Genet. Res., Camb. (2005), **85**, *pp.* 183–193. *With 3 figures.* © 2005 Cambridge University Press
doi:10.1017/S0016672305007536 Printed in the United Kingdom

Effect of genes, social experience, and their interaction on the courtship behaviour of transgenic *Drosophila* males

NICOLAS SVETEC†, BENJAMIN HOUOT† AND JEAN-FRANÇOIS FERVEUR*
Unité de Recherche 5548 Associée au Centre National de la Recherche Scientifique, Faculté des Sciences, Université de Bourgogne, 6, Bd Gabriel, 21000 Dijon, France

(*Received 4 January 2005 and in revised form 30 March 2005*)

Summary

Behaviour depends (a) on genes that specify the neural and non-neural elements involved in the perception of and responses to sensory stimuli and (b) on experience that can modulate the fine development of these elements. We exposed transgenic and control *Drosophila melanogaster* males, and their hybrids, to male siblings during adult development and measured the contribution of genes and of experience to their courtship behaviour. The transgene *CheB42a* specifically targets male gustatory sensillae and alters the perception of male inhibitory pheromones which leads to frequent male–male interactions. The age at which social experience occurred and the genotype of tester males induced a variable effect on the intensity of male homo- and heterosexual courtship. The strong interaction between the effects of genes and of social experience reveals the plasticity of the apparently stereotyped elements involved in male courtship behaviour. Finally, a high intensity of homosexual courtship was found only in males that simultaneously carried a mutation in their *white* gene and the *CheB42a* transgene.

1. Introduction

Deciphering the genetic basis of complex behaviours is a difficult task given that most behavioural variations involve polygenic effects (Greenspan, 2004). Moreover, each gene can be involved in multilayered networks and change several distinct characters (pleiotropy; Greenspan, 2001). *Drosophila* is one of the most amenable species for studying the relation between genes and behaviour: for example, geotactic behaviour has been shown to be under polygenetic control following a transcriptomic study (Toma *et al*., 2002). The *white* (*w*), *yellow* (*y*) and *ebony* (*e*) genes, which were initially identified because their mutation has a strong effect on the eye or cuticle colour (Lindsley & Zimm, 1992), also induce pleiotropic effects on behaviour. The defective pigmentation is caused by the abnormal biosynthesis of monoamines (dopamine for *y* and *e*; serotonin for *w*) which also affect neurotransmission in the central nervous system and during courtship (Bastock, 1956; Wilson *et al*., 1976; Kyriacou, 1981; Zhang & Odenwald, 1995; Rosato *et al*., 1997). *y* and *w* also interact with the *fruitless* gene (*fru*; Nilsson *et al*., 2000; Drapeau *et al*., 2003), altered expression of which increases male homosexual courtship (Goodwin, 1999).

The interaction of social experience with genes and their mutual effect on behaviour is difficult to measure. In *Drosophila*, the sensory signals received from conspecifics following adult eclosion can change the neuronal density in the brain (Heisenberg *et al*., 1995; Barth *et al*., 1997) and fly behaviour. Male flies raised in groups or in isolation show significant variation in the intensity of male heterosexual courtship and mating frequency (McRobert & Tompkins, 1988; Hirsch *et al*., 1995), as well as in male–male aggressivity during the establishment of a territory (Hoffman, 1990). Adult experience also changes the homosexual behaviour of transgenic males partly defective (i) for their expression of a male-specific factor (*CheB42a*) restricted to a few chemosensory tarsal hairs, and (ii) for their responses to male-inhibitory cuticular pheromones (Xu *et al*., 2002; Svetec & Ferveur, 2005).

* Corresponding author. Tel: +33 3 80393782. Fax: +33 3 80396289. e-mail: jean-francois.ferveur@u-bourgogne.fr
† Both authors contributed equally to this work.

Here, we have used two control strains and the transgenic *CheB42a* strain to measure the respective effects of genotype, social experience, and their interaction on male homo- and heterosexual courtship behaviours. First, the behavioural consequences of homosexual experience during adult life were compared between related genotypes. This revealed an interaction between the effects of genes and experience. The role of the principal hereditary components that change male homosexual courtship was then determined by hybridization between variant strains.

2. Materials and methods

(i) Drosophila *strains and crosses*

All experiments and fly husbandry took place at $24{\cdot}5 \pm 0{\cdot}5\ ^{\circ}C$ and 65% humidity, under a 12 : 12 dark : light cycle. All *D. melanogaster* strains were raised in glass vials containing standard cornmeal and yeast medium. The two wild-type strains Canton-S (Cs) and Dijon2000 (Di2) are considered as controls: Cs is widely used and Di2 was set up using a small number of inseminated females caught at Dijon, in 2000. The w1118 strain carries a mutation in the *white* gene (w^{1118}) linked with the X chromosome (Lindsley & Zimm, 1992). The w1118;CheB42a-Ga14;UAS-GFP strain ('B42';a gift from Claudio Pikielny) carries the w^{1118} mutation and two transgenes: *CheB42a-Ga14* contains the promoter of a male-specific factor expressed only in a few tarsal sensillae (*CheB42a*; Xu *et al*., 2002) fused to the yeast *Gal*4 sequence; *UAS*-GFP is the Green Fluorescent Protein sequence fused downstream of the yeast *UAS* (upstream activating sequence), specifically recognized by *Gal4* (Fisher *et al*., 1988; Brand & Perrimon, 1993). We initially used B42 transgenic male flies because they show intense homosexual courtship activity, probably due to their partly defective response to male-inhibitory cuticular pheromones (Svetec & Ferveur, 2005). However, we do not know whether this behavioural defect is caused by the abnormal expression in male tarsal hairs of the *CheB42a* male-specific factor, or of Gal4. We also used nine other B42 lines (A–I; also provided by Claudio Pikielny) that contain only the *CheB42a-Gal4* transgene inserted on various chromosomes and in the w^{1118} background. The exact chromosomal position of the B42 transgene in these 10 transgenic strains was not determined.

All combined genotypes shown represent the progeny of a mother × father cross. For example, *Cs*/*B42* is the progeny of Cs virgin females mated with B42 males. For each cross, 5 pairs of flies were kept in a food vial and transferred every 2–3 days to vial with fresh food. Male and female flies were sexed 0–2 h after emergence under light CO_2 anaesthesia and aged (either in isolation or in small groups) in standard food vials until the test.

Inheritance of the intensity of courtship behaviour was studied in various crosses carried out between Di2, Cs, B42, and w1118 parental strains, based on flies bred in similar conditions and analysed within a short period of time. For the genetic procedure inducing 14 hybrid lines, the two parental strains Cs and B42 were reciprocally mated to yield reciprocal F1 flies (*Cs*/*B42* and *B42*/*Cs*; crosses 3 and 4 in Table 1). Both F1 females and males were then reciprocally backcrossed to the Cs parents (#5–8), or to the B42 parents (#9–12), or between themselves (#13–16). For each male genotype, the origin of the principal hereditary components (X and Y chromosomes, autosomes, and maternal heredity) were estimated with Mendelian models (Wahlsten, 1979).

(ii) *Behaviour*

Courtship tests were performed on 5-day-old flies, 1–4 h after lights on, which is the period during which flies are most sexually active (Tauber *et al*., 2003). Flies tested as objects (5-day-old Cs females and males) were kept in groups of 5. Subject males were kept either in isolation until the day of the test (day 5), or with 4 other same-age sibling males for a period that always started at emergence and lasted 1–5 days. One hour before the test, tester males were aspirated from the vial (without anaesthesia) and kept isolated in a food vial. Each tester male was aspirated into an observation chamber ($3{\cdot}5\ cm^3$), and after 5 min one decapitated object fly (a Cs male or female) was introduced. Decapitation standardizes behavioural observations (Ferveur *et al*., 1995). We used only flies that had recovered their posture after anaesthesia and decapitation (standing in the vial). We measured the courtship index (CI) that the tester male directed toward the object for a period of 10 min. CI is the cumulative period of time that the male spends in active courtship (wing vibration, licking and attempting copulation) relative to the total observation period.

(iii) *Statistics*

The 10-bin frequency distribution of male homosexual CIs was 10/0/3/0/0/0/14/17/35/21 for B42 ($n = 29$), and 45/40/0/3/9/3/0/0/0/0 for Cs ($n = 35$). For the sake of clarity, and because most of the parental males had either extremely high or low CIs, we pooled the distribution into four histogram bars (see Figs. 2 and 3). To determine the hereditary components involved in the variation of homosexual CI, we divided this quantitative phenotype into two discrete classes: high or low CIs. The cut-off point was an empirical value derived from the parental distributions to reduce the misclassification probability. With a cut-off value at

https://doi.org/10.1017/S0016672305007536 Published online by Cambridge University Press

Table 1. *Mendelian distribution of factors inherited from two parental strains in 16 male lines*

	Cross		Hereditary components			
	Female	Male	X-Chr	Y-Chr	Autosomal	Maternal
	Parental strains					
#1	B42		■	■	■	■
#2	Cs		□	□	□	□
	Reciprocal F1					
#3	B42 /	Cs	■	□	▪	■
#4	Cs /	B42	□	■	▪	□
	Backcrosses to Cs					
#5	Cs /	(Cs/B42)	□	■	◧	□
#6	(Cs/B42) /	Cs	◧	□	◧	□
#7	Cs /	(B42/Cs)	□	□	◧	□
#8	(B42/Cs) /	Cs	◧	□	◧	■
	Backcrosses to B42					
#9	B42 /	(Cs/B42)	■	■	◨	■
#10	(Cs/B42) /	B42	◧	■	◨	□
#11	B42 /	(B42/Cs)	■	□	◨	■
#12	(B42/Cs) /	B42	◧	■	◨	■
	Reciprocal F2					
#13	(B42/Cs) /	(Cs/B42)	◧	■	◧	■
#14	(Cs/B42) /	(B42/Cs)	◧	□	◧	□
#15	(Cs/B42) /	(Cs/B42)	◧	■	◧	□
#16	(B42/Cs) /	(B42/Cs)	◧	□	◧	■

Next to the line#, the simplified genotype is indicated with the maternal (left) and paternal (right) contributions. The rectangles represent the principal hereditary factors (X-Chr, X chromosome; Y-Chr, Y chromosome; Autosomal, autosomes; Maternal, maternal cytoplasmic heredity). The genetic origin of each factor is indicated by a colour code: (B42, black; Cs, white; hybrid, shaded) and its contribution to each factor is represented by the coloured fraction of the rectangle. For example, #5 males are the progeny of Cs females and *Cs/B42* males; they theoretically carry 100% 'Cs' X chromosomes, 100% 'B42' Y chromosomes, 50% autosomes from Cs and 50% autosomes from hybrid lines, and 100% 'Cs' maternal cytoplasmic factors. In F2 males, 25% of autosomes were always inherited from Cs, 25% from B42 and 50% from hybrid lines.

CI = 50, the misclassification probability was reduced (0·069): all Cs males showed a 'low' CI phenotype (CI < 50), and 86·2% of B42 males showed a 'high' CI phenotype (CI > 50).

In males from the 14 hybrid lines resulting from the reciprocal crosses between Cs and B42, the distribution of low and high CIs, obtained for each genotype, was compared with that predicted by simple Mendelian models, using a homogeneity test (chi-square). Models involved either one factor (X chromosome, Y chromosome, maternal heredity, autosomes) or two factors (autosomes combined with any of the three former factors). The model fitted the observed distribution only when the chi-square value was above the threshold of significance (at $P > 0{\cdot}05$), indicating that no difference was detected between the observed and predicted distributions.

For each male genotype, a comparison was carried out with a Mann–Whitney test (i) between the homo- and heterosexual CIs of males with a similar experience, (ii) between naive (×1) and experienced (×5) males paired with same-sex object flies, and (iii) between reciprocal F1 males paired with a male object fly. CI values were logarithmically transformed (log CI + 1) to normalize the distribution, and were then analysed with ANOVA to measure the interaction between the effect of experience, and that of male genotype, toward a similar sex object (Table 2B). Pairs of male genotypes were compared to test the interaction between experience and either (i) the

https://doi.org/10.1017/S0016672305007536 Published online by Cambridge University Press

genetic background, (ii) the asymmetrically inherited genetic factors from the B42 strain, or (iii) the two types of genetic factors combined. ANOVA and Bonferroni post-hoc tests were also used to compare, within each genotype, the effect of different periods of social exposure on the intensity of male homo- and heterosexual courtship.

3. Results

We principally used *B42*/*Di2* males (resulting from the cross between transgenic B42 mothers and control Di2 fathers) because the intensity of their homosexual courtship varied with social experience (Svetec & Ferveur, 2005). This contrasts with *Di2*/*B42* males (produced by the reciprocal cross between Di2 mothers and B42 fathers), which showed a much weaker homosexual courtship. In this study, the courtship index (CI) of a single tester male (the subject) was always measured towards a decapitated male or female object of the control Cs strain.

(i) *The critical period of exposure varies with genotype*

We first tried to determine the period of adult development during which exposure to male siblings affected the intensity of male homo-and heterosexual courtship. CIs were measured in *Di2*/*B42* and *B42*/*Di2* males that were grouped with 4 same-age siblings immediately after adult eclosion for a variable period of between 1 and 5 days (Fig. 1). The comparison between homo- and heterosexual CIs shows that *Di2*/*B42* males better discriminated the sex of their partner than the reciprocal *B42*/*Di2* males, for all treatments except when grouping lasted 5 days.

Other differences were detected between reciprocal F1 males with regard to the period of social exposure. *B42*/*Di2* males that were communally raised during the first 3 days of adult life showed no effect of exposure. Their homo- and heterosexual CIs decreased, compared with those of isolated siblings, when exposure lasted more than 3 days. Conversely, *Di2*/*B42* males showed a consistently low homosexual CI whereas their heterosexual CI increased on day 1, remained relatively constant between days 2–4, and dramatically decreased after a 5 day exposure. This suggests that social experience during equivalent developmental periods can affect male courtship behaviour in a genotype-dependent manner.

(ii) *Interaction between the effects of experience and genotype*

As social experience during 5 days versus isolation induced a very strong effect, we compared this effect (i) in parental strains (=the two control Cs and Di2 strains and the B42 homozygous transgenic strain)

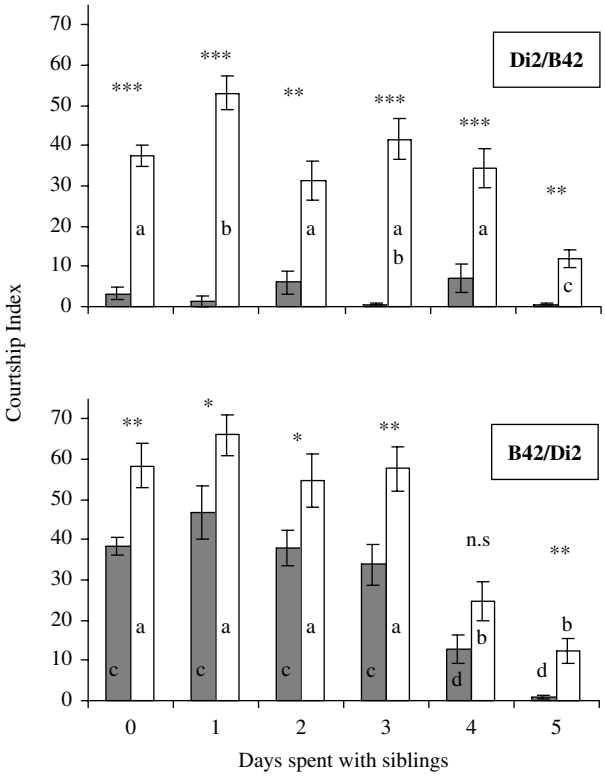

Fig. 1. Effect of experience on homo- and heterosexual courtship performance (as courtship index, CI) in two male genotypes. Male CIs are shown as a mean (±SEM). All 5-day-old males resulted from two crosses: (1) between Di2 mothers and B42 fathers (*Di2*/*B42*), and (2) between B42 mothers and Di2 males (*B42*/*Di2*). Males were either kept in isolation from emergence until the day of the test ('0 day'), or grouped with 4 same-age sibling males between emergence until day 1, day 2, day 3, day 4, or day 5. For each genotype and treatment, homosexual (shaded bars) and heterosexual (white bars) CIs were measured towards single decapitated Cs object flies. Within each male genotype, CI variation was compared (i) between different experience durations to the same sex object (with ANOVA and Bonferroni post-hoc tests; intra-sex differences are represented by different letters), and (ii) between homo- and heterosexual CIs for males with a similar experience (with a Mann–Whitney test: $P<0{\cdot}001$***, $P<0{\cdot}01$**, $P<0{\cdot}05$*; n.s., not significant). $24<n<40$, except for the homosexual CI of isolated *B42*/*Di2* males ($n=94$). The data shown for 'day 0' and 'day 5' correspond to the values shown in Table 2A.

and (ii) in reciprocal hybrid F1 males between Cs and B42. For all genotypes, subject males were either isolated (×1), or grouped with 4 other same-age siblings (×5), throughout their complete adult development (Table 2A).

In all cases, subject males showed a higher CI to the female object than to the male object (df=1; $F=206{,}97$; $P<0{\cdot}001$). Social experience generally decreased the intensity of male behaviour. The absolute amount of homosexual CI was strongly decreased in experienced males with a B42 mother (*B42*;*B42*/*Cs*;*B42*/*Di2*); it was less significantly decreased in the two control males (Cs and Di2),

https://doi.org/10.1017/S0016672305007536 Published online by Cambridge University Press

Table 2. *Intensity of homo- and heterosexual courtship in various male genotypes with different social experiences*

(A)		Sex object					
		Male			Female		
Male genotype	Experience	*n*	CI	Test	*n*	CI	Test
Cs	×1	20	4·3 (1·2)	*	30	30·6 (2·9)	n.s.
	×5	24	1·1 (0·6)		27	27·0 (5·7)	
Di2	×1	19	2·0 (0·4)	**	32	36·2 (6·1)	*
	×5	20	0·6 (0·2)		34	17·4 (4·2)	
B42	×1	31	43·1 (5·1)	***	27	70·0 (3·6)	**
	×5	27	19·3 (6·0)		28	36·2 (6·7)	
Cs/B42	×1	56	5·6 (1·2)	n.s.	28	53·7 (5·3)	n.s.
	×5	30	10·6 (3·8)		29	53·5 (6·1)	
B42/Cs	×1	22	37·0 (4·0)	***	30	64·6 (4·2)	***
	×5	26	2·2 (1·1)		43	22·6 (4·8)	
Di2/B42	×1	40	3·2 (1·6)	n.s.	93	37·4 (2·6)	***
	×5	31	0·6 (0·2)		85	12·0 (2·1)	
B42/Di2	×1	94	38·5 (2·3)	***	29	58·4 (5·5)	***
	×5	34	0·9 (0·3)		34	12·5 (3·0)	

Male courtship intensity (or courtship index, CI) is shown as a mean (±SEM) in various genotypes. Each 5-day-old tester male was paired once with a single decapitated object male or female fly of the control Cs strain, and its CI measured over 10 min. Both control Canton-S and Dijon2000 strains (Cs; Di2) were combined with the B42 transgenic strain to produce four reciprocal F1 lines (for example, *Cs/B42* had Cs mothers and B42 fathers). Males of each genotype were kept either in isolation (×1), or grouped with 4 same-age sibling males (×5), throughout adult development. The effect of conditioning (×1 vs ×5), tested with a Mann–Whitney test, is shown with its significance at $P<0{\cdot}001$***, $P<0{\cdot}01$** or $P<0{\cdot}05$*; n.s., not significant.

(B)	Male			Female		
Compared genotypes	Gen	Exp	G × E	Gen	Exp	G × E
Cs vs Di2	n.s.	**	n.s.	*	***	n.s.
Cs vs Cs/B42	n.s.	n.s.	n.s.	**	**	n.s.
Di2 vs Di2/B42	n.s.	**	n.s.	n.s.	***	n.s.
Cs/B42 vs Di2/B42	***	n.s.	n.s.	***	***	**
B42/Cs vs B42/Di2	n.s.	***	n.s.	n.s.	***	n.s.
Cs vs B42/Cs	***	***	***	n.s.	***	*
Di2 vs B42/Di2	***	***	***	n.s.	***	*
Cs/B42 vs B42/Cs	***	***	***	*	***	***
Di2/B42 vs B42/Di2	***	***	***	*	***	n.s.

Effect of the tester male genotype (Gen), of its experience (Exp), and of the interaction between genotype and experience (G × E) on male courtship. Log-transformed values of homo- and heterosexual CIs were compared between pairs of male genotypes with a two-factor ANOVA ($P<0{\cdot}001$***, $P<0{\cdot}01$** or $P<0{\cdot}05$*; n.s., not significant).

and was not affected in *Cs/B42* and *Di2/B42* males. Social experience also decreased the absolute values of heterosexual CIs in all males except those with a Cs mother (Cs and *Cs/B42*).

Given that the effect of experience on male homo- and heterosexual CIs differed between genotypes, we tested the effect of genotype, of experience and their interaction on behaviour (Table 2B). Pairs of genotypes were compared in order to assess the effect of different genetic backgrounds either in their wild-type context, or when combined with B42 factors maternally or paternally inherited. With regard to homosexual courtship, a very strong interaction between genes and experience was detected between males that had, and those that had not, a B42 mother. With regard to heterosexual courtship, the strongest interaction was detected between *Cs/B42* males and both *Di2/B42* and *B42/Ds* males, implying the respective roles of (i) the genetic background of control strains, and (ii) the genetic B42 factors asymmetricallly inherited. A slight interaction was also found between Cs versus *B42/Cs* and between Di2 versus *B42/Di2*.

(iii) *Additive role of two genetic factors*

In the second part of our study, we tried to determine the hereditary components involved in the large

https://doi.org/10.1017/S0016672305007536 Published online by Cambridge University Press

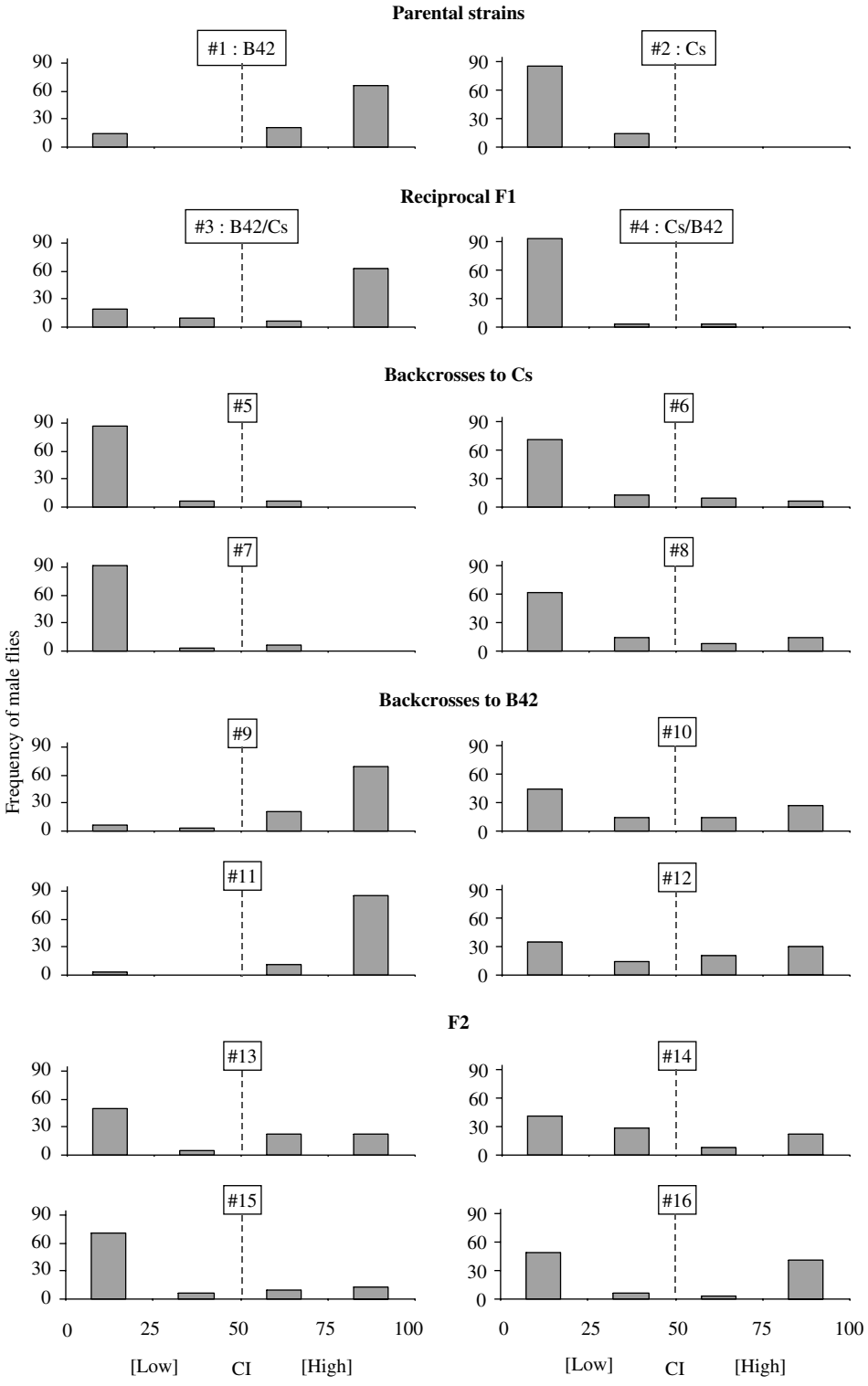

Fig. 2. Variation of male homosexual courtship intensity in 16 lines. The distribution of values of courtship intensity (CI) obtained for tester males was organized into four discrete categories obtained by the equal division of the full CI range (0–100; bottom of the figure). The two courtship phenotypes were defined (see the broken line) by a cut-off point at CI = 50 (low CI < 50 < high CI; see Section 2). Reciprocal crosses were made between two parental strains: the B42 transgenic strain and the Canton-S (Cs) control strain yielded reciprocal F1 males (*B42*/*Cs*: with B42 mothers and Cs fathers; *Cs*/*B42*: with Cs mothers and B42 fathers). Reciprocal F1 females and males were reciprocally backcrossed to each parent (Backcrosses to Cs, Backcrosses to B42), and between themselves (F2). The numbers shown above each histogram refer to the crosses listed in Table 1. $27 < n < 49$.

https://doi.org/10.1017/S0016672305007536 Published online by Cambridge University Press

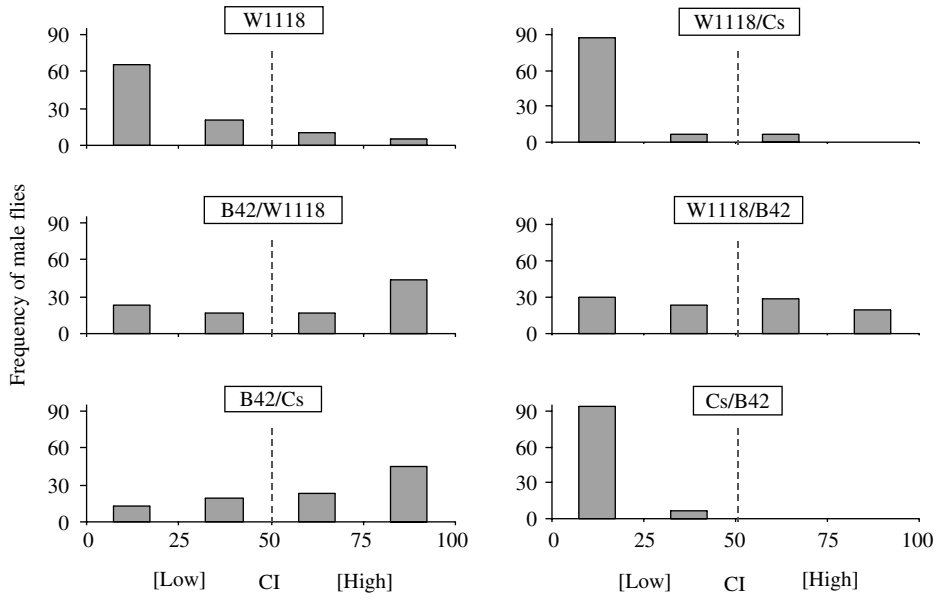

Fig. 3. Variation of male homosexual courtship intensity in various lines. Crosses were carried out between the mutant w1118 strain, the B42 transgenic stain and the control Cs strain. For example, *B42/w1118* indicates that these F1 males had B42 mothers and w1118 fathers. For all parameters, see Fig. 2. $40 < n < 57$.

variation in homosexual CI observed between reciprocal F1 subject males (*B42/Cs* and *Cs/B42*), independently of social experience. We measured male homosexual courtship in 14 hybrid lines resulting from various crosses between Cs and B42 strains (Table 1). As the two parental strains (Cs and B42) showed highly contrasted distributions of male homosexual CIs, we empirically determined a cut-off value (at CI = 50) to distinguish 'low' and 'high' homosexual CI phenotypes (see Section 2). The distribution of the CI phenotypes obtained with subject males of the 16 genotypes is shown in Fig. 2. The highly contrasted distributions of 'low' phenotypes between *B42/Cs* (30%) and *Cs/B42* (96·6%) suggests that male homosexual courtship intensity depends on a small number of sex-specific factors which are asymmetrically distributed between parental strains.

Using simple models of Mendelian inheritance, the proportion of the principal hereditary factors – X and Y chromosomes, autosomes, and maternal effect – inherited either from the Cs or from the B42 parent was estimated for each male genotype (Table 1), and statistically compared with the distribution obtained for the low-CI and high-CI phenotypes in the 16 genotypes (Table 3). The influence of each asymmetrically inherited component (X chromosome, Y chromosome, maternal heredity) was tested either separately or jointly with the autosomes (one- or two-factor models). With one-factor models, the observed distribution matched the prediction only for the frequency of the low-CI phenotype that followed the inheritance of 'Cs' maternal cytoplasmic factor(s). However, this was not the case for the frequency of high-CI phenotype and the transmission of 'B42' maternal cytoplasmic factor(s). Using two-factor models, the distribution observed for the two CI phenotypes fitted the segregation of the autosomes combined with that of the X chromosome. This strongly suggests that the variation in homosexual courtship noted between B42 and Cs strains is caused by at least two dominant factors segregating with the X chromosome and with an autosome.

(iv) *Role of the* white *mutation associated with the X chromosome*

The previous result suggests that the high level of homosexual courtship shown by *B42/Di2* and *B42/Cs* naive males segregates with the X chromosome of the B42 strain (Table 2). Because this chromosome carries a mutant allele of the *white* gene (w^{1118}) that was initially present in the B42 transgenic strain, we dissociated the effects of the X chromosome (with w^{1118}) and those of the autosomes (with the *B42* transgene) to measure their respective effects on the variation in homosexual CI in naive males. We therefore crossed the w1118 strain (carrying a w^{1118} X chromosome) to the B42 strain or to the Cs strain.

Parental w1118 males showed a very reduced homosexual CI, with only 15% high-CI males. This distribution was close to that of (i) *w1118/Cs* males (with a maternally inherited w^{1118} X chromosome), which showed only 6·4% high-CI, and (ii) *Cs/B42* males, with no high-CI phenotype. The comparison of the absolute CI values between these three genotypes revealed only a slight difference between w1118 and *Cs/B42* males (Bonferroni test; $P = 0{\cdot}033$). The w^{1118}

https://doi.org/10.1017/S0016672305007536 Published online by Cambridge University Press

Table 3. *Statistical evaluation of the effect caused by the principal hereditary factors on male homosexual behaviour*

	CI Phenotype	df	Chi-Square Observed	Chi-Square Threshold
One-Factor Models				
Autosomal	Low	8	138·3 ***	15·5
	High	14	125·1 ***	23·7
X-Chr	Low	11	23·2 *	19·7
	High	11	26·5 **	19·7
Y-Chr	Low	7	54·2 ***	14·1
	High	7	109·9 ***	14·1
Maternal	Low	7	12·2 n.s.	14·1
	High	7	64·8 ***	14·1
Two-Factor Models				
X + Autosomal	Low	11	3·3 n.s.	19·7
	High	11	8·8 n.s.	19·7
Y + Autosomal	Low	10	115·8 ***	18·3
	High	7	78·8 ***	14·1
Maternal + Autosomal	Low	10	45·3 ***	18·3
	High	7	30·3 ***	14·1

Hereditary factors were tested alone (one-factor), or combined two by two (two-factors) and tested with Mendelian models (see Table 1). Male homosexual phenotypes were regrouped in two classes according to their CI (Low < 50 < High), and their distribution, obtained from a variable number of hybrid lines ($n = df + 1$), was compared with the significance threshold provided by a chi-square test. A non-significant effect (n.s.) means that the prediction fitted the observed distribution.

mutation carried by the X chromosome seems to be involved in the high-CI phenotype because when this chromosome was maternally inherited and combined with B42 autosomes, 47·4% of the *w1118*/*B42* males showed a high CI. Elevated frequencies of high-CI phenotypes were also found in *B42*/*w1118* and *B42*/*Cs* males (60·5% and 67·9%, respectively). These three genotypes showed very similar CI values, and only one significant difference was detected between *w1118*/*B42* and *B42*/*Cs* males (Bonferroni test; $P = 0{\cdot}001$). This experiment confirms that the w^{1118} mutation linked with the X chromosome is involved in the high intensity of homosexual courtship that was initially detected in males of the B42 strain.

(v) *Role of the* B42 *transgene*

To clearly assess the behavioural implications of the *B42* transgene, 9 independent lines containing the B42 transgene inserted on different autosomes (at unknown chromosomal locations) in a w^{1118} background were reciprocally crossed with the Cs control strain. The level of homosexual courtship was measured in all reciprocal F1 males produced by these crosses (Table 4). In 8 *B42*/*Cs* lines, F1 males showed higher homosexual CI than in the *Cs*/*B42* reciprocal

Table 4. *Homosexual courtship in reciprocal F1 males of various genotypes*

B42 strains	Cs/B42	B42/Cs	Test
A	11·2 (1·4)	32·9 (5·2)	***
B	10·8 (1·9)	21·5 (2·8)	**
C	9·4 (2·7)	24·2 (3·3)	**
D	8·9 (1·9)	28·8 (2·5)	***
E	4·7 (1·5)	40·2 (4·4)	***
F	12·1 (1·3)	33·7 (4·5)	***
G	8·7 (2·3)	53·0 (4·6)	***
H	13·0 (2·9)	13·4 (3·1)	n.s.
I	10·1 (2·5)	36·9 (6·4)	**

All tested males were induced by reciprocal crosses between the Cs strain and each of the nine B42 transgenic strains (A–I). Male CIs are shown as a mean (±SEM), and their variation was tested between reciprocal crosses involving a similar B42 strain using a Mann–Whitney test ($P < 0{\cdot}001$***, $P < 0{\cdot}01$**; n.s., not significant). For each treatment, $n = 15$.

lines ($0{\cdot}006 < P < 0{\cdot}0001$). Only one transgenic line (H) showed a relatively low homosexual CI that was not different between reciprocal F1 males. This experiment clearly indicates that the *B42* transgene is involved in the high intensity of homosexual courtship. It also confirms that this behavioural effect was induced when the *B42* transgene was combined with the X chromosome maternally inherited from the w^{1118} mutant.

4. Discussion

Our data show that the intensity of male courtship behaviour can be modulated by (i) experience with same-age siblings during adult life, (ii) two genetic sequences with the genetic background, and (iii) their interaction. We found that social experience can induce a variable influence on male behaviour depending on the genotypes of the tester male and of the sex object, and on the period of social exposure.

(i) *Relationship between experience and sensory perception*

The effect of social interactions during the exposure period depends on the quality of sensory communication. We principally used B42 males, because they showed an abnormally high homosexual activity in the absence of visual and acoustic stimulation (in red light and with immobilized objects; Svetec & Ferveur, 2005). Given that the B42 transgene is expressed only in a few leg chemosensory hairs (Xu *et al.*, 2002), male homosexual behaviour may result from the defective perception of male inhibitory pheromones. Although we cannot formally exclude the possibility that the varying effect of experience between male genotypes was also caused by altered sensory stimuli emitted by

https://doi.org/10.1017/S0016672305007536 Published online by Cambridge University Press

the sibling males used for conditioning, it seems unlikely: male inhibitory pheromones apparently showed no difference between genotypes (Sureau & Ferveur, 1999; data not shown).

Two experimental parameters related to sensory communication could account for the variation in the behavioural effect induced by experience: (1) the period of adult development during which experience occurred and (2) the age of the siblings used for conditioning. In the male golden hamster, repeated exposure to social stress can alter the development of agonistic male behaviour depending on the age at which exposure occurs: repeated contact during adult age inhibits aggression and increases submissive and avoidance behaviours while a similar experience during puberty enhances offensive aggression (Wommack *et al*., 2004). In the male cockroach *Leucophea cinerea*, exposure to a new social experience (with same-age males) increased agonistic activity when these males were placed with males from their original group (Moore *et al*., 1988). Contact during early adulthood with older aggressive male cockroaches delayed the development of aggressive behaviour whereas contact with another immature male tended to accelerate it (Manning & Johnstone, 1970). During the first day of adult life, *D. melanogaster* flies undergo a dramatic change both in the plasticity of brain centers involved in integrated behaviours (Heisenberg *et al*., 1995; Barth *et al*., 1997) and in the production of sensory signals required for sexual communication (cuticular pheromones: Antony & Jallon, 1981; acoustic signals: Moulin *et al*., 2001). These data suggest that the variable effect induced by conditioning males could be related to their age difference: (i) with immature conditioners, male homosexual behaviour was not altered and heterosexual courtship was only slightly increased (Svetec & Ferveur, 2005; this study) whereas older conditioning males strongly enhanced heterosexual male courtship (Zawistowski & Richmond, 1985; McRobert & Tompkins, 1988).

(ii) *Interaction between genes and with experience*

The intensity of male homosexual courtship increased when the *B42* transgene segregated with the X chromosome carrying a *white* mutation (w^{1118}), but no effect was induced when only one of these two factors was present. This indicates the existence of an epistatic interaction between the two genetic sequences.

The genetic background of transgenic *D. melanogaster* strains often contains the w^{1118} mutation, which allows the transgene to be detected (because it carries a minimal *w* sequence = *mini-w*). The conditional activation of *mini-w* has been correlated with high levels of male homosexual courtship in both mass experiments and between pairs of transgenic males (Zhang & Odenwald, 1995; Hing & Carlson, 1996). This phenomenon was initially explained by decreased levels of serotonin and/or of dopamine caused by the *white* mutation (Zhang & Odenwald, 1995). However, increased homosexual behaviour was in fact correlated with the insertional position of the transgene (when homozygous) rather than with an elevated amount of *mini-w* in the male fly (An *et al*., 2000). This behavioural defect was then interpreted as a consequence of the perturbation caused by *w* on the DNA binding activity of other genetic factors involved in male courtship. For example, *w* interacts with the *fruitless* gene (*fru*) to induce male homosexual behaviour (Nilsson *et al*., 2000). However, we have no information about the biological basis of the interaction between *w* and the *B42* transgene. We therefore assume that the increased level of male –male courtship is caused by the direct effect of the transgene, which contains the promoter of a male-specific factor (Xu *et al*., 2002). We hypothesize that the abnormal expression of this factor, or of Gal4, in male tarsal hairs together with its interaction with mutated White product(s) leads to altered male pheromonal perception (Svetec & Ferveur, 2005).

The multiple behavioural consequences of the *w* mutant gene, or the *mini-w* transgene, are difficult to characterize with regard to sensory communication: the activation of *mini-w* (Zhang & Odenwald, 1995) did not alter the overall auditory, olfactory and visual electrophysiological responses of males showing a high homosexual behaviour. Moreover, the bilateral ablation (i) of their antennae and/or palps (that detect auditory and olfactory signals) or (ii) of their wings (that produce acoustic signals) did not decrease their strong homosexual behaviour. However, in darkness these transgenic males showed a reduced homosexual CI that was not modified by the activation of the *mini-w* transgene (Hing & Carlson, 1996). This supports the hypothesis that the *w* gene may affect pleiotropic characters required for courtship behaviour including the perception of sex-specific visual and pheromonal stimuli and/or their processing in the central nervous system.

The genetic background that diverged between the two control strains (Cs and Di2) also affected male behaviour in relation to social experience. For example, the fact that the interaction noted for heterosexual courtship varied between *Cs*/*B42* and *Di2*/*B42* – but not between Cs and Di2 – genotypes suggests that the genetic background, diverging between the two control strains, interacts differently with B42 factors. This interpretation is supported by the finding that the interaction was significant between reciprocal *B42*/*Cs* and *Cs*/*B42* males but not between reciprocal *B42*/*Di2* and *Di2*/*B42* males (Table 2B). However, males of the latter two

https://doi.org/10.1017/S0016672305007536 Published online by Cambridge University Press

genotypes revealed a subtle difference: in *B42*/*Di2* males, heterosexual CI was depressed by social experience 1 day earlier than in *Di2*/*B42* males. If these behavioural variations are consistent, they could segregate either with the X chromosome, with cytoplasmic factors, or with both types of hereditary components. The effect of the genetic background on behaviour is not surprising given that complex behaviours, including *Drosophila* male courtship, can be affected by multiple genes with pleiotropic effects (Gerlai, 1996; Greenspan, 2001, 2004; Moehring & Mackay, 2004). Moreover, the variation in brain expression of a mutant gene between wild-type strains (DeBelle & Heisenberg, 1996) suggests that the genetic context can have wide effects on the neural functions underlying complex behaviours.

The Y chromosome might also be responsible for some of the behavioural effects observed if it carries characters that are similar in the two control strains, and different from the B42 strain. This seems unlikely since (i) Cs and Di2 strains have a very different life history, and (ii) the Y chromosome of B42 and of Cs had no effect on the variation in male homosexual CI, either when tested alone or when combined with the autosomes (Table 3). Finally, maternal cytoplasmic heredity was also implicated in the inheritance of homosexual CI, but we suspect this was an artefact: the X chromosome form the Cs strain could have a confounding effect with that of Cs cytoplasmic heredity because (i) the two factors often segregated together, and (ii) the inheritance of the Cs X chromosome showed a close fit to the frequency of low CIs (one-factor model; Table 3).

In conclusion, we found that social experience changes the intensity of *Drosophila* male courtship depending on the male's genotype. Future studies dealing with male courtship should rigorously control male experience to evaluate the contribution of genes to this 'stereotypical' behaviour.

We thank Claudio Pikielny for the generous gift of all B42 transgenic lines before publication, two reviewers for fruitful comments and Matthew Cobb for correcting the manuscript. This work was partly funded by the CNRS and by the Burgundy Regional Council (J.F.F.).

References

An, X., Armstrong, J. D., Kaiser, K. & O'Dell, K. M. (2000). The effects of ectopic *white* and *transformer* expression on *Drosophila* courtship behavior. *Journal of Neurogenetics* **14**, 227–243.

Antony, C. & Jallon, J. M. (1981). Evolution des hydrocarbures comportementalement actifs de *Drosophila melanogaster* au cours de la maturation sexuelle. *Compte Rendus l'Académie des Science de Paris* **292**, 239–241.

Barth, M., Hirsch, H. V., Meinertzhagen, I. A. & Heisenberg, M. (1997). Experience-dependent developmental plasticity in the optic lobe of *Drosophila melanogaster*. *Journal of Neuroscience* **17**, 1493–1504.

Bastock, M. (1956). A gene mutation that changes a behaviour pattern. *Evolution* **10**, 421–439.

Brand, A. H. & Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401–415.

Bray, S. & Amrein, H. (2003). A putative *Drosophila* pheromone receptor expressed in male-specific taste neurons is required for efficient courtship. *Neuron* **39**, 1019–1029.

Cobb, M. & Ferveur, J. F. (1996). Evolution and genetic control of mate recognition and stimulation in *Drosophila*. *Behavioural Processes* **35**, 35–54.

de Belle, J. S. & Heisenberg, M. (1996). Expression of *Drosophila* mushroom body mutations in alternative genetic backgrounds: a case study of the *mushroom body miniature* gene (*mbm*). *Proceedings of the National Academy of Sciences of the USA* **93**, 9875–9880.

Drapeau, M. D., Radovic, A., Wittkopp, P. J. & Long, A. D. (2003). A gene necessary for normal male courtship, *yellow*, acts downstream of *fruitless* in the *Drosophila melanogaster* larval brain. *Journal of Neurobiology* **55**, 53–72.

Ferveur, J. F., Stortkühl, K. F., Stocker, R. F. & Greenspan, R. J. (1995). Genetic feminization of brain structures and changed sexual orientation in male *Drosophila*. *Science* **267**, 902–905.

Fischer, J. A., Giniger, E., Maniatis, T. & Ptashne, M. (1988). GAL4 activates transcription in *Drosophila*. *Nature* **332**, 853–856.

Gailey, D. A. & Hall, J. C. (1989). Behavior and cytogenetics of fruitless in *Drosophila melanogaster*: different courtship defects caused by separate, closely linked lesions. *Genetics* **121**, 773–785.

Gerlai, R. (1996). Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends in Neurosciences* **19**, 177–181.

Goodwin, S. F. (1999). Molecular neurogenetics of sexual differentiation and behaviour. *Current Opinion in Neurobiology* **9**, 759–765.

Greenspan, R. J. (2001). The flexible genome. *Nature Reviews in Genetics* **2**, 383–387.

Greenspan, R. J. (2004). *E pluribus unum, ex uno plura*: quantitative and single-gene perspectives on the study of behavior. *Annual Review of Neuroscience* **27**, 79–105.

Heisenberg, M., Heusipp, M. & Wanke, C. (1995). Structural plasticity in the *Drosophila* brain. *Journal of Neuroscience* **15**, 1951–1960.

Hing, A. L. & Carlson, J. R. (1996). Male–male courtship behavior induced by ectopic expression of the *Drosophila white* gene: role of sensory function and age. *Journal of Neurobiology* **30**, 454–464.

Hirsch, H. V. B., Barth, M., Luo, S., Sambaziotis, H., Huber, M., Possidente, D., Ghiradella, H. & Tompkins, L. (1995). Early visual experience affects mate choice of *Drosophila melanogaster*. *Animal Behaviour* **50**, 1211–1217.

Hoffman, A. A. (1990). The influence of age and experience with conspecifics on territorial behaviour in *Drosophila melanogaster*. *Journal of Insect Behaviour* **3**, 1–12.

Kyriacou, C. P. (1981). The relationship between locomotor activity and sexual behavior in ebony strains of *Drosophila melanogaster*. *Animal Behaviour* **29**, 462–471.

Lindsley, D. L. & Zimm, G. G. (1992). *The genome of* Drosophila melanogaster. London: Academic Press.

Manning, A. & Johnstone, G. (1970). The effect of early adult experience on the development of aggressiveness in

males of the cockroach, *Nauphoeta cinerea*. *Revue du Comportement Animal* **4**, 12–16.
Marcillac, F., Grosjean, Y. & Ferveur, J.-F. (2005). The production and the discrimination of *Drosophila* sex pheromones are affected by a single mutation in the same gene. *Proceedings of the Royal Society of London, Series B* **272**, 303–309.
McRobert, S. P. & Tompkins, L. (1988). Two consequences of homosexual courtship performed by *Drosophila melanogaster* and *Drosophila affinis* males. *Evolution* **42**, 1093–1097.
Moehring, A. J. & Mackay, T. F. (2004). The quantitative genetic basis of male mating behavior in *Drosophila melanogaster*. *Genetics* **167**, 1249–1263.
Moore, A. J., Ciccione, W. J. & Breed, M. D. (1988). The influence of social experience on the behaviour of male cockroaches, *Nauphoeta cinerea*. *Journal of Insect Behaviour* **1**, 157–168.
Moulin, B., Rybak, F., Aubin, T. & Jallon, J. M. (2001). Compared ontogenesis of courtship song components of males from the sibling species, *D. melanogaster* and *D. simulans*. *Behavior Genetics* **31**, 299–308.
Nilsson, E. E., Asztalos, Z., Lukacsovich, T., Awano, W., Usui-aoki, K. & Yamamoto, D. (2000). *Fruitless* is in the regulatory pathway by which ectopic *mini-white* and *transformer* induce bisexual courthip in *Drosophila*. *Journal of Neurogenetics* **13**, 213–232.
Rosato, E., Piccin, A. & Kyriacou, C. P. (1997). Circadian rhythms: from behaviour to molecules. *Bioessays* **19**, 1075–1082.
Sureau, G. & Ferveur, J.-F. (1999). Co-adaptation of pheromone production and behavioural responses in *Drosophila melanogaster* males. *Genetical Research* **74**, 129–137.
Svetec, N. & Ferveur, J.-F. (2005). Social experience and pheromonal perception can change male–male interactions in *Drosophila melanogaster*. *Journal of Experimental Biology* **208**, 891–898.
Tauber, E., Roe, H., Costa, R., Hennessy, J. M. & Kyriacou, C. P. (2003). Temporal mating isolation driven by a behavioral gene in *Drosophila*. *Current Biology* **13**, 140–145.
Toma, D. P., White, K. P., Hirsch, J. & Greenspan, R. J. (2002). Identification of genes involved in *Drosophila melanogaster* geotaxis, a complex behavioral trait. *Nature Genetics* **31**, 349–353.
Wahlsten, D. (1979). A critique of the concepts of heritability and heredity in behavioral genetics. In *Theoretical Advances in Behavioral Genetics* (ed. J. R. Royce & L. Mos), pp. 424–481. Germantown, MD: Sijhoff and Nordhoff.
Wilson, R., Burnet, B., Eastwood, L. & Connolly, K. (1976). Behavioural pleiotropy of the *yellow* gene in *Drosophila melanogaster*. *Genetical Research* **28**, 75–88.
Wommack, J. C., Salinas, A., Melloni, R. H., Jr & Delville, Y. (2004). Behavioural and neuroendocrine adaptations to repeated stress during puberty in male golden hamsters. *Journal of Neuroendocrinology* **16**, 767–775.
Xu, A., Park, S. K., D'Mello, S., Kim, E., Wang, Q. & Pikielny, C. W. (2002). Novel genes expressed in subsets of chemosensory sensilla on the front legs of male *Drosophila melanogaster*. *Cell and Tissue Research* **307**, 381–392.
Zawistowski, S. & Richmond, R. C. (1985). Experience-mediated courtship reduction and competition for mates by male *Drosophila melanogaster*. *Behavior Genetics* **15**, 561–569.
Zhang, S. D. & Odenwald, W. F. (1995). Misexpression of the *white* (*w*) gene triggers male–male courtship in *Drosophila*. *Proceedings of the National Academy of Sciences of the USA* **92**, 5525–5529.