type flies resets the locomotor activity rhythms, changes the blend of pheromones, and increases mating frequency of the wild-types [14–16] as well as modulates gene expression in their brain, and in their pheromone-producing cells [15]. Social interactions within D. melanogaster groups are thus clearly subjected to IGEs [16], allowing for the phenotype of others to affect the behaviour of an individual. However, little is known about the fitness consequences of these group interactions.

Genotypic frequency in a population is affected by mate preference. In the laboratory, mate choice is often tested by observing interactions between a single male and female. However, genetic monogamy is rare; both males and females of many species mate with multiple individuals in their lifetime [17–19]. In species where females have large broods, such as in D. melanogaster, multiple matings in one reproductive episode may lead to multiple paternities, but each male may not sire equal amounts of offspring [20,21]. For instance, when a female re-mates, the second male sires a majority of her offspring by out-competing his predecessor, a phenomenon called last male sperm precedence [20,21]. Because of male sperm competition, remating could have little benefit on females [22–24]. Yet, females mating with many males in a single reproductive episode and polyandry have been regularly reported in D. melanogaster [15,25–28], so the adaptive significance of this behaviour remains unclear. In other species, multiple matings and/or polyandry are reported to have a positive effect on female fitness, including an increase in the number of offspring produced [17] and offspring genetic diversity [18].

We have previously reported high incidence of remating when flies are housed continuously for 24 h in groups of six
males and six females [5]. This high mating frequency is dramatically higher than what is observed in most labora
tory assays of remating in D. melanogaster, which include a
single male and female [22,29,30]. This discrepancy
between assays suggests that social context affects mating
frequency in D. melanogaster [31] and provides a possible
example of social selection—if group composition affects
variance in reproductive success.

Here, we show that the social environment affects the
reproductive success of individual flies. Group assays with
six males and six females demonstrate high frequencies of
remating. We show that housing males from different strains
to increase social heterogeneity further increases remating
frequency and affects female mate choice. Females are cen
tral to this effect of the social environment, assessing group
composition by olfactory mechanisms. We also report that
females can block fecundity with certain males or cause a
breakdown in last male sperm precedence in a social con
text-dependent manner. This study shows that the social
environment can affect variance in reproductive success
and thus cause an impact on the evolutionary process.

2. MATERIAL AND METHODS

(a) Fly rearing
Flies were reared on a medium containing agar, glucose,
sucrose, yeast, cornmeal, wheat germ, soya flour, molasses,
propionic acid and Tegosept in a 12 L : 2 D cycle at 25
C. Virgin adults were collected using CO2 anaesthesia and
aged in same-sex groups of 20 in food vials for 5–6 days.

(b) Group-mating assays
Group-mating assays were performed in disposable 55 x
8 mm Petri dishes containing a fly food slice (22 x 5 mm).
Assays were set up by sequentially introducing six virgin
females followed by different types and numbers of
virgin males using a mouth pipette. We report four different
variations on this assay.

Experiment 1 tests the effect of group composition on
individual mating and reproductive success over 8 h.
Two common laboratory wild-type strains, Canton-S and
Oregon-R, were assayed in groups of six females housed with
six males. We tested these strains for male cuticular hydro
carbons [15]: our Canton-S are characterized by a ratio of
7-tricosene to 7-pentacosene of 5:1 and Oregon-R of 2:1.
Five group compositions were tested: groups of six males com
prising only Canton-S or Oregon-R males (homogeneous
groups) or heterogeneous groups comprising both Canton-S
and Oregon-R males at ratios of 2:4, 3:3 and 4:2, respec
tively. Flies were marked with acrylic paint (pink, blue,
yellow, gold, white or black) on the thorax upon collection
for identification. Assays ran from Zeitgeber Time (ZT) ZT1
(10.00 h) to ZT18 (18.00 h) and were continuously recorded
using a Sony AVCHD camcorder. An observer, blind to the
flies’ colour code, scored the time to mating, and identity of
mating partners using the Picture Motion Browser software
(Sony, Inc.). Females were extracted after the assays and
placed individually in food vials for egg-laying at 25 C. Vials
were changed daily for 5 days; females were left in the last
vial for another 5 days. Freshly eclosed adult offspring were
counted daily to determine female fecundity, then aged in
fresh food vials for 3–7 days before paternity analysis (see $3e$).

The unit of replication is the group. Individual measure
ments in a given group were averaged by sex, strain or
mating history to generate one value per replicate. Diff
erences in reproductive behaviour in different social contexts
were tested using the following mixed effect linear model
run with JMP v. 9.0 (SAS):

$$X_{ijkl} = \text{const.} + S_i + \text{So}_{ij} + \text{StSo}_{ij} + \text{Gr}_{ij} + \text{error}.$$ 

where $X$ is the number of matings, reproductive success or
offspring genotype in figure 1 a–c, respectively. $S_i$ is the
effect of male strain, $\text{So}_{ij}$ is the effect of social context
(male strain ratios), $\text{StSo}_{ij}$ is the effect of the interaction
of strain with social context and $\text{Gr}_{ij}$ is the effect of replicate
groups. Group was modelled as a random effect whereas all
other factors were fixed.

Experiment 2 tests the effect of group composition on
mating frequency over 24 h [15]. Frequency and time of
mating of Canton-S and Oregon-R, as well as Orco mutants
(née Or83b) [32] in a Canton-S genetic background were
assayed. Orco encodes an odorant co-receptor required for
the functioning of most olfactory receptor neurons [32].
The original $y^{1118}$ + Orco$^2$ was outcrossed to a Canton-S
isogonized white stock for five generations and the X and
second chromosomes were replaced by those of Canton-S.
Six females were housed with groups of males composed
either of Canton-S or Oregon-R males, or of four males of
these strains with two males mutant for the yellow and white
genes ($S_{y, w}$ males; unknown genetic background). Assays
were started at ZT8 (17.00 h) in an incubator set at 25 C
and at 12 L:12 D. The ZoomBrowser EX software
(Canon, Inc.) controlled a Canon S10 digital camera to
take images of the assays at 2 min intervals for 24 h. Red
light (wavelength above 620 nm) was used to monitor
mating during the dark phase. The lighter body and eye col
ours of $S_{y, w}$ mutants allowed discrimination from wild-type
flies by the observer scoring mating events. Images were sur
veyed for copulating pairs and scored if a pair was observed
for at least four consecutive frames.

Experiment 3 assesses the effect of multiple female mat
ings on last male sperm precedence in groups over 8 h. Six
Canton-S females were housed with three GFP-marked and
three non-GFP-marked males. The GFP-marked males
express the green fluorescent protein (GFP) ubiquitously
via a homoygous Ubiquitin::GFP transgene. The non-GFP
males do not have this transgene, but are otherwise in the
same foraging wild-type background. These males were
chosen because they had been shown to be equivalent despite
the GFP transgene [33]. This experiment was otherwise per
formed and scored as in experiment 1. Colour tags permitted
the identification of mate choice and mating order. Females
were extracted at the end of the assay as in experiment 1
for fecundity and offspring paternity analysis, but paternity
was determined through presence or absence of GFP using
a Leica MZ10F fluorescence stereomicroscope. Maximum
one female per group was used for statistical analysis.

Experiment 4 (figure 4) assays mating frequency of seven
wild-type strains: Canton-S, Oregon-R, C0–3, TW-1, Amhert-
3, Florida-9 and Urbana-S (the last five are from the
Bloomington Drosophila stock centre) in groups composed of
six females and six males, either all from the same strain, or
in conditions where the sexes were from different strains.
Experiments were set-up and scored as in Experiment 2.

(c) Paternity analysis
Abdominal segments A6 and A7 are more darkly pigmented
in Canton-S than in Oregon-R females. Daughters from an
**3. RESULTS**

(a) **Experiment 1: social context modulates male-mating success, reproductive success and offspring genotype**

We assayed mating behaviour of six females from Oregon-R or Canton-S wild-type strains housed with groups of six males comprising different mixes of Oregon-R or Canton-S to determine effects of the social environment on reproductive behaviour (see experiment 1 in §2). In 8 h, Oregon-R females mated on average twice and Canton-S females thrice (electronic supplementary material, figure S1a1,2). Housing females in different social contexts neither affected the mating frequency of Oregon-R ($F_{3,90,7}=1.47, p=0.22$), nor of Canton-S ($F_{2,56.82}=0.68, p=0.51$) females (electronic supplementary material, figure S1a1,2), but did affect male-mating success (figure 1a). The interaction of male strain and social context had a significant effect ($F_{3,88.07}=8.4, p<0.0001$), indicating that a male’s mating success is not only determined by his strain identity, but also by the identity of other males in his group (figure 1a1). The social environment mainly affected Canton-S males, which gained more mating when in a 2:4 minority than in any other social contexts (estimate = 0.81, s.e. = 0.18, $p<0.001$; figure 1a1). The influence of the social context on a male’s mating success depends on the females’ strain because the effect was not observed when males were housed with Canton-S females (figure 1a2). Male-mating success was similar for both strains ($F_{1,60.48}=0.05, p=0.82$), was unaffected by social context ($F_{2,54.73}=0.67, p=0.52$) and there was no significant interaction between strain and social context ($F_{2,60.51}=1.29, p=0.28$; figure 1a2).

Changes in social context had dramatic effects on reproductive success. In homogeneous groups, Oregon-R...
females had similar numbers of offspring with Canton-S and Oregon-R males, indicating equal male fecundity and no reproductive incompatibility (electronic supplementary material, figure S1b). However, Oregon-R females’ fecundity changed in different social contexts (F_{5,52.02} = 3.47, p = 0.022; electronic supplementary material, figure S1b). Comparison of females who mated solely with one male type (Canton-S or Oregon-R) in different social contexts revealed that the strain of the male they mated with has a significant effect on their fecundity (F_{3,39.02} = 28.84, p < 0.0001). This effect is dependent on social context (F_{4,45.85} = 4.42, p = 0.0081), and social context and the strain of the males interact to determine reproductive success (F_{3,44.88} = 4.42, p = 0.0146). The fecundity of Oregon-R females was affected by the social context both when mating only with Canton-S males (F_{5,33.72} = 10.60, p < 0.001), or only with Oregon-R (F_{3,26} = 3.67, p = 0.0250; figure 1b). Oregon-R females who mated with Oregon-R males in the presence of Canton-S males, were on average 70 per cent less fecund than when they mated with Oregon-R males in the absence of Canton-S males (figure 1b). The social context in which individuals mate can be as important as mating itself because mating does not ensure reproductive success in all social environments (figure 1b). Interaction between social context and male strain affecting reproductive success was not observed when similar groups of males were housed with Canton-S females (F_{2,39.97} = 0.50, p = 0.60; figure 1b), indicating that influence of the social context on male reproductive success depends on the females. Decreased fecundity of Oregon-R and not Canton-S females in heterogeneous groups is not linked to a general fecundity problem because Oregon-R females are significantly more fecund than Canton-S females when mating with either Canton-S or Oregon-R in homogeneous groups (two-way ANOVA F_{1,41} = 8.0, p = 0.007; electronic supplementary material, figure S1d,12).

We determined paternity ratios in the offspring of females that mated in different social contexts to determine the fitness of different male strains (see §2). In heterogeneous groups, Canton-S males consistently sired approximately 80 per cent of offspring and Oregon-R males 20 per cent (figure 1c). There was a significant deviation in paternity success from a predicted ratio of 50:50 in all social contexts (for 2:4, \( \chi^2(1,n = 281.93) = 136.26, p < 0.0001; \) for 3:3 ratio, \( \chi^2(1,n = 293.44) = 110.29, p < 0.0001; \) for 4:2 ratio, \( \chi^2(1,n = 398.03) = 193.14, p < 0.0001 \). Despite changes in mating and reproductive success (figure 1a1 and b1), we observed no significant difference in the pattern of offspring produced by Oregon-R females across mixed social contexts (F_{4,30} = 1.95, p = 0.16). We highlight that this stable percentage is a group property and is not found in any one female. This effect was not observed when males were housed with Canton-S females (figure 1c2). Offspring genotypic frequency of Canton-S females was similar in different social contexts (F_{1,13.92} = 2.94, p = 0.10) and offspring were sired equally by Canton-S and Oregon-R males in the 4:2 context (\( \chi^2(1, n = 342.26) = 171.13, p = 0.24; \) but not in the 2:4 context (\( \chi^2(1, n = 293.06) = 40.09, p < 0.0001; \) figure 1c2). We conclude that social environment can influence mate choice and fecundity, and notably genotypic diversity in the next generation.

(b) Experiment 2: Social heterogeneity affects mating frequency

Male strain diversity strongly influences mating behaviour of Oregon-R but not Canton-S females, leaving the generality of this phenomenon unclear. We extended the assay from 8 to 24 h to determine a potential long-term effect of the social environment on Canton-S female-mating behaviour, and created socially heterogeneous groups by housing mutant males with wild-type males and female Canton-S or Oregon-R (see experiment 2, §2). Mutant males (\( y,w \)) are sluggish courters with low mating success [34], making them unlikely to directly influence mating frequency. This allows testing their indirect effect on the mating success of the wild-type males by comparing mating patterns in homogeneous groups composed of six females and males from either the Canton-S or the Oregon-R strain with that of socially heterogeneous groups comprising four males (either all Canton-S or Oregon-R) and two \( y,w \) mutant males (figure 2).

The presence of \( y,w \) males indirectly increased Canton-S males’ mating success (figure 2a1). Canton-S males mated 38 per cent more in socially heterogeneous groups than in groups comprising no \( y,w \) males (figure 2a1), indicating that social heterogeneity also affects Canton-S females’ reproductive behaviour. Because the mating success of the two \( y,w \) males is negligible (less than 3% of all matings in Canton-S groups), we reasoned that the mating frequency of four Canton-S males with whom they are housed is better compared with that of four Canton-S males without \( y,w \) males, but that changes social density. We ruled out a confound of social density because homogeneous groups of Canton-S flies with either four or six Canton-S males were not different in terms of individual male-mating frequency (figure 2a1).

No significant increase was observed in mating frequency when Oregon-R flies were mixed with mutant males (\( p = 0.11; \) figure 2a2). Differences in response to social heterogeneity between Canton-S and Oregon-R between experiments 1 and 2 may be partially caused by a temporal difference in behaviour. Graphing the temporal distribution of mating over the 24 h of experiment 2 reveals that the effect of mixing \( y,w \) males in Canton-S groups becomes apparent 10–12 h after the start of the experiment (after ZT18; figure 2b). Further, Canton-S and Oregon-R have different temporal distributions of mating (figure 2b1,2). Increased mating frequency in socially heterogeneous groups of Canton-S flies (figure 2a1) may be caused by Canton-S females perceiving male diversity and increasing receptivity. Because flies use chemical communication during social interactions [35], we hypothesized that smell-impaired Canton-S females would not increase mating frequency between homogeneous and heterogeneous groups. We re-tested the mating behaviour of Canton-S flies in the presence of \( y,w \) males, using Canton-S females carrying a null Orco allele, impairing their olfaction. Unlike groups including Canton-S females (figure 2a1), groups including Orco females did not show increased mating frequency (figure 2a3) indicating that social context perception is mediated by an olfactory cue perceived by females. Orco females behaved like Canton-S females in homogeneous groups in terms of mating frequency (figure 2a1,3) and temporal distribution of mating (figure 2b1,3) indicating no basic reproductive
(c) Experiment 3: female mating frequency affects last male sperm precedence

The function of Canton-S increased mating frequency in heterogeneous groups is unclear (figure 2a1). Females produce large broods (approx. 100), so increasing number of mates could increase offspring genetic diversity. However, males possess mechanisms ensuring sperm precedence [21] predicting that female mating will not achieve greater offspring diversity because the last male will sire most offspring [24]. We tested this prediction by examining the percentage of offspring sired by the last male when mating with Canton-S females who had already mated with one, two, three or four males (see experiment 3, §2). Groups were composed of three males genetically marked with a GFP transgene and three unmarked housed with six Canton-S females, allowing determination of paternity in offspring by observing GFP. We observed classical instances of last male sperm precedence in twice-mated females, with the last male siring a majority of offspring (figure 3a1). Last male precedence was, however, not observed in females that mated more than twice, and the portion of offspring sired by the last male was reduced to one-third or less (figure 3a2). This rejects the prediction that increased remating does not change offspring diversity. A one-way ANOVA with the proportion of offspring sired by the last male as the dependent variable showed that last male sperm precedence is significantly affected by the number of times a female mated (for GFP male: $F_{2,52} = 17.52$, $p < 0.0001$; for non-GFP males: $F_{2,52} = 13.35$, $p < 0.0001$). The higher percentage of offspring sired by non-GFP (figure 3a1) compared with GFP (figure 3a2) last males is probably linked to an effect of the ubiquitous expression of GFP, which may reduce the ability of the sperm from GFP males to compete with sperm from non-GFP males [21]. Female fecundity was not significantly affected by numbers of mating or male types, indicating that multiple matings do not affect female fecundity (electronic supplementary material, figure S3).

Males reportedly transfer fewer sperm in successive copulations, until exhaustion after four to five matings [26]. We observed on average 3.89 mating per female (s.e.m. = 0.08; $n = 464$), each spaced by an average 135 min (s.e.m. = 3 min). GFP males mated 3.4 times (s.e.m. = 0.12; $n = 217$) and non-GFP males mated 4.9 times (s.e.m. = 0.13; $n = 230$). This remating frequency may allow females to affect male–male sperm competition through sperm depletion. We indirectly checked sperm depletion in males who had mated last with three times mated females by comparing those males who had previously mated with no more than two females (‘fresh’ males) and those who had mated with three or more (‘exhausted’ males). The ability of ‘exhausted’ non-GFP males to sire offspring was reduced when compared with ‘fresh’ ones, suggesting that sperm depletion is a factor in last male precedence reduction (figure 3b). However, it cannot be the only factor as the percentage of defects caused by the mutation. We conclude that effects of social heterogeneity on mating frequency are mediated by females.

Figure 2. Social heterogeneity affects mating frequency and is perceived by female olfaction. (a) Mean number of matings per male in 24 h, excluding y,w males, in groups of six females whose genotype is indicated above each graph in either homogenous groups of males (light grey bars) or in mixed groups comprising four wild-type males and two y,w mutant males (black). The four males were from the same strain as the females in (a1,2). In the Orco females’ experiment (a3), the males were Canton-S. Number of matings per male was estimated by dividing the total number of matings by the number of wild-type males (four or six). (a1) Bars labelled by the same letter (A or B) are not statistically different (ANOVA followed by Tukey–Kramer test, $p > 0.05$). (a2,3) n.s. indicates no significant difference between groups determined by a two-tailed Student’s t-test ($p > 0.05$). (b) Temporal distribution of mating in groups of the indicated genotypes and composition as in (a). Data points indicate the mean number of matings observed per 2 h time bins over 24 h. Numbers of group replicates are between brackets. Error bars: ±s.e.m.
Figure 3. Female mating affects pattern of last male sperm precedence. (a) Mean percentage of offspring sired by the last male in a mating series ranging from one to five matings. Mating series are indicated below the graphs. Numbers between brackets indicate females recorded with this particular mating series. Histograms labelled by different letters (A, B or C) are statistically different (ANOVA followed by Tukey–Kramer test, p > 0.05). (b) Effect of male mating history on last male precedence in thrice mated females. Offspring percentages are those presented in (a) for thrice-mated females. Data were separated between offspring sired by males having mated with other females twice or less before and those that had mated more, as indicated below the x-axis. Asterisks indicate significant differences determined by a two-tailed Student’s t-test. Error bars: ± s.e.m. Number of broods from individual female tested is between brackets. Black bars, non-GFP male; grey bars, GFP male.

**Experiment 4: variation in remating behaviour between wild-type strains**

As discussed in §1, the high female remating frequencies we observed are at odds with several reports of low female remating frequency in *D. melanogaster*. We tested seven laboratory wild-type strains to better characterize between-strain variability in remating behaviour in our assay.

Mating frequency is strain-specific and ranges on average from two to five matings per individual (figure 4a). The percentage of individuals that mated more than once is also strain-specific and ranged from 50 to 100 per cent (figure 4b). We also observed strain differences in timing between the first and second mating (figure 4c). Canton-S male for the second time (first remating) 4h after the virginal mating, while other strains, such as Oregon-R, mate for the second time 10h later (figure 4c). Temporal distributions of remating over 24h of the two wild-type strains with the highest remating frequencies (Oregon-R and Canton-S) are clearly different (repeat-measure ANOVA: F1.7.5 = 19.08, p < 0.001; figure 4d). Canton-S re-mated at the highest frequency in the first half of the night, while Oregon-R peaked in the second half of the night and in the early morning (figure 4d).

We investigated the contribution of each sex to mating frequency. We selected three strains with high, medium and low mating frequency (Canton-S, Oregon-R and CO-3, respectively; figure 4a) and quantified mating in groups composed of the nine possible permutations of males and females (figure 4f). In this factorial design, Canton-S females showed the highest mating frequency, while Oregon-R and CO-3 showed medium and low frequency, respectively (figure 4f). Oregon-R males mated more than Canton-S or CO-3 males with females from a given strain (figure 4f). A two-way ANOVA revealed that the strain of females significantly affects mating frequency and accounted for 47 per cent of the variance between groups (F2.124 = 133.87, p < 0.0001). Strain of the male also significantly affects mating frequency but accounts for only 11 per cent of the variance (F2.124 = 30.33, p < 0.0001). The male-by-female genotypic interaction was small and accounted for only 3 per cent of the variance (F3.124 = 4.77, p = 0.001). Temporal distribution of mating also tracks the distribution observed in the strain of the female and not that of the male (figure 4e) and so does the time to first remating (figure 4g). We conclude that remating is a common behaviour in groups, but both the frequency and temporal distribution of mating vary between strains. Although males exert some influence, females are the main determinant of when and how often remating occurs.

4. DISCUSSION

We investigated the effect of group composition on mating behaviour and offspring production to determine the connection between an individual’s social environment and its reproductive success. In groups, male reproductive success was predicted not only by strain identity, but also by the strain of the other males (figures 1 and 2). Thus, the contribution of the strain of an individual male to his own reproductive success can be small relative to the contribution of group composition. Because effects of group composition on male reproductive success were dependent on the type of females they were housed with (figure 1), we suggest that females may assess all potential mates in their social environment and adjust mate preference and mating frequency as...
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Figure 4. Remating differs in (a,b) frequency and (c–e) temporal distribution between wild-type strains because of strain differences in female behaviour (f,g). (a) Mean number of matings over 24 h per individual in groups of six males and six females. Number of matings was estimated by dividing the sum of matings in each experiment by six (the number of potential couples). Each replicate was taken as a single data point. (b) Fraction of individuals that mated more than once. (c) Time to first remating. Fraction of the total mating observed over 24 h binned per 2 h. (d) Temporal distribution of mating. Fraction of the total mating observed over 24 h binned per 2 h. (e) Mean number of mating per individual in groups of six males and six females from different strains. Asterisks indicate significant differences determined by ANOVA followed by Tukey–Kramer post hoc test (**p < 0.001; ***p < 0.0001). (f) Temporal distribution of mating as in (d), but with males and females from different strains. (g) Time to first remating measured as in (c), in combinations of females and males of the indicated genotypes. Box plots are as in (c). White or grey boxes indicate significant differences (ANOVA, p < 0.0001). Sample size is between brackets. Error bars: ± s.e.m. except for (c,g).

As well as fecundity accordingly (figures 1 and 2). The social environment can thus influence sexual selection.

A general result across our assays is that increase in social heterogeneity is accompanied by changes in female reproductive behaviour resulting in an increase in offspring genetic diversity. This happens either via disassortative mate choice and selective fecundity (figure 1), or by increase in remating frequency diluting last male sperm precedence (figures 2 and 3). Several studies have indicated that female remating may increase the production of genetically diverse progeny, which may increase offspring fitness, for instance, through greater resistance to parasites [18,19]. One interpretation of our data may be that females adapt reproductive behaviour to male diversity to maximize offspring fitness via a greater progeny genetic diversity.

Females from different strains behave differently in similar conditions, perhaps reflecting different adaptations in the natural populations from which they were originally derived, or could have been picked up in the laboratory owing to different levels of genetic heterogeneity among stocks (figures 1, 2 and 4) [36,37]. However, females from the same strain also change reproductive behaviour when housed with a mixture of males, indicating that females can assess male diversity. Female ability to assess the genetic diversity of potential mates...
would offer an interpretation for our observations. For instance, Canton-S females increase mating frequency with Oregon-S males in the presence of y,w mutant males, but Oregon-R females do not (figure 2); y,w males may not be perceived as different enough from Oregon-R males to warrant extra mating. Oregon-R females blocking offspring production when mating with males from their own strain in the presence of unrelated males may stem from the ability to determine genetic relatedness (figure 1b). Alternatively, both direct and indirect contributions of the mating to these effects are difficult to reconcile with our observations. In the context of the blocking of offspring production by Oregon-R females, indirect male–male interactions like sperm competition are not possible because we report on females who only mated with Oregon-R males (figure 1b). Direct male–male interactions, such as Canton-S males interrupting Oregon-R mating, preventing ejaculate transfer and causing a reduction in female fecundity, can be excluded because mating length does not differ between male strains or between social contexts (electronic supplementary material, figure S4). A focus on female mechanisms, such as the active discarding of sperm [21,38], appears more relevant to explain the offspring production block. This post-mating phenomenon recalls the ‘Bruce effect’ in which female rodents terminate pregnancy if exposed to a male from a different strain than the original stud male [39]. This similarity is intriguing because the Bruce effect is mediated by the smell of the ‘other’ male [39]. Smell-impaired Drosophila females do not adapt to a mix of males in their group (figure 2), indicating that male discrimination occurs via smell in Drosophila as well. It remains to be determined what other similarities are shared between the phenomenon we report in Drosophila and the Bruce effect.

We studied the effect of the social context on reproductive success by measuring the frequency of remating. The observation of high remating frequency contrasts with several studies reporting low levels of remating in D. melanogaster. These studies employ assays where a female is mated with a single male, isolated for 24 h and subsequently housed with a different male, typically resulting in very low remating frequencies [22,29–31]. Our assay differs in the number of flies, length and presence of food, explaining discrepancies between reports. Our observation of high female remating frequencies does not appear to be an experimental artefact because it matches data on polyandry in the wild. Wild-caught D. melanogaster females commonly carry sperm from several males and will have offspring from four to five different sires [25,27], indicating that our assay may better model the natural situation than classical assays. Differences in mating frequency may also be linked to genetic variation between populations [36], as implied by the strain specificity of this phenotype (figure 4). Strain differences also extend to temporal distribution of mating (figure 4). Temporal preference in mating may create reproductive barriers between species [40]. We may speculate that strain-specific temporal differences in remating may also limit reproduction between populations, to the extent that patterns of variance observed in laboratory strains reflect differences between wild populations [37].

Drosophila melanogaster is a model for last male sperm precedence, but this phenomenon has mainly been explored in the context of females mating twice and with long intervals between copulations (1–2 days) [21,24]. In this context, females mating three times failed to show an effect of increased mating on last male sperm precedence [24]. That high remating frequency in our assay dilutes last male precedence (figure 3) is therefore at odds with previous publications. This discrepancy may be partially owing to ejaculate depletion resulting from fast mating frequency [19], which would not be an issue in classical assays. However, this factor is not sufficient to fully explain the breakdown in sperm precedence we observe. Studies in other arthropods have shown that last male sperm precedence breaks down when females mate more than twice, or at high frequencies [41,42], showing precedents for this effect.

Observations in this study support the evolutionary theory of IGEs, which states that the reproductive success of an individual can be influenced by the genotype of another, thereby affecting allelic distribution in the next generation and in populations [3,4]. Evolutionary changes may occur whenever the breeding value of one individual covaries with the phenotype of its social partners [5]. Here, we show that the reproductive success of a given male can depend more on the genotypes and abundance of other males in the group than his own genotype. Females appear to mediate this social effect creating a behavioural equivalent of other variation-generating mechanisms such as meiotic recombination and sex itself.

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