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The genetics of mating recognition between *Drosophila simulans* and *D. sechellia*

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Summary

During courtship, visual and chemical signals are often exchanged between the sexes. The proper exchange of such signals ensures intraspecific recognition. We have examined the genetic basis of interspecific differences in male mating behaviour and pheromone concentration between *Drosophila simulans* and *D. sechellia* by using *Drosophila simulans*/*D. sechellia* introgression lines. Our results show a majority of quantitative trait loci (QTLs) explaining variation in both male mating behaviour and pheromone concentration to be located on the third chromosome. One QTL found on the third chromosome explains variation in time needed to start courtship and copulation as well as time spent courting. The position of such QTL (approximately 84A–88B) with effects on courtship and copulation aspects of mating includes the candidate sex determination gene *doublesex* (84E5–6) and *Voila* (86E1–2), a gene that affects male courtship in *D. melanogaster*. One additional third chromosome QTL explained variation in 7-tricosene pheromone concentrations among males. The interval mapping position of this QTL (approximately 68E–76E) did not overlap with the position detected for differences in mating behaviour and the intervals did not include candidate genes previously identified as having an effect on *D. melanogaster* cuticular hydrocarbon production. We did not detect any directionality of the effect of *Drosophila sechellia* allele introgressions in male mating recognition.

1. Introduction

Courtship in Drosophila follows a series of well-characterized steps that start with the male orienting towards the female and starting to track her, often touching her abdomen with his forelegs. Next, the male vibrates his wings to generate a specific courtship song, licks the female's genitalia and attempts copulation. These steps usually occur in the same sequence with the female rejecting courtship by a series of behaviours such as kicking and extrusion of her ovipositor, or accepting the courting male by raising her wings and opening her vaginal plate (Hall, 1994).

There is a strong genetic basis to mating behaviour in *Drosophila melanogaster*. Hall (1994) and Yamamoto & Nakano (1998) describe mutations that dramatically alter components of male mating behaviour. Quantitative genetic analyses of phenotypic variation in mating behaviour have shown extensive genetic variability across inbred lines with no X chromosome effect and a genetic system characterized by both additive and dominant effects (Collins & Hewitt, 1984; Casares *et al.*, 1993). Different levels of pre-mating isolation have been found between male and female *Drosophila melanogaster* originated from different populations (Wu *et al.*, 1995; Capy *et al.*, 2000; Korol *et al.*, 2000). The use of lines with whole cosmopolitan versus African chromosome substitutions has established a major role of the third chromosome in mating isolation between Drosophila populations in incipient steps of speciation (Hollocher *et al.*, 1997; Colegrave *et al.*, 2000; Ting *et al.*, 2001). These studies examined pre-mating isolation using mating choice designs (Hollocher *et al.*, 1997; Ting *et al.*, 2001) or by analysis of differences in courtship songs produced by male wing vibrations (Colegrave *et al.*, 2000).

Studies on the genetics of interspecific mating isolation among closely related species of the simulans clade have focused on explaining differences in their pheromone profiles. The waxy compounds secreted

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by the epidermis of Drosophila include hydrocarbons, with pheromonal properties that are exchanged during the mating process for the purpose of recognition of conspecific members of the opposite sex. During mating, the forelegs pick up chemical signals by touch, while volatile pheromones released during courtship are received by olfactory receptor neurons in the antennae (de Bruyne *et al*., 1999). Among species of the simulans clade, *Drosophila simulans* and *D. mauritiana* show similar pheromone profiles with the pheromone profile of *D. sechellia* being quite distinct. The predominant pheromone in males and females is 7-tricosene for *D. simulans* and *D. mauritiana* but 7,11-heptacosadiene among female *D. sechellia*. *D. sechellia* males are also unique in having a larger amount of the 6-tricosene isomer than other members of the simulans species clade. 7-Pentacosene is present in both *D. simulans* and *D. sechellia* females and males and shows no distinctive pattern between species. The concentration of 7-pentacosene can be higher or lower in *D. simulans* than in *D. sechellia* depending on the strain used (Jallon & David, 1987; Cobb & Jallon, 1990). The differences in pheromone profiles seem to affect courtship, *D. simulans* males do not court *D. sechellia* females but do court F1 female hybrids to some extent (Cobb & Jallon, 1990; Coyne, 1992; Coyne *et al*., 1994). By using mutagenesis and crossing protocols using strains that carry phenotypically visible markers, *kété* (X chromosome) and *Ngo* (second chromosome) where singled-out as candidate loci responsible for polymorphism in pheromone production in *D. simulans* (Ferveur, 1991; Ferveur & Jallon, 1993). Comparisons between *D. simulans* and *D. sechellia* species have established that divergence in pheromone profiles is affected by all chromosomes, with the major effect being assigned to the third chromosome (Coyne *et al*., 1994; Coyne, 1996; Ferveur, 1997).

The genetic basis of differences in visual and/or auditory signals between species has been explored by the use of backcrosses between species. Pre-mating sexual isolation between *Drosophila simulans* and either *D. mauritiana* or *D. sechellia* reveals a pattern of no X-linked chromosome loci (Coyne, 1989, 1992). Carracedo *et al*. (1995) used *D. melanogaster* chromosome substitution lines to show mainly a second and third chromosome role in mating isolation, while assays of mating choice between F2 progeny from crosses between a *Drosophila simulans* line capable of hybridization with *D. melanogaster* and laboratory stock carrying phenotypic markers on the three major chromosomes established X and second chromosome effects on mating isolation between *D. simulans* and *D. melanogaster* (Carracedo *et al*., 1998).

Most studies have focused on the search for candidate genes responsible for intraspecific polymorphism in mating behaviour or pheromone production, while only the broad chromosomal basis of interspecific differences has been examined. Here, we use a combination of molecular markers and statistical approaches to narrow down the genetic mapping of interspecific differences to a small number of quantitative trait loci (QTLs) with major phenotypic effects. We use a set of homozygous *Drosophila simulans* lines with an average 12·5% of their genome introgressed from *Drosophila sechellia* (Dermitzakis *et al*., 2000; Civetta *et al*., 2002). For each introgressed line, we combined information on 27 molecular markers spread across the three major Drosophila chromosomes with phenotypic information on male mating behaviour and both male and female pheromone production. Using this information we identify QTLs responsible for differences in mating recognition between *D. simulans* and *D. sechellia* and establish whether the introgression of different *D. sechellia* alleles into a *D. simulans* genetic background causes predictable changes in the phenotype (i.e. changes in the same direction).

2. Materials and methods

(i) Drosophila *stocks and maintenance*

We used lines of *Drosophila simulans* with random chromosome introgression from *D. sechellia* that were constructed by backcrossing F1 hybrid females to *Drosophila simulans* males and then crossing the male progeny to *D. simulans* females. The progeny were sib-mated for 14 generations to create independent homozygous lines (Dermitzakis *et al*., 2000; Civetta *et al*., 2002). *D. simulans* flies from the original stock used to generate the introgressed lines (sim2, Winters, CA) were also used in this study. Flies from each introgressed (IG) line and the sim2 stock were set up in large plastic bottles containing a cornmeal–molasses medium and kept at 24 °C in a 12 h cycle of light and darkness. Parental flies were removed after 3–4 days and virgin progeny flies were collected from these bottles three times a day over a period of 3 days. Collected flies were sexed and groups of five females or males were placed into plastic vials containing cornmeal–molasses food medium. The flies were then stored at 24 °C under the same conditions and allowed to age for 5–7 days before phenotyping.

(ii) *Mating behaviour assays*

We scored male mating behaviour indices from 99 different IG lines. One virgin female from the sim2 stock was placed in a glass vial (diameter 15×45 mm) together with one virgin male from an IG line. Groups of 5 vials for each IG line were videotaped for 2 h. All IG lines were scored over a 4 month period. Scores for each line were replicated using a randomized

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design so that measures for each IG line were randomly recorded in two trials on different days during the experiment. On each of two random days, at least one group of 5 vials was scored. Videotapes were examined and times for the following behaviour were recorded: Male starts tracking the female, male turns away or starts copulation, copulation starts, copulation ends. Five different mating behaviour indices were calculated as follows: Courtship latency (CL) is the time from when the male is introduced into a vial until he starts orienting and/or tracking; courtship duration (CD) is the time spent tracking until the male turns away or achieves copulation; copulation latency (CpL) is the time spent in the vial until copulation starts; copulation duration (CpD) is the time spent *in copula*; and courtship intensity (CI) is the proportion of time spent courting from when courtship starts until it ends (CI = CD/(CpL − CL)) (Coyne, 1996).

(iii) *Hydrocarbon extraction and gas chromatography–mass spectrometry*

Cuticular hydrocarbon profiles were obtained from males and females from 69 different IG lines and fully replicated in a second trial. Five virgin females or males from each IG line were slightly anaesthetized with CO_2, placed in a 7 ml glass vial with 400 μl of hexane and vortexed for 1 min. A small amount of sodium sulfate drying agent was added to the vial to remove any water from solution. The organic solvent was transferred to a 1·5 ml glass gas chromatography vial and stored at −20 °C until ready for gas chromatography–mass spectrometry (GC-MS). Before running GC-MS, the samples were evaporated under a slow stream of nitrogen gas, dissolved in 25 μl hexane and placed in 150 μl glass inserts that sat inside each GC vial. To measure the concentration of the hydrocarbon extractions, 25 μl of a 40 μg/ml hexacosane internal standard was added to each sample and the concentration of each pheromone was quantified relative to the hexacosane standard.

Samples (1 μl each) were automatically injected into a Hewlett Packard 5980 GC and analysed using a Hewlett Packard 5970 MS. The program used for hydrocarbon data collection was MS ChemStation G1034C (version C.03.00). The pheromone profile of each IG line was analysed separately by a function that automatically estimates the area under each significant peak recorded. The identity of each peak was determined by comparing the mass spectrum of each peak with the mass spectrum of a database of known compounds (Wiley 138, MS ChemStation software).

(iv) *Genotyping*

To establish possible gene-trait associations, we combined information on the phenotypic scores obtained from different IG lines with previously available information on the genetic makeup of IG lines for 23 different molecular markers spread across the three major chromosomes of Drosophila (Civetta *et al*., 2002). Four new molecular markers were added to the map by polymerase chain reaction (PCR) using primers to amplify genes that differ in an insertion/deletion or a restriction fragment length polymorphism (RFLP) between *D. simulans* and *D. sechellia*. Table 1 lists all 27 molecular markers used in this study, their map position and type of polymorphism scored. PCR amplification conditions for the four new molecular markers are as follows: *Anp* and *rux*: 45 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1·5 min followed by 72 °C extension for 1·5 min; *Acp63F*: 46 cycles of 95 °C for 1 min, 45 °C for 1 min and 72 °C for 1·5 min followed by 72 °C extension for 2 min; and *DM22F11T*: see MacDonald & Goldstein (1999).

The oligonucleotide primers used to amplify new markers are as follows: *Anp*: forward 5′-TGC TTC TCA GTC TAA ATC AT-3′, reverse 5′-GTA TTG CGT TTT CCT GTA G-3′; *rux*: forward 5′-CAT ACC CCT GGA AGA GCA G-3′, reverse 5′-GAT TGT CTG TTG TCG TTT CC-3′; *Acp63F*: forward 5′-CTT GCG TAG AAA CTC ACT CAG A-3′, reverse 5′-GTT TTA CTT TTA CAT CCC CTT TAG-3′. *DM22F11T* primers are described elsewhere (Colson *et al*., 1999).

(v) *Statistical and QTL analysis*

Flies from every line were tested in different trials on different days under the same food and temperature conditions. Phenotypic variation among IG lines was tested using analysis of variance with line and trial nested within lines as factors and assessing the significance of line effects when using trials as the error mean square. Average male mating behaviour indices (CL, CD, CpL, CpD and CI) were estimated for each IG line. Phenotypic variation among male and female IG lines in concentration of detectable pheromones relative to the hexacosane standard was tested using analysis of variance with lines as a factor. All statistical analyses were done using SPSS version 10.1.

QTL mapping analysis was done using QTL Cartographer version 2.0 (Wang *et al*., 2002). For each trait, single marker–trait associations were first explored using linear regression analysis. Interval mapping and different composite interval mapping models using alternative window sizes between markers and number of background markers were used to test whether variation in these parameters affected our ability to detect the presence of significant QTLs at different intervals between markers. The significance of any potential QTL was calculated by comparing the likelihood scores of any given QTL with the

Table 1. *Molecular markers used for QTL mapping. Genetic positions in IG lines* (*sim-sec*) *were calculated as in Civetta* et al. (*2002*)

GMarker	Cytological position	Genetic position (mel)	Genetic position (*sim-sec*)	Differences scored
white	3C2	1–1.5	1–3.6	Microsatellite
Cdk7	4F1–2	1–11	1–8.9	Microsatellite
rux	5D5	1–15	1–11.2	Insertion/deletion
dec-1	7C4–5	1–20.7	1–14.3	Insertion/deletion
sev	10A2–4	1–33.4	1–23.7	Microsatellite
sog	13E3–8	1–53	1–42.7	Microsatellite
Sh	16E4–F1	1–57.6	1–49	Microsatellite
shakB	19E3	1–64	1–57.9	Microsatellite
aop	22C3–D1	2–12	2–13	Microsatellite
Acp26Ab	26A5	2–18	2–22	Restriction site
ninaC	27F5–6	2–22	2–28.5	Microsatellite
da	31D11–E1	2–41.3	2–47.8	Microsatellite
Su(*h*)	35B10–C1	2–50.5	2–64	Microsatellite
cad	38E5–6	2–54	2–71.9	Microsatellite
mam	50C23–D3	2–70.3	2–100.5	Microsatellite
Amy-d	53F13–54A2	2–77.9	2–115.3	Insertion/deletion
AC004365	58A4–B1	2–107.6	2–146.5	Microsatellite
Cdc37	62B4	3–5	3–8.8	Microsatellite
Acp63F	63F1	3–9	3–11.9	Restriction site
ple	65C3	3–18	3–20.3	Microsatellite
Lanb2	67B10	3–28	3–30.8	Microsatellite
DM22F11T	73A1–B7	3–44	3–56.7	Microsatellite
Cat	75D7–E1	3–47	3–71.7	Insertion/deletion
AC001655	84C1–4	3–48	3–134.6	Microsatellite
cpo	90C10–D1	3–62	3–97	Insertion/deletion
pnt	94E11–F1	3–79	3–141.5	Microsatellite
Anp	99D5	3–100	173.6	Restriction site

Table 2. *Analysis of variance for differences in mating behaviour indices among IG lines*

Trait	Source of variation	d.f.	Adjusted mean squares	*F*	*P*
CL	Line	98	0·580	1·50	0·023
	Trial (within line)	99	0·387	2·08	<0·001
	Error	746	0·186		
CD	Line	98	1·045	1·59	0·011
	Trial (within line)	99	0·659	1·62	<0·001
	Error	655	0·407		
CpD	Line	96	0·039	2·42	<0·001
	Trial (within line)	96	0·016	2·16	<0·001
	Error	580	0·007		
CpL	Line	95	0·427	1·53	0·019
	Trial (within line)	96	0·280	1·78	<0·001
	Error	595	0·157		
CI	Line	95	0·262	1·25	0·139
	Trial (within line)	96	0·210	1·29	0·108
	Error	571	0·163		

distribution of likelihood scores obtained from 1000 random permutations of the trait data among markers (Doerge & Churchill, 1996). We searched for any candidate gene within a significant QTL interval by using the cytosearch tool available at FlyBase (http://flybase.bio.indiana.edu/).

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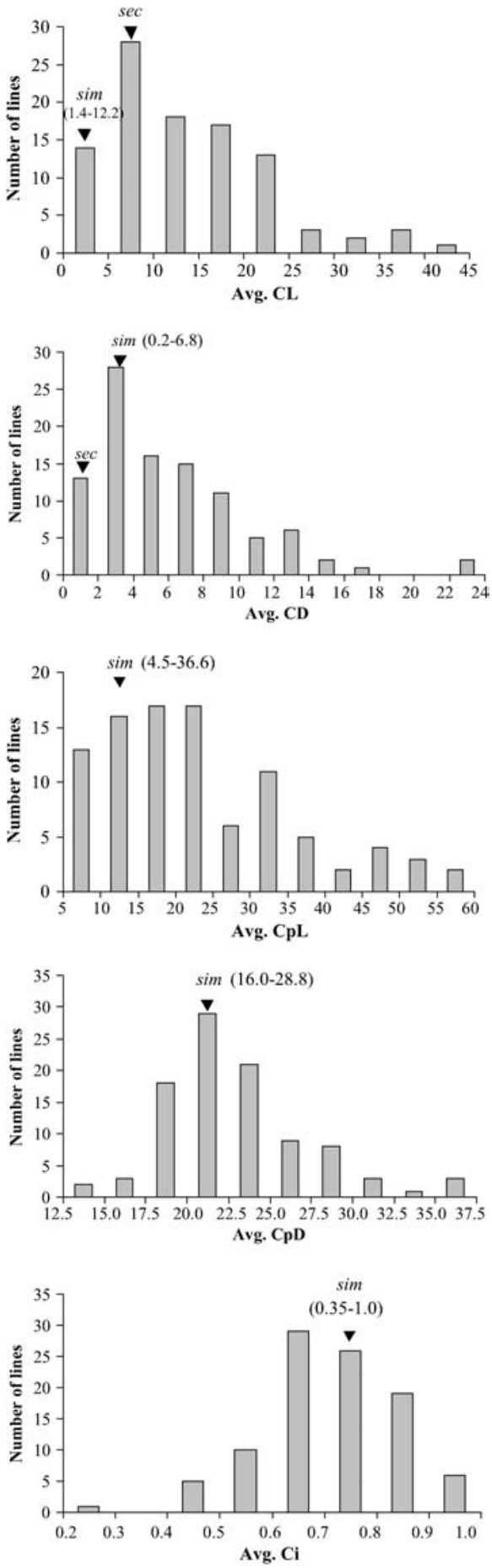

3. Results

(i) *Differences in mating behaviour and pheromone production among IG lines*

Average mating behaviour indices and pheromone concentrations were obtained for 99 and 69 different IG lines respectively. To fit normality and homoscedasticity assumptions mating behaviour indices were log-transformed, and an angular transformation was used for CI scores and 7-pentacosene concentrations, while 7-tricosene concentrations required no transformation of the data.

All IG lines were scored using a randomized design and each line was recorded in two trials at different times. With the exception of CI index, analysis of variance detected significant variation among IG lines and trials within IG lines (Table 2).

Fig. 1 shows the phenotypic distribution of mating behaviour indices among IG lines. Courtship latency ranged between 2·3 and 43·6 min, while copulation latency ranged between 5·6 and 56·4 min among IG lines. The two time duration indices, courtship and copulation, ranged from 46 s to 23·7 min, and from 13 min to 37·3 min, respectively. Males that spent less time courting were able to copulate for a longer period of time ($R=-0{\cdot}21$; $P=0{\cdot}041$; $N=97$) while longer courting was positively correlated with courtship latency ($R=0{\cdot}30$; $P=0{\cdot}003$; $N=99$). Courtship intensity ranged between 29 % and a maximum of 97 % and showed a significant negative correlation only with copulation latency ($R=-0{\cdot}42$; $P<0{\cdot}001$; $N=96$).

We were able to detect measurable concentrations of both 7-tricosene and 7-pentacosene among the introgressed lines but 7,11-heptacosadiene (common in *D. sechellia* females) and 6-tricosene (common in *D. sechellia* males) were not detected. Phenotypic variation in the 7-tricosene to hexacosane ratio was significant among IG females ($F_{(1,68)}=2{\cdot}10$; $P=0{\cdot}001$) and males ($F_{(1,64)}=1{\cdot}84$; $P=0{\cdot}008$). We also detected significant variation among IG lines for the 7-pentacosene to hexacosane ratios (females: $F_{(1,68)}=2{\cdot}12$; $P=0{\cdot}001$; males: $F_{(1,64)}=1{\cdot}64$; $P=0{\cdot}025$).

More variation was found in pheromone content among females than males. The 7-tricosene to hexacosane ratio in females ranged between 2·6 and 10·4 and the 7-pentacosene to hexacosane ratio ranged between 0·12 and 0·72 (Fig. 2). The same ratios in males ranged only between 3·2 and 8·0, and 0·16 and

Fig. 1. Distribution of IG line means for male mating index phenotypes in minutes. The values of parental species are indicated by arrows, except for *D. sechellia* copulation and CI indices because only one *D. sechellia* male out of 44 tested mated with a *D. simulans* female. Values in parentheses show the full range of time scores for *D. simulans* parental species.

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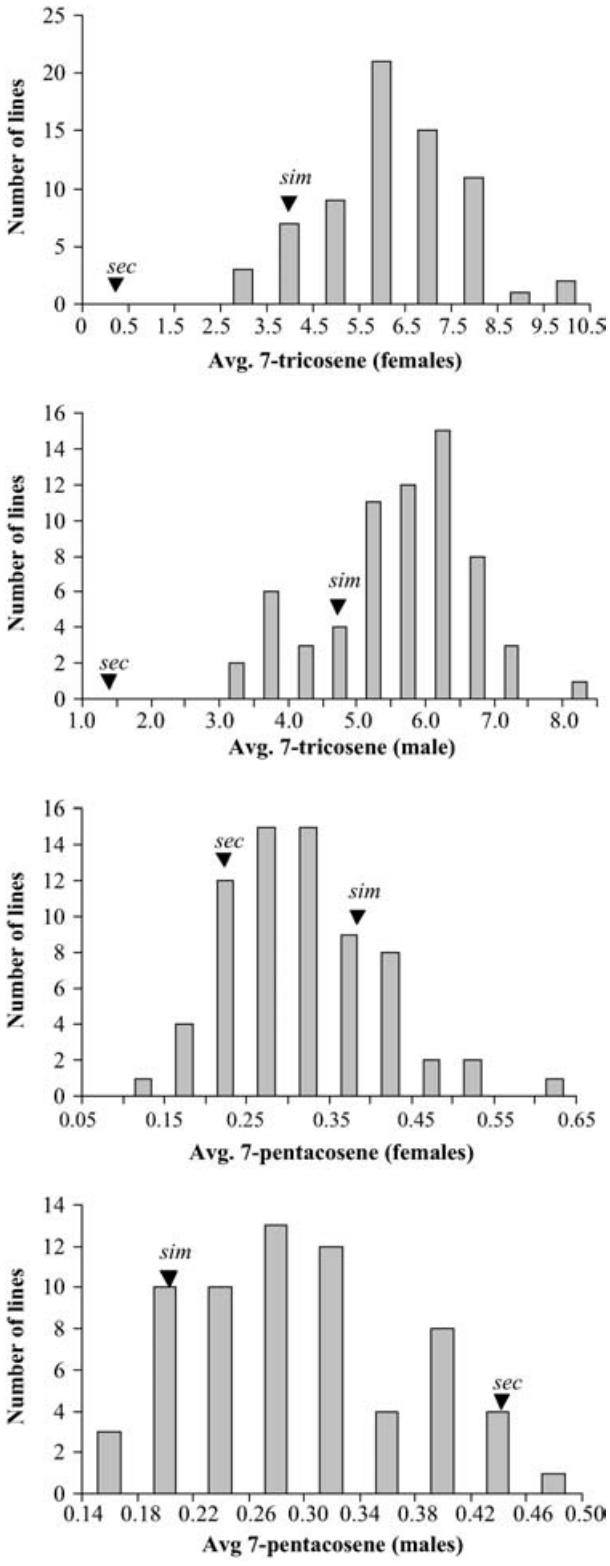

Fig. 2. Distribution of average IG male and female pheromone concentrations. Arrows indicate the values of parental species.

0·46 respectively (Fig. 2). Levels of 7-tricosene and 7-pentacosene between females and males were significantly correlated among lines (7-tricosene: $R=0{\cdot}352$; $P=0{\cdot}011$; $N=51$, and 7-pentacosene: $R=0{\cdot}44$; $P=0{\cdot}001$; $N=51$) and significant positive correlations were also detected between pheromones within sexes (males: $R=0{\cdot}630$; $P<0{\cdot}001$; $N=65$, and females: $R=0{\cdot}617$; $P<0{\cdot}001$; $N=68$).

(ii) *Marker–trait associations and QTL mapping*

Associations were first established between specific molecular markers and variation at each phenotype by regressing phenotypic values against genotype at a specific marker. The critical values for each chromosome were obtained by Bonferroni corrections with $P'=P/n$, where n is the number of markers per chromosome. A significant association was found between variation in courtship latency and a third chromosome marker (*AC001655*) at cytological position 84C1–4 ($F_{(2,97)}=11{\cdot}19$; $P=0{\cdot}0010<0{\cdot}05/10$). The same marker was significantly associated with copulation latency and courtship duration ($F_{(2,94)}=14{\cdot}92$; $P=0{\cdot}0001$ and $F_{(2,97)}=9{\cdot}36$; $P=0{\cdot}0025$ respectively).

To narrow down the position of the third chromosome QTL responsible for variation in CL, CpL and CD, we used interval and composite interval mapping approaches. Likelihood ratios of having a QTL responsible for variation in the phenotypes scored were tested by comparison with a random distribution of likelihood ratios obtained from permutation of the phenotypic values. CpL, CD and CL were significantly associated ($P<0{\cdot}05$) with overlapping genomic regions on the third chromosome and a maximum LR score at position 3-134·6 (Fig. 3). A 2 LOD support interval maps this QTL between 3-118 and 3-138·6, corresponding approximately to the 84A–88B cytological position in *D. melanogaster* (Civetta *et al.*, 2002). The detection of a significant QTL for these three indices in this region is in agreement with the association detected with marker *AC001655* (84C1–4) and the significant correlation detected among mating indices. This is most likely a single QTL that results in flies being slower at courting and needing longer courtship before achieving copulation. The assumption that this might be a single QTL is supported by the fact that the direction of the average phenotypic value of carriers of a *D. simulans* allele minus that for carriers of a *D. sechellia* allele at molecular marker *AC001655* is the same for all three indices. IG males that carry a *D. simulans* allele at molecular marker *AC001655* were quicker to start and spent less time courting (*sim-sec* allele effect $-0{\cdot}130\pm0{\cdot}026$ and $-0{\cdot}136\pm0{\cdot}036$ respectively), and their copulation latency was also shorter (*sim-sec* $=-0{\cdot}185\pm0{\cdot}025$).

Other marker–trait associations were established by linear regression analysis. Marker *Cdk7* on the X chromosome (4F1–2) and marker *da* on the second chromosome (31D11–E1) showed a significant association with variation in CI ($F_{(2,94)}=4{\cdot}78$; $P=0{\cdot}030$; $F_{(2,94)}=4{\cdot}48$; $P=0{\cdot}035$). Markers *aop* and *Acp26Ab*

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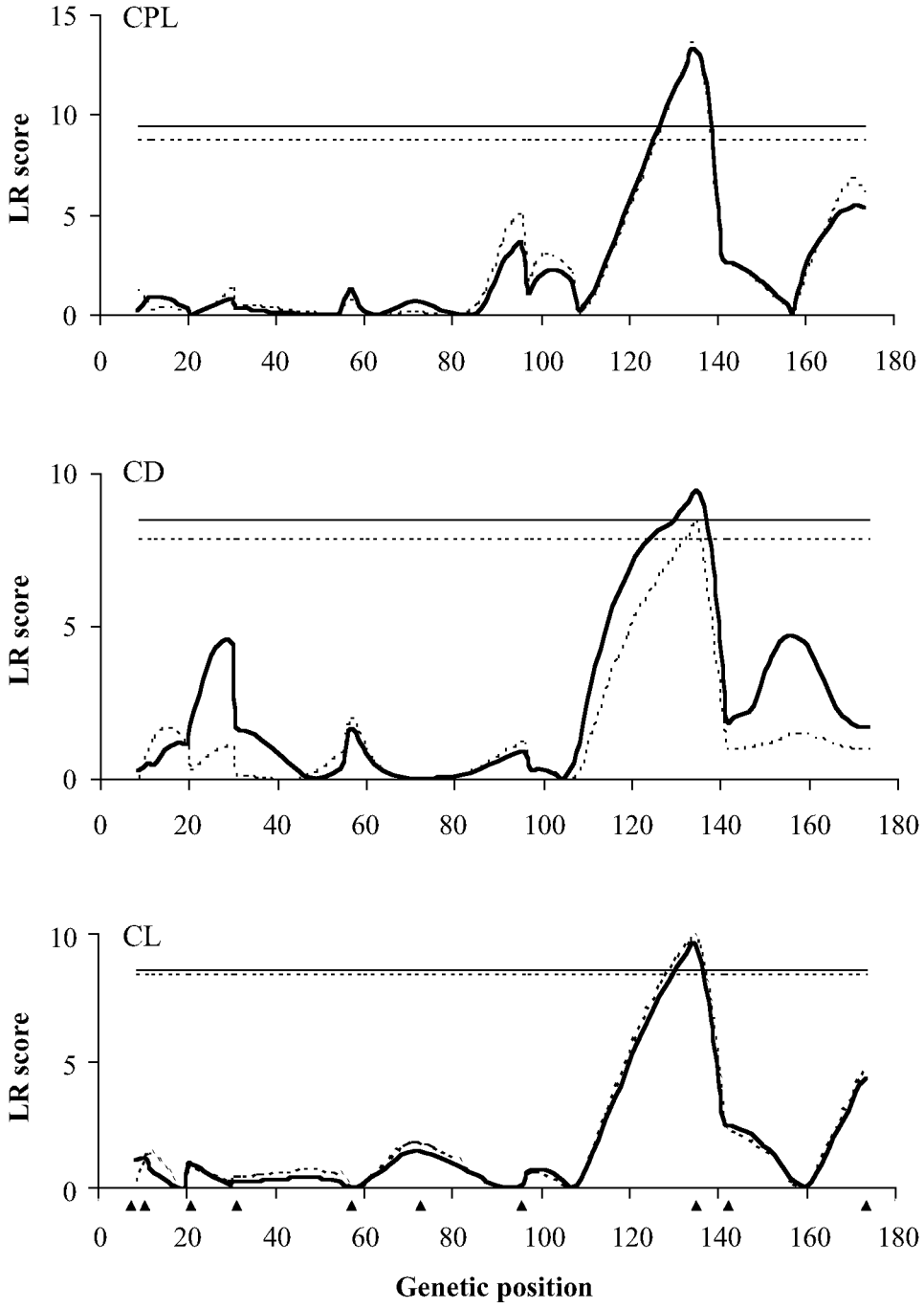

Fig. 3. Third chromosome QTLs for differences in male mating behaviour using interval (dashed lines) and composite interval mapping (continuous lines) models. Experiment-wise significance levels ($P<0{\cdot}05$) are shown across genetic positions. Marker positions are shown as triangles on the *x*-axis.

on the second chromosome showed a significant association with variation in CD ($F_{(2,97)}=4{\cdot}09$; $P=0{\cdot}044$; $F_{(2,97)}=6{\cdot}22$; $P=0{\cdot}0133$) and marker *cad* on the second chromosome associated with CL ($F_{(2,97)}=4{\cdot}33$; $P=0{\cdot}039$).

The direction of allele effects at these marker positions was not consistent. Males carrying a *D. simulans* allele at molecular marker *Cdk7* were less intense at courting (*sim-sec* $=-0{\cdot}293\pm0{\cdot}032$) while those carrying a *D. simulans* allele at the second chromosome marker *da* were more intense at courting (*sim-sec* $=0{\cdot}324\pm0{\cdot}057$). Second chromosome markers affecting courtship duration also had opposite effects (*aop*: *sim-sec* $=0{\cdot}313\pm0{\cdot}130$; *Acp26Ab*: *sim-sec* $=-0{\cdot}432\pm0{\cdot}170$). IG lines carrying *D. simulans* alleles at chromosome 2 marker *cad* (38E5–6) took longer to start courting ($0{\cdot}307\pm0{\cdot}026$).

For variation in cuticular pheromone concentrations, no single marker trait association or QTLs were detected for variation in 7-pentacosene. Weak

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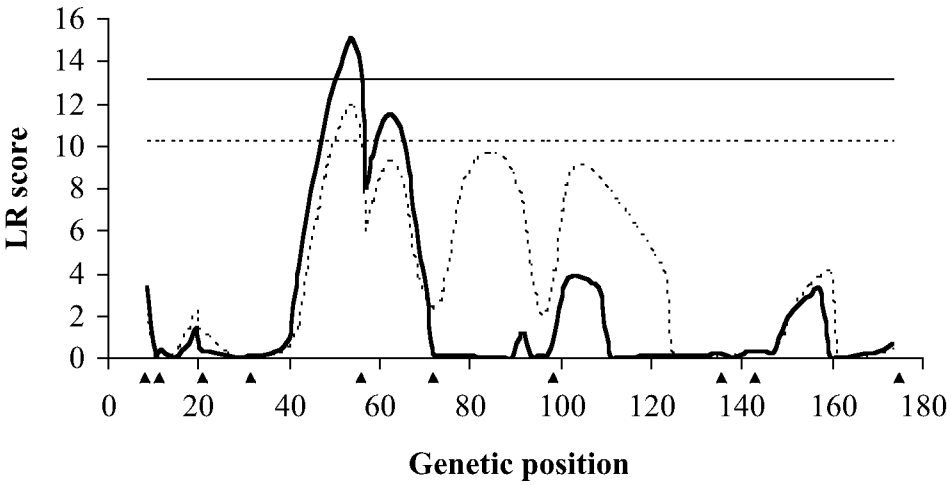

Fig. 4. Third chromosome QTL for differences in 7-tricosene concentration among males using interval (dashed lines) and composite interval mapping (continuous lines) models. Levels of significance and markers position are shown as in Fig. 3.

associations were detected between marker *Sh* at cytological position 16E4–F1 ($F_{(2,67)}=4{\cdot}74$; $P=0{\cdot}031$) and *Amy-d* (53F13–54A2; $F_{(2,67)}=5{\cdot}55$; $P=0{\cdot}019$) for variation in 7-tricosene concentration in females. IG lines carrying the simulans allele at *Sh* showed a lower concentration (*sim-sec* $=-0{\cdot}843\pm0{\cdot}361$) of 7-tricosene and those carrying the simulans allele at *Amy-d* showed a higher concentration (*sim-sec* $=1{\cdot}213\pm0{\cdot}395$) of 7-tricosene. Marker *DM22F11T* (73A1–B7) showed a significant association with variation in male 7-tricosene concentrations ($F_{(2,63)}=4{\cdot}17$; $P=0{\cdot}042$) and the *simulans* allele led to higher concentrations of the pheromone (*sim-sec* $=1{\cdot}745\pm0{\cdot}143$). Only one significant QTL for variation in male 7-tricosene concentration was found with maximum LR score at position 3-54.3. A 2 LOD support interval maps the QTL in the genetic interval 3-44 to 3-68 (Fig. 4). This position corresponds approximately with cytological position 68E–76E in *D. melanogaster* (Civetta *et al*., 2002).

4. Discussion

The distribution of mating behaviour phenotypes among IG lines is closer to that of the *D. simulans* parental line as their genome is mainly from this species. However, the distribution exceeds the range of the parental species for at least some of the behaviours analysed. Such phenotypic transgression has been shown for recombinant inbred lines of *Drosophila melanogaster* in QTL studies of traits such as mean lifespan, bristle number and wing interpulse intervals (Nuzhdin *et al*., 1997; Gurganus *et al*.,1998; Gleason *et al*., 2002) as well as for interspecific hybrids (Dobzhansky, 1952). Between closely related species such a phenomenon could be a result of complementation between alleles from different species creating a heterosis-like effect or misregulation of behaviour if the genes controlling the phenotype have diverged beyond the point of being able to interact properly. In our case, the introgression of *D. sechellia* alleles has disrupted the phenotypes, creating a distribution beyond the values observed for parental species.

We have shown that variation in the behavioural aspects of mating recognition between *Drosophila simulans* and *D. sechellia* involves at least one locus on the third chromosome. Males carrying a *D. sechellia* allele at this site were slower to start courting and achieve copulation, and they spent more time courting. The directionality of the effect could suggest that the introgression of a *D. sechellia* allele becomes detrimental for the males in terms of the amount of time and energy spend courting. However, the lack of fitness experiments and/or more QTLs to test directionality of allele effects (Orr, 1998) make this assumption difficult to support. If we consider all alleles at markers with some marginally significant effect on any aspect of courtship and copulation, the introgression of *D. sechellia* alleles does not show any directionality. While *D. sechellia* alleles at a QTL on the third chromosome with an effect on different aspects of courtship behaviour lead to these flies spending more time courting than carriers of a *D. simulans* allele, other marker–trait associations (on the X and second chromosome) showed the opposite direction. Carrying a *D. sechellia* allele at markers with an effect on mating behaviour can lead to shorter time courting (*aop* and *cad*) and more intense courting (*Cdk7*) than carrying a *D. simulans* allele. Although it is clear that the introgression of *D. sechellia* alleles into a *D. simulans* background disrupts male mating behaviour in terms of releasing phenotypic variance, it does not appear to disrupt mating behaviour in any particular direction that might imply a tightly co-adapted genetic system. Rather, *D. sechellia* introgressions seem to affect the behavioural phenotype in a random manner.

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The third chromosome QTL with an effect on mating behaviour is approximately within the interval 84A–88B, which includes *doublesex* (84E5–6), a gene of the sex determination pathway that also affects courtship song (Villella & Hall, 1996), and *Voila* (86E1–2), a gene that affects taste and male courtship behaviour in *D. melanogaster* (Balakireva *et al*., 1998; Grosjean *et al*., 2001). It has been shown in *Drosophila melanogaster* that misexpression of sex determination genes in the male's brain is responsible for triggering bisexual behaviour and that this is mediated via sex-specific splicing of *dsx* and *fru* (An *et al*., 2000; Nilsson *et al*., 2000).

None of the distinctive pheromones produced by *D. sechellia* (6-tricosene and 7,11-heptacosadiene) could be detected in the introgression lines, suggesting that there is a polygenic basis for the species difference in production of these pheromones or that a major gene(s) responsible for the production of these pheromones in *D. sechellia* was not introgressed into the *D. simulans* genome. The distribution of phenotypes for 7-tricosene and 7-pentacosene concentrations showed a similar pattern to that of mating behaviour indices, with a distribution beyond the parental species values but closer to *D. simulans*.

Our QTL approach found a single locus on the third chromosome but does not rule out a polygenic basis to explain interspecific differences in male tricosene production (Coyne, 1996), it simply narrows down the mapping to a locus with major phenotypic effect. Our result of a single third chromosome QTL is in agreement with Coyne's result (1996) as he detected a twice as large an effect attributable to the third chromosome. The introgression of *D. sechellia* alleles at the QTL responsible for variation in male 7-tricosene caused a reduction in 7-tricosene concentrations. Although the third chromosome QTLs with an effect on mating behaviour and pheromone production did not overlap, the localization of the QTLs affecting interspecific differences in mating behaviour and pheromone production on the third chromosome is in agreement with previous studies that analysed the genetics of pre-mating isolation barriers between cosmopolitan and African populations of *D. melanogaster* and suggested a major role for the third chromosome (Hollocher *et al*., 1997; Colegrave *et al*., 2000; Ting *et al*., 2001). Further dissection of the genetic basis of pre-mating isolation barriers between divergent populations *of D. melanogaster* and closely related species of Drosophila could therefore benefit from a closer examination of third chromosomes. The genetic intervals detected as having an effect on variation in 7-tricosene production among IG lines did not include the *desaturase* genes (87B9) or the Antennapedia/Bithorax complexes that have previously been shown to affect intraspecific polymorphism in cuticular hydrocarbon production in *D. melanogaster* (Wicker-Thomas *et al*., 1997; Coyne *et al*., 1999; Wicker-Thomas & Jallon, 2001; Labeur *et al*., 2002).

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