Genet. Res., Camb. (2006), **87**, *pp.* 93–107. © 2006 Cambridge University Press
doi:10.1017/S0016672306008111 Printed in the United Kingdom

Genetic architecture of variation in sex-comb tooth number in *Drosophila simulans*

HARUKI TATSUTA[1]† AND TOSHIYUKI TAKANO-SHIMIZU[1,2]*
[1] *Department of Population Genetics, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan*
[2] *School of Advanced Sciences, The Graduate University for Advanced Studies (SOKENDAI), Shonan Village, Hayama, Kanagawa 240-0193, Japan*

(*Received 7 June 2004 and in revised form 3 January 2006*)

Summary

The sex comb on the forelegs of *Drosophila* males is a secondary sexual trait, and the number of teeth on these combs varies greatly within and between species. To understand the relationship between the intra- and interspecific variation, we performed quantitative trait locus (QTL) analyses of the intraspecific variation in sex-comb tooth number. We used five mapping populations derived from two inbred *Drosophila simulans* strains that were divergent in the number of sex-comb teeth. Although no QTLs were detected on the X chromosome, we identified four QTLs on the second chromosome and three QTLs on the third chromosome. While identification and estimated effects of the second-chromosome QTLs depend on genetic backgrounds, significant and consistent effects of the two third-chromosome QTLs were found in two genetic backgrounds. There were significant epistatic interactions between a second-chromosome QTL and a third-chromosome QTL, as well as between two second-chromosome QTLs. The third-chromosome QTLs are concordant with the locations of the QTLs responsible for the previously observed differences in sex-comb tooth number between *D. simulans* and *D. mauritiana*.

1. Introduction

The maintenance and significance of intraspecific variation are two major unsolved issues in studies of phenotypic evolution. One hypothesis is that most variants contributing to quantitative phenotypic variation are deleterious and are quickly removed from a population by natural selection. Intraspecific variation rarely contributes to interspecific divergence, and therefore it may require some additional mechanisms. One such mechanism is a population bottleneck, which may cause breakdowns of balanced genetic systems and allow genetic revolution (e.g., Mayr, 1954; Carson, 1975; Templeton, 1980). According to this view, phenotypic evolution is irregular and a small number of genes with large and epistatic effects are assumed to be involved in speciation and interspecific divergence. Alternatively, intraspecific variants may be neutral or maintained by balancing selection and serve as a potential for future evolutionary response. In this scenario, intraspecific variants may be fixed in a population by random genetic drift or selection, and interspecific divergence is viewed as the accumulation of such fixations. As a result, a relatively large number of genes with small effects are expected to be involved in interspecific divergence.

Comparisons of the genetic architectures of intraspecific variation and interspecific divergence for the same character will lead to a better understanding of the evolutionary process. We are now able to genetically dissect trait variation with increased resolution due to the availability of dense molecular markers and the development of quantitative trait locus (QTL) mapping methods (e.g., Lander & Botstein, 1989; Zeng, 1994). These genetic approaches naturally focus on closely related species pairs for the following two reasons: (i) requirement of cross-fertility and (ii) resolution of individual evolutionary changes. The relevance of the latter lies in the fact that the longer the divergence time of the two species studied, the

* Corresponding author. Tel: +81 55 9816781. Fax: +81 55 9816784. e-mail: totakano@lab.nig.ac.jp
† Present address: Laboratory of Ecological Risk Assessment, National Institute for Environmental Studies, Tsukuba, Ibaraki, 305-8506, Japan.

more mutations they would have accumulated. We have a better chance of identifying the causative gene from a small number of such genes if we focus on closely related species pairs. In this regard, special attention is paid to rapidly evolving characters, such as sexually dimorphic characters under sexual selection. Kopp *et al*. (2000) studied the sexually dimorphic abdominal pigmentation pattern in *Drosophila* and found that expression of the *bric-a-brac* (*bab*) gene was a key element for the evolution of sexually dimorphic pigmentation pattern. This *bab* gene is also a putative candidate gene responsible for the variation in female abdominal pigmentation within *D. melanogaster* (Kopp *et al*., 2003). In this case, both intra- and interspecific variation may be governed by the same gene (Kopp *et al*., 2000).

A good example of a rapidly evolving secondary sexual trait is the male sex comb of *Drosophila melanogaster*. The sex comb, a specific row of enlarged bristles on the foreleg of males, is a recently derived trait that is found almost exclusively in the *melanogaster* and *obscura* species groups in the subgenus *Sophophora*. Its structure varies greatly among species with respect to the numbers of sex combs and their teeth, and orientation of the sex comb (Kopp & True, 2002; Schawaroch, 2002). For instance, whereas *D. melanogaster* and its relatives in the *melanogaster* species subgroup have a sex comb only on the first tarsal segment of the foreleg, most species of the *montium* subgroup have one sex comb on each of the first and second tarsal segments. In contrast, *D. lucipennis* in the *suzukii* species subgroup lacks the sex comb entirely.

Behavioural studies suggest that in some species the sex comb plays an important role in mating success (Spieth, 1952; Cook, 1977; Coyne, 1985). The number of teeth in the sex combs in *D. mauritiana* is significantly greater than in *D. simulans* (13·9 vs 10·3 per foreleg in True *et al*., 1997), and surgical removal of the foreleg tarsi (including sex combs) reduces the frequency of copulatory success to a larger degree in *D. mauritiana* than in *D. simulans* (Coyne, 1985).

A small number of studies have examined the genetic basis for the variation in sex-comb tooth number, especially in *D. melanogaster* and its sister species from the *simulans* clade of the *melanogaster* species complex: *D. simulans*, *D. mauritiana* and *D. sechellia* (Coyne, 1985; True *et al*., 1997; Macdonald & Goldstein, 1999; Nuzhdin & Reiwitch, 2000; Kopp *et al*., 2003). True *et al*. (1997) studied differences in sex-comb tooth number between *D. simulans* and *D. mauritiana*, and identified two QTLs responsible for the interspecific differences on the third chromosome. In the same backcross design, Macdonald & Goldstein (1999) found one X-chromosome and three second-chromosome QTLs responsible for the differences in tooth number between *D. simulans* and *D. sechellia*. No evidence for shared QTLs was observed in these two studies. Based on the results of True *et al*. (1997), Nuzhdin & Reiwitch (2000) undertook a QTL analysis using recombinant inbred strains between two *D. melanogaster* strains with different sex-comb tooth numbers to test whether the same genes were responsible for intra- and interspecific variation. Despite a small difference in the mean number of teeth on the sex combs between the two strains (9·1 vs 10·5 teeth per foreleg), Nuzhdin & Reiwitch (2000) identified two QTLs on the X chromosome. More recently, Kopp *et al*. (2003) used a panel of recombinant inbred lines of *D. melanogaster* to identify a new third-chromosome QTL. Except for several weak and suggestive QTLs, the positions of these QTLs responsible for the within-*melanogaster* variation differed from those of the interspecific differences. These analyses, however, suffer from the fact that the comparisons have not involved a common species. The intraspecific variation was genetically studied within the context of *D. melanogaster* (Nuzhdin & Reiwitch, 2000; Kopp *et al*., 2003), while the interspecific differences in the number of sex-comb teeth were studied in the sister clade of *D. simulans*, *D. mauritiana* and *D. sechellia* (True *et al*., 1997; Macdonald & Goldstein, 1999). Therefore, an analysis of within-*simulans* variation should provide valuable data for comparisons with the interspecific variation.

Here we present the results of our QTL analyses for the variation in sex-comb tooth number between two *D. simulans* strains. We performed QTL mapping on a whole-genome F_2 and four specialized populations: two second-chromosome and two third-chromosome mapping populations in homozygous backgrounds. Background homogeneity should reduce genetic complexity and thus increase the power for detecting QTLs (e.g., Shrimpton & Robertson, 1988; Long *et al*., 1995). What is important is that this study allows an independent and phylogenetically closer comparison of the genetic architectures of intra- and interspecific variations in this character.

Coyne (1985) reported low intraspecific variation for tooth number for five *D. melanogaster* strains (9·5–10·7 per foreleg) and six *D. simulans* strains (9·9–10·5 per foreleg); however, we observed considerable variation among *D. simulans* strains. The two parental strains used in the present study had significantly different numbers of sex-comb teeth (18·5 vs 24·7 per individual). The strain difference divided by the square root of the variance within strains (strain difference in environmental standard deviation units) was 4·3, which was even larger than those in the interspecific comparisons for this character (3·5 in the *D. simulans* – *D. mauritiana* comparison in True *et al*., 1997; 3·1 in the *D. simulans* – *D. sechellia* comparison in Macdonald & Goldstein, 1999). All else being equal, the large difference should provide similar

analytical power to that of the interspecific comparisons. In this study, we analysed 27 markers for approximately 200 flies in each mapping population. These numbers are also comparable to those in True *et al*. (1997) and Macdonald & Goldstein (1999) (18 and 38 markers respectively, for about 200 flies in each of two backcross analyses). True *et al*. (1997) and Macdonald & Goldstein (1999) both used backcross designs; in contrast, we used inter-cross designs of F_1 progeny. In this study, we found a significant dominance-by-dominance interaction effect between QTLs on different chromosomes. In a backcross design, dominance effects and additive-by-dominance and dominance-by-dominance interaction effects cannot be tested, because there are only two genotypes at each locus. In addition to the whole-genome intercross F_2 analysis, we studied the second-chromosome effects in two isogenic backgrounds of the strains with high and low sex-comb tooth number; a backcross design cannot produce recombinant homozygotes (e.g., homozygote for a high strain allele in the homozygous background of a low strain allele). From the experiments, we identified further QTLs and an additive-by-additive interaction.

2. Materials and methods

(i) Drosophila *strains*

Six inbred and three isofemale strains of *D. simulans* and four inbred strains of *D. mauritiana* were used to study variation in the number of sex-comb teeth (Takano, 1998; Takano-Shimizu, 2000). The *D. simulans* inbred strains were: A1 (Australia), Sim-3 (Raleigh, North Carolina), Sim-5 (Brazzaville, Congo), Sim-11 (Ethiopia), Tananarive (Madagascar) and Shira-2 (Kofu, Japan). *D. simulans* isofemale strains were: SAvo-15 (Madagascar) and Sey-4 (Seychelles) provided by S. I. Chigusa, and Sim-12 (Madagascar). *D. mauritiana* inbred strains were: g76, 75, Robertson and Les Galets.

For subsequent QTL mapping experiments, we used two inbred strains of *D. simulans* – Sim-3 (Raleigh, North Carolina) and Tananarive (Madagascar) – which were established by sib mating for 20 generations (Takano-Shimizu, 2000). We performed four (Sim-3) and two (Tananarive) further generations of single-pair matings for homozygosity tests. Homozygosity at all 27 marker loci (Table 1) was confirmed by genotyping both female and male parental flies of the single-pair matings.

(ii) *DNA markers*

We examined, in total, 27 markers, of which five molecular markers were located on the X chromosome, 10 on the second chromosome and 12 on the third chromosome (Table 1). We used four PCR-based genotyping methods: single-strand conformation polymorphism (PCR-SSCP; Orita *et al*., 1989; Takano-Shimizu, 1999), length polymorphism, allele-specific oligonucleotide (ASO) hybridization assay (modified from Maekawa *et al*., 1995 and Saiki *et al*., 1986) and restriction fragment length polymorphism (PCR-RFLP). PCR reactions were carried out with 32 cycles, each cycle comprising 30 s at 94 °C, 30 s at 60 °C or 55 °C, and 30 s at 72 °C. Annealing temperatures and Mg^{2+} concentrations specific to each primer pair and wash temperature in the ASO assay are provided in Table 1.

(iii) *Sex-comb tooth number score of inbred and isofemale strains of* D. simulans *and* D. mauritiana

Two vials were set up for the study of the number of sex-comb teeth for each of the nine *D. simulans* strains, and one vial for each of the four *D. mauritiana* strains. The vials each contained three to five females and three to five males. Five males were collected from each vial and counted for the number of sex-comb teeth on both legs, except for five vials (two or three males per vial). We did not detect a significant vial effect for the *simulans* strains. Among-strain and between-species variation was studied by two-level mixed model nested analysis of variance after pooling the data from two vials for the *simulans* strains. We tested between-locality differences by using strain means as replicates.

(iv) *Sex-comb tooth number score of Sim-3, Tananarive and* F_1 *flies*

Sex-comb tooth numbers were counted again for the Sim-3 and Tananarive strains and for the F_1 progeny in both directions. Five vials each were set up for crosses of Sim-3 × Sim-3, Tananarive × Tananarive, Sim-3 females × Tananarive males and Tananarive females × Sim-3 males, in which each vial contained five females and five males. One vial of crosses of Sim-3 females with Tananarive males did not produce any progeny. Ten male progeny were collected from each vial, and the numbers of sex-comb teeth on both forelegs were pooled and analysed.

(v) *Five mapping populations*

We established five different mapping populations with the two strains Sim-3 and Tananarive. For the whole-genome F_2 mapping population, we first made crosses between Sim-3 and Tananarive in both directions (i.e., Sim-3 females × Tananarive males = S*T and Tananarive females × Sim-3 males = T*S) and then made 50 crosses of five F_1 males and five F_1 females (12 crosses of females from S*T × males from

https://doi.org/10.1017/S0016672306008111 Published online by Cambridge University Press

Table 1. *Molecular markers*

Marker	Cytological position	PCR primers	PCR condition (annealing temperature, °C; magnesium concentration, mM)	Typing method[a]
X chromosome				
shaggy (*sgg*)	3A8–B2	TCCAGCAATCACAGCAAACT ACTTCATCTTCGCCTCCTCT[b]	60; 2·0	Length variation
G01498	8A1–2	GTGGTATGTTGGACACCTTCC AAGTGAGCGTTCTCCTTCTCC[c]	60; 2·0	SSCP
Dm1865	10F2–11	TACAACGTCGTCATGTTTGG GCCCACATAGCTATGGGTATT[c]	60; 2·0	SSCP
Dm0478	13A8–9	AGACTCAGCTCGTTGGTCAC GGATAGTGCCATACTGCTCAA[c]	60; 2·0	SSCP
runt (*run*)	19E2	CAGTGGGATAGAAGGATAAAC ATGAGTCCGAGCATAAAACTT[b]	60; 2·0	SSCP
Second chromosome				
decapentaplegic (*dpp*)	22F2–3	CTGCCAGATACGAAGAGTTGG AGCACTGCGAGGAGTAGAAGC	60; 2·5	*Tru*9 I
Phosphoglycerate kinase (*Pgk*)	23A3	GGCAAGCGGGTGTTGATGCG TTGGCAGGATCGGCCTTGAC[d]	60; 2·0	*Alu* I
neither inactivation nor afterpotential C (*ninaC*)	27F3	GAAGTCCATCTTCCAGGTCC TATGGGCACTGGCAGTGGTC[d]	60; 1·5	*Alu* I
paired (*prd*)	33C1–2	GATGCAAGGTGAGTGTCTATCG GAGGAAGATGAGGAAATGGAA	60; 2·0	Length variation through *Bgl* II digestion
Dopa decarboxylase (*Ddc*)	37C1	TGGCTGACGAGAAGAAGAAC CAAGATTCCGGAAGACGACG[d]	60; 2·0	*Fok* I
even skipped (*eve*)	46C3–11	TTGTGGACCTCTTGGCCACC AACTCCTTCTCCAAGCGACC[d]	60; 1·5	*Rsa* I
vestigial (*vg*)	49E1	GCTCCAAAGGTGAAACTAATC GCGAGTGCTTCGTCGGAAAT	60; 2·0	Length variation
slit (*sli*)	52C9–D1	TTACCAGCTTTAAGGGCTGC CATTTGTTCTCCAGGCAAGG[d]	60; 1·5	*Scr*F I
Polycomblike (*Pcl*)	55B8	TTTGTCGCCAGTTGCCCTACC GCTGATGCTTTCGCAGATTGT	60; 2·0	CGAGATTCATCTCAGTTT (45/47) CTAAACTGAAATGAATCTC (45/47)
twist (*twi*)	59C2	TCCCTGCAGCAGATCATCCC ATCACTCGAGCTGAGCATGC[d]	60; 1·5	CCCTTAAACTTGCCA (41/45) TGGCCAGTTTGAGGG (41/46)
Third chromosome				
veinlet (*ve*)	62A1–2	GAGAACCCAACGCAGAATGT ATATCCTCCGACTCCGGAAG[d]	55; 1·5	GGTGGCACGCTCCTC (45/56) GAGGAGCATGCCACC (45/50)
hairy (*h*)	66D15	ACTCAAGACTCTGATTCTGG TGTCTTCTCCAGAATGTCGG[d]	55; 1·5	*Tru*9 I
Ecdysteroid-inducible polypeptide encoded in 71CD (*Eip71CD*)	71C3–D2	CCTGTATGGAGCCACCCG GGGGCTGAGATTTAGCGATG[e]	60; 2·0	*Scr*F I
Serotonin receptor 2 (*5-HT2*)	82C5	TGACGATTCCCCTCCTCC CGCCCACTGATAGGAATTTG[e]	60; 2·5	*Taq* I

Antennapedia (*Antp*)	84B1–2	ACGGACGTTGGAGTTCCCGA ACATGCCCATGTTGTGATGG[d]	60; 2·5	*Tru*9 I	
neither inactivation nor afterpotential E (*ninaE*)	92B6–7	CTACATCTGGTTCATGTCGAGC CCACAGCGTGATGGTGAC[e]	60; 2·0	*Hinc* II	
Male-specific RNA 87F (*Msl87F*)	87F14	TCCTTTGCCTCTTCAGTCCG TCCACAGGCATAGCATGGTC[d]	60; 2·5	*Rsa* I	
hunchback (*hb*)	85A7	TCTGCCCATCTAATCCCTTG CTGCGTCGAGTTTTTTCCTC[d]	60; 1·5	*Scr*F I	
Aldolase (*Ald*)	97A6	CCAACCATTGATTCCATTTCC AAGTATCTTTTCCAGCGAACC	60; 2·5	Length variation	
Myosin light chain 1 (*Mlc1*)	98A14–15	ACCCTGTTCGCTGACTGC AAGTAGACATCCAGAGATCGGC[e]	60; 2·0	ACATTAGATCAGGATCGT (46/51) GACGATCCTAATCTAATG (46/51)	
janus A (*janA*)	99D3	CGCATTGAACACAATCCCGA CTCATCGGAGATTTCGATCG[d]	60; 2·0	*Fok* I	
Elongation factor 1α100E (*Ef1α100E*)	100D2	GACTGGTCTCCTCAAGCCAG AGCCTCGTGGTGCATCTC[e]	60; 2·0	*Hae* III	

[a] Restriction enzyme for RFLP or two probe-oligonucleotide sequences for ASO with hybridization and wash temperatures are given.
[b] From Takano-Shimizu (2000).
[c] Berkeley Drosophila Genome Project sequence tagged sites (STSs).
[d] From Liu *et al.* (1996).
[e] From Zeng *et al.* (2000).

S*T; 13 crosses of S*T females × T*S males; 13 crosses of T*S females × S*T males; 12 crosses of T*S females × T*S males). We randomly selected four F_2 male progeny from each cross for genetic analysis for a total of 200 F_2 males.

The analysis of the whole-genome F_2 mapping population uncovered multiple sex-comb QTLs on both the second and third chromosomes. Genetic dissection of the variation in a completely homozygous background reduces the genetic complexity and increases the precision of phenotyping for each genotype. For this purpose, we made two second-chromosome and two third-chromosome mapping populations in homozygous backgrounds as follows.

The second-chromosome F_2 mapping population in the Sim-3 background (IIs mapping population) was generated by the following procedure (Fig. 1*a*). F_1 males of Sim-3 × Tananarive crosses were backcrossed to Sim-3 females. Each F_2 offspring possessed one of the four possible second- and third-chromosome combinations (*S*/*T*; *S*/*T*, *S*/*T*; *S*/*S*, *S*/*S*; *S*/*T* and *S*/*S*; *S*/*S*). Forty F_2 males were then backcrossed individually to Sim-3 females. A few days later, we extracted genomic DNA from the males and determined the genotypes for *prd* on the second chromosome and *Ald* on the third chromosome. Four of the 40 F_2 males were hemizygous for the Sim-3 X chromosome, heterozygous for the second chromosome, and homozygous for the Sim-3 third chromosome (*S*/Y; *S*/*T*; *S*/*S*). Only these four crosses were kept for the subsequent crosses and all the others were discarded. Fifty one-pair matings between F_3 progeny from these four crosses were made, and the chromosome constitutions of the parental flies were individually examined in the same manner. We obtained 20 G_3 crosses of *S*/*S*; *S*/*T*; *S*/*S* × *S*/Y; *S*/*T*; *S*/*S*, and the male offspring were examined for sex-comb tooth number and marker genotypes. Similarly, we made 29 G_3 crosses of *T*/*T*; *S*/*T*; *T*/*T* × *T*/Y; *S*/*T*; *T*/*T* to produce the second-chromosome F_2 mapping population in the Tananarive background (IIt mapping population). The total sample size was 212 for the IIs mapping population (2–12 males from each of 20 crosses) and 194 for the IIt population (4–7 males from each of 29 crosses).

Two third-chromosome congenic strains were made in the Sim-3 background (IIIs-1 and IIIs-2) as shown in Fig. 1*b*. We repeated the following selection-and-backcross procedure six times: select males with high sex-comb tooth number, backcross to Sim-3 females, and perform mass mating of offspring to produce homozygotes. This was followed immediately by three or five generations of sib mating (a single female or two females × a single male cross) to make congenic strains. During this process, we identified two significant QTLs on the third chromosome (one between *Antp* and *ninaE* and the other between

https://doi.org/10.1017/S0016672306008111 Published online by Cambridge University Press

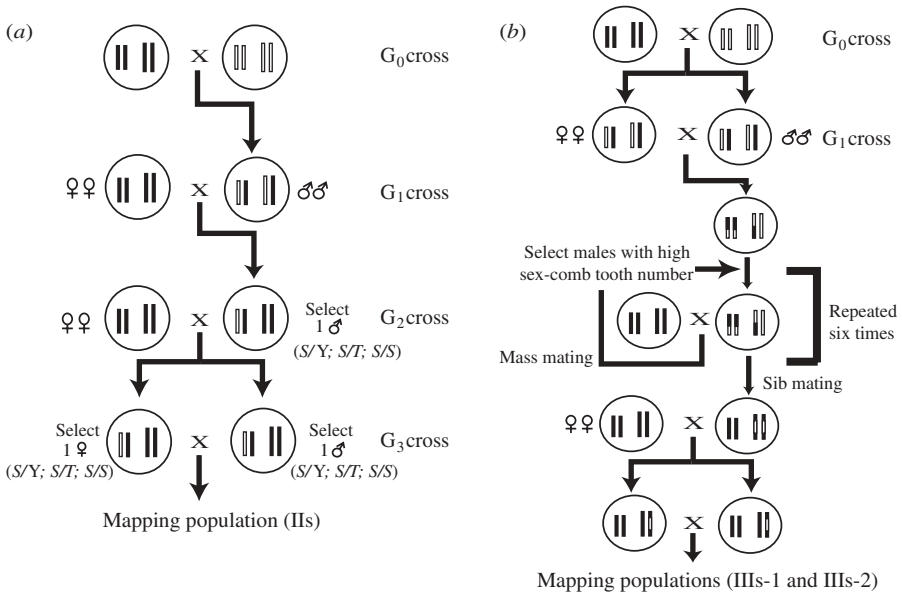

Fig. 1. Mating scheme for construction of the second-chromosome F_2 mapping population in Sim-3 background and third-chromosome congenic strains. (*a*) The scheme for second-chromosome F_2 mapping population in the Sim-3 background (IIs). (*b*) The scheme for third-chromosome congenic-strain mapping populations in the Sim-3 background (IIIs-1 and IIIs-2). Short bars in circles represent the second chromosomes and long bars represent the third chromosomes. The filled and open bars indicate the Sim-3 and Tananarive chromosomes, respectively. See Section 2 for a detailed explanation.

hb and *Ef1α100E*) from a survey of the whole-genome F_2 mapping population, and therefore we concentrated on the construction of the following two congenic strains. The IIIs-1 congenic strain carried Sim-3 alleles at all marker loci studied except *Antp* and *ninaE*, which were from Tananarive. The IIIs-2 congenic strain carried Tananarive alleles only at *hb*, *Ald*, *Mlc1*, *janA* and *Ef1α100E*. For each of the congenic strains, we set up 20 vials simultaneously, each containing five female and five male F_1 progeny of the congenic strains with Sim-3. Ten male progeny per vial were studied for sex-comb tooth number and genotypes of specific third-chromosome markers, except for one vial of the IIIs-2 (two males). The total sample size was 200 for the IIIs-1 congenic-strain mapping population and 192 for the IIIs-2 population.

(vi) *Construction of genetic maps of* D. simulans *chromosomes*

We calculated crossover frequencies in all pairwise combinations of two markers from the 5 X-chromosome, 10 second-chromosome and 12 third-chromosome markers in the whole-genome F_2 mapping population. Map distances were calculated with the map function described in Foss *et al.* (1993; with $m=4$), which accounted very well for the pattern of interference on the X chromosome of *D. melanogaster*. The resulting map was used for all subsequent analyses.

(vii) *QTL mapping*

The numbers of sex-comb teeth on both forelegs were pooled and used in all mapping analyses. We applied two types of QTL mapping analyses: composite interval mapping (CIM) and multiple interval mapping (MIM) analyses.

First, QTLs were identified by CIM (Zeng, 1994). We used the Windows QTL Cartographer program (v2.0; Wang *et al.*, 2004) to calculate the likelihood ratio (LR) for hypothesis testing under Model 6 of Zmapqtl (with a window size of 10 cM as default value; Basten *et al.*, 2002). Different window sizes (1 cM, 5 cM and 15 cM) did not affect the number of QTLs. For the two autosomes, the LR statistic was $-2\ln(L_0/L_3)$, where L_0 is the maximum likelihood under the null hypothesis (H_0) of a (additive effect of putative QTL)$=0$ and d (its dominance effect)$=0$, and L_3 is the maximum likelihood under the alternative hypothesis (H_3) of $a\neq0$ and $d\neq0$. For the X chromosome, the LR statistic was $-2\ln(L_0/L_1)$ where L_1 is the maximum likelihood under the null hypothesis (H_1) of $a\neq0$ and $d=0$. Using the program implemented in QTL Cartographer (v2.0; Wang *et al.*, 2004), we estimated experiment-wise significance levels ($\alpha=0{\cdot}05$) for QTL detection from 1000 permutations in which the phenotypic data were shuffled against genotypic data (Churchill & Doerge, 1994). For the IIIs-1 and IIIs-2 mapping populations, we used the critical value estimated from the whole-genome F_2 mapping population because the numbers of markers in IIIs-1 and IIIs-2 were too few to calculate practical

https://doi.org/10.1017/S0016672306008111 Published online by Cambridge University Press

critical values with the randomization procedure. In fact, the critical value used (13·0) was larger than that estimated from the permutation tests of IIIs-1 and IIIs-2 data (6·4 and 7·9, respectively), implying that our tests are conservative. To estimate the confidence intervals for the QTL positions, we calculated the 2LOD support interval of each QTL position as in Lander & Botstein (1989).

We also performed a MIM analysis (Kao *et al*., 1999) using the MImapqtl in QTL Cartographer v2.0 (Wang *et al*., 2004). This evaluates both the main effects and epistatic interactions between QTLs (Zeng *et al*., 2000). We used two information criteria (ln(n) and 2ln(ln(n)), where ln indicates the natural logarithm function and n is the sample size) as the threshold for adding or deleting parameters. The MIM analysis was applied to the entire genome, IIs and IIt F_2 mapping populations.

Epistatic interactions between QTLs were also studied by analysis of variance (ANOVA) based on the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{k(ij)},$$

where Y_{ijk} is the phenotypic value of the kth individual carrying ith genotype at the first locus ($i = 1, 2, 3$) and jth genotype at the second locus ($j = 1, 2, 3$), μ is the overall mean, α_i and β_j the main effects of ith and jth loci, respectively, $(\alpha\beta)_{ij}$ the interaction effect and $e_{k(ij)}$ the residual.

3. Results

(i) *Variation in* D. simulans *and* D. mauritiana *strains*

The mean numbers of sex-comb teeth for the nine strains of *D. simulans* and four strains of *D. mauritiana* are given in Table 2, and show a highly significant among-strain difference in the tooth number ($F = 27{\cdot}8$, d.f. = [11, 85], $P < 0{\cdot}001$). As noted previously (Coyne, 1985; True *et al*., 1997), the number of sex-comb teeth was larger in *D. mauritiana* than in *D. simulans* ($F = 5{\cdot}7$, d.f. = [1, 11], $P < 0{\cdot}05$). The four *simulans* strains originating from Madagascar and Seychelles (SAvo-15, Sim-12, Tananarive and Sey-4) had significantly larger sex-comb tooth number (mean 23·5) than the other *simulans* strains (mean 19·8; $t = 3{\cdot}6$, $P < 0{\cdot}01$).

Table 2. *Results of the survey of sex-comb tooth number variation in nine* D. simulans *and four* D. mauritiana *strains*

	Strains	Mean (standard error of mean)
D. simulans	Sim-5	17·7 (0·5)
	Sim-3	18·5 (0·4)
	Shira-2	20·4 (0·5)
	Sim-11	20·9 (0·4)
	A1	21·7 (0·6)
	SAvo-15[a]	22·5 (0·3)
	Sim-12[a]	22·6 (0·4)
	Tananarive	23·7 (0·4)
	Sey-4[a]	25·1 (0·3)
D. mauritiana	g76	24·4 (0·4)
	75	26·4 (0·2)
	Les Galets	26·5 (0·5)
	Robertson	28·5 (0·5)

[a] Isofemale strains; all the others are inbred strains.

(ii) *Sex-comb tooth number of parental strains and* F_1 *flies*

We used Tananarive and Sim-3 strains for the QTL mapping experiments. Tananarive was a strain with a high sex-comb tooth number (mean ± standard error of mean from 50 fly data = 24·7 ± 0·2, which was slightly different from the mean from the first survey data in Table 2; see Section 2), while Sim-3 was a low number strain (18·5 ± 0·2). The difference between the two means was highly significant ($t = 21{\cdot}6$, $P < 0{\cdot}001$), and the overlap in distributions was very small (Fig. 2). Because of the high homozygosity of the parental inbred strains, the variances within strains were largely attributable to environmental variances and those did not differ significantly between the two parental strains (1·72 in Sim-3 and 2·43 in Tananarive; $F_s = 1{\cdot}4$, d.f. = [49, 49], $P > 0{\cdot}1$). The strain difference divided by the square root of the mean of the variances within strains was 4·3 (strain difference in environmental standard deviation units; True *et al*., 1997).

The mean (± SEM) sex-comb tooth number of F_1 progeny from crosses of Tananarive females and Sim-3 males was 22·2 ± 0·2 and that of F_1 progeny from the reciprocal crosses was 21·9 ± 0·2 (Fig. 2). The difference between these two means was not statistically significant, and the latter mean was not significantly different from the mid-parent value (21·6 ± 0·1). However, the mean sex-comb tooth number of the F_1 progeny from the cross of Tananarive females with Sim-3 males was significantly larger than that of the mid-parent even after the correction for multiple tests ($t = 2{\cdot}9$, $P < 0{\cdot}01$), suggesting either an X-chromosomal or a maternal effect.

(iii) *Whole-genome QTL mapping*

With the CIM analysis on the whole-genome F_2 mapping population, we detected two second-chromosome and three third-chromosome QTLs: one between *Ddc* and *eve*, one between *sli* and *Pcl*, one between *Antp* and *ninaE*, one between *hb* and *Ald* and

https://doi.org/10.1017/S0016672306008111 Published online by Cambridge University Press

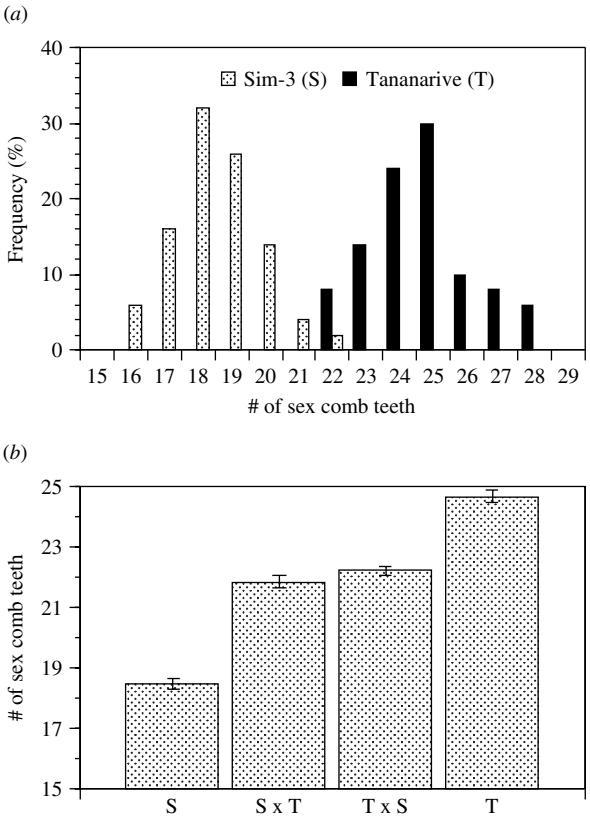

Fig. 2. The number of sex-comb teeth in two parental strains, Sim-3 and Tananarive, and their F_1 progeny. (*a*) Distribution in the parental strains. (*b*) Mean number with standard errors. S and T stand for Sim-3 and Tananarive, respectively. S × T designates F_1 progeny of Sim-3 female and Tananarive male crosses, and T × S designates F_1 of the reciprocal crosses. The sample size was 50, except for S × T, in which the sample size was 40.

one between *Mlc1* and *janA* (Table 3; Fig. 3). The QTL between *Ddc* and *eve* showed a highly over-dominant effect (Table 3). Unlike the other QTLs identified in the whole-genome F_2 mapping population, the QTL between *sli* and *Pcl* showed a negative additive effect, indicating the Tananarive allele reduced the number of sex-comb teeth (Table 3). No significant QTL was detected on the X chromosome (Fig. 3).

With the MIM analysis under the information criterion of 2log(log(*n*)), we confirmed four of the five QTLs detected by CIM (Fig. 3) (*Ddc-eve* and *sli-Pcl* on the second chromosome and *Antp-ninaE* and *hb-Ald* on the third chromosome), although the estimated positions of the two second-chromosome QTLs were slightly different (1 cM or less) from those by CIM (*eve-vg* in MIM instead of *Ddc-eve*, and *vg-sli* instead of *sli-Pcl*; see Table 3). Their estimated QTL effects in MIM were similar to those in CIM (Table 3). Even under the higher threshold, that is the criterion of log(*n*), MIM detected three QTLs (*vg-sli*, *Antp-ninaE* and *hb-Ald*; data not shown).

(iv) *Second-chromosome QTL mapping in homozygous backgrounds*

We further analysed second-chromosome QTLs in two homozygous backgrounds. By applying CIM to the IIs F_2 mapping populations (Sim-3 background), only one QTL was found in the *prd-Ddc* region, and the LR test was only marginally significant (Fig. 4*a*). In contrast, in the IIt F_2 mapping populations (Tananarive background), we found four QTLs: one between *Pgk* and *ninaC*, one between *prd* and *Ddc*, one between *eve* and *vg*, and one between *vg* and *sli* (Fig. 4*b*). The additive effect of the *Pgk-ninaC* QTL was negative, whereas the additive effects of the other three QTLs were positive (Table 3).

By MIM under the information criterion of 2 log(log(*n*)), we found no QTL in IIs, but three QTLs (*Pgk-ninaC*, *prd-Ddc* and *vg-sli*) in IIt (Table 3; Fig. 4*c*). When the critical value of CIM was adopted, one QTL (*Pgk-ninaC*) was still significant in IIt (Fig. 4*c*).

Based on the 2LOD support intervals, the *prd-Ddc* QTL detected in IIt may be identical to the *prd-Ddc* QTL in IIs, and the *eve-vg* and *vg-sli* QTLs in IIt may be identical to the *Ddc-eve* and *sli-Pcl* QTLs in the whole-genome F_2, respectively. However, the intervals of *Pgk-ninaC* QTL in IIt did not overlap with any other QTL intervals, suggesting that this QTL had background-dependent effects (Table 3).

(v) *Third-chromosome QTL mapping in homozygous background*

We introgressed two segments (*Antp-ninaE* and *hb-Ef1α100E*) of the high tooth number strain (Tananarive) into the genetic background of the low tooth number strain (Sim-3). By using these recombinant congenic strains (IIIs-1 and IIIs-2; see Fig. 3), we studied third-chromosome effects on the number of sex-comb teeth in a Sim-3 homozygous background. We confirmed the significant effects of both the *Antp-ninaE* and *hb-Ef1α100E* regions by CIM (Table 3). Fig. 5 illustrates the mean number of sex-comb teeth for three genotypes at *ninaE* for IIIs-1 and at *Ald* for IIIs–2. The combined effect of the two introgressed regions (2·7 + 3·0) could explain most of the Tananarive/Sim-3 parental difference (6·2). However, because the QTL peaks in the CIM analysis were not very clear (data not shown), the exact number and positions of QTLs in the *hb-Ef1α100E* region could not be established in IIIs-2. As mentioned above, the CIM analysis on the whole-genome F_2 mapping population revealed the two QTLs in this region (*hb-Ald* and *Mlc1-janA* in Fig. 2). This might be because these two QTLs are very close (about 10 cM; see Table 3) and because the sample size in the experiments (~200) was not large enough to identify multiple QTLs.

https://doi.org/10.1017/S0016672306008111 Published online by Cambridge University Press

Table 3. *Positions and effects of QTLs responsible for the difference in the sex-comb tooth number between Sim-3 and Tananarive*

Mapping population	Position (cM)	Flanking markers	Support interval[a]	Additive effect	Dominance effect	Epistasis
Second-chromosome						
Whole-genome	57·7	*Ddc-eve*	53·7–61·2	0·10	0·81	
	(58·3)	(*eve-vg*)		(0·60)	(0·29)	
	76·2	*sli-Pcl*	72·2–82·2	−1·01	1·02	
	(75·2)	(*vg-sli*)		(−1·03)	(0·75)	*
IIs	40·6	*prd-Ddc*	32·6–65·2	0·60	0·15	
IIt	9·0	*Pgk-ninaC*	2·0–14·0	−0·99	0·24	
	(8·0)	(*Pgk-ninaC*)		(−1·15)	(−0·70)	#
	48·6	*prd-Ddc*	33·6–52·7	0·56	0·63	
	(37·6)	(*prd-Ddc*)		(0·61)	(0·77)	
	65·2	*eve-vg*	58·2–66·2	0·81	0·50	
	71·2	*vg-sli*	67·2–80·2	0·39	0·56	
	(72·2)	(*vg-sli*)		(0·67)	(−0·13)	#
Third-chromosome						
Whole-genome	61·5	*Antp-ninaE*	52·9–67·0	0·96	0·60	
	(61·5)	(*Antp-ninaE*)		(0·92)	(0·60)	
	111·3	*hb-Ald*	101·3–117·3	1·78	−0·17	
	(111·3)	(*hb-Ald*)		(1·77)	(−0·23)	*
	122·4	*Mlc1-janA*	117·4–127·0	1·02	−0·21	
IIIs-1	63·5	*Antp-ninaE*		1·36	0·46	
IIIs-2	118·4	*hb-Ef1α100E*		1·49	−0·24	

For the whole-genome and second-chromosome mapping populations (IIs and IIt), we applied the CIM and MIM methods; for the third-chromosome mapping populations (IIIs-1 and -2), we used only the CIM method. Units of effects are in tooth number. Positions and effects of QTLs detected by MIM are shown in parentheses. As indicated by * and #, MIM detected a dominance-by-dominance interaction between the *vg-sli* and *hb-Ald* QTLs (effect = −1·33) and an additive-by-additive interaction between the *Pgk-ninaC* and *vg-sli* QTLs (effect = −0·76). The penalty function used for QTL detection in MIM was $c(n) = 2\ln(\ln(n))$, where n is the sample size. Even under a high stringent criterion, $c(n) = \ln(n)$, MIM identified three QTLs (*vg-sli*, *Antp-ninaE* and *hb-Ald*) and an epistasis between *vg-sli* and *hb-Ald* QTLs for the whole-genome F_2 mapping population and two QTLs (*Pgk-ninaC* and *eve-vg*, position = 65·2, additive effect = 0·88 and dominance effect = 0·03) and an epistasis between *Pgk-ninaC* and *eve-vg* QTLs for the IIt mapping population.

[a] 2LOD support interval for QTL position (see Lander & Botstein, 1989). For the IIIt-1 and IIIt-2 populations, the support intervals covered over all the markers involved and thus were not shown here.

(vi) *Epistatic interactions between QTLs*

By MIM, we found a dominance-by-dominance interaction between the *vg-sli* and *hb-Ald* QTLs in the whole-genome F_2 mapping population (LR = 6·7; Table 3). In Fig. 6*a*, the phenotypic values are plotted according to the genotypes of the markers close to those QTLs (*sli* and *hb*), in which *hb* was chosen instead of *Ald* because of an insufficient number of flies that were doubly homozygous for *T* allele at *sli* and *Ald*. We estimated the dominance effects for the three *sli* genotypes separately by $\{T/S - (T/T + S/S)/2\}$, where T/S, T/T and S/S are the mean tooth number for three *hb* genotypes. The estimates of dominance effect were $2{\cdot}0 \pm 0{\cdot}4$ for *hb* S/S, $0{\cdot}1 \pm 0{\cdot}3$ for *hb* S/T and $1{\cdot}4 \pm 0{\cdot}5$ for *hb* T/T. The two-way analysis of variance also showed a significant interaction between the two loci *sli* and *hb* ($F = 3{\cdot}17$, $P < 0{\cdot}02$).

Furthermore, we found an additive-by-additive interaction between the *Pgk-ninaC* and *vg-sli* QTLs in IIt (LR = 1·87; Table 3). The mean tooth numbers are graphically presented for the *ninaC* and *sli* genotypes in Fig. 6*b*.

4. Discussion

(i) *Genetic architecture of intraspecific variation*

Using two *D. simulans* inbred strains, we performed QTL mapping analyses examining male sex-comb tooth number and found, in total, four second-chromosome and three third-chromosome QTLs. Strikingly, the number of second-chromosome QTLs varied among the three mapping populations of different genetic backgrounds: two QTLs in the whole-genome F_2 mapping population, one in IIs and four in IIt. The *prd-Ddc*, *eve-vg* and *vg-sli* QTLs in IIt may match the *prd-Ddc* QTL in IIs and *Ddc-eve* (*eve-vg* by MIM) and *sli-Pcl* (*vg-sli* by MIM) QTLs in the whole-genome F_2, respectively. However, the remaining *Pgk-ninaC* QTL in IIt was localized in a different interval. This discrepancy among the mapping

https://doi.org/10.1017/S0016672306008111 Published online by Cambridge University Press

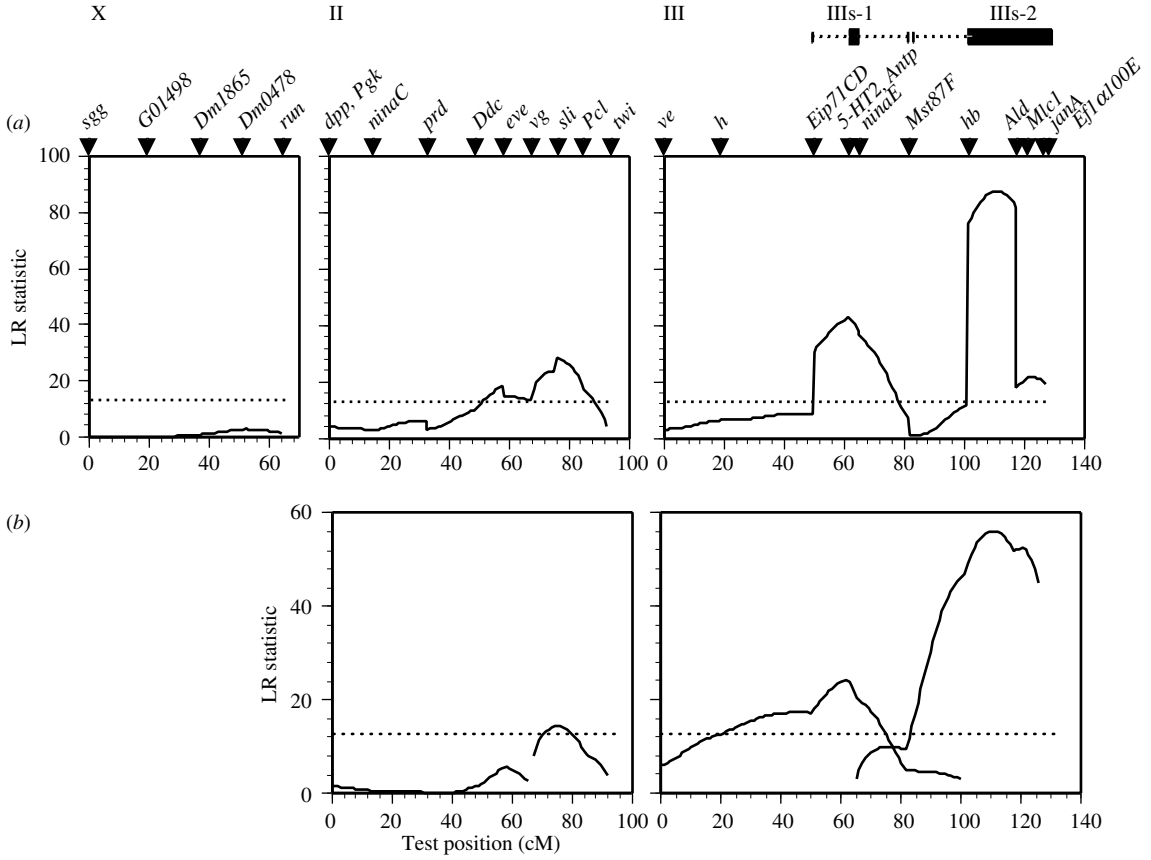

Fig. 3. QTL mapping results of sex-comb tooth number variations for the whole-genome F_2 mapping population. (*a*) CIM analysis. (*b*) MIM under the information criterion of 2ln(ln(*n*)). Neither CIM nor MIM detected any QTL on the X chromosome and the MIM figure for the X chromosome is not shown. Likelihood ratio test statistic (LR; Zeng, 1994) is plotted at every 1 cM position. The likelihood ratio test statistic = 2 × (ln10) × LOD (= 4·61 × LOD). The critical value for the CIM analysis is 12·96, which was estimated from 1000 times randomization of phenotypic value against genotype (dotted line). Arrowheads above graphs in (*a*) indicate positions of markers. Black boxes above the third-chromosome map indicate the introgressed segments in the third-chromosome congenic strains (IIIs-1 and IIIs-2) and their boundaries are in the regions shown by dotted lines.

populations may simply reflects a low power of detection in each mapping population (Dilda & Mackay, 2002; Cornforth & Long, 2003) or be caused by some other factors. We discuss different causes of the discrepancy, especially a significance of epistasis, in the following.

In the *prd-Pcl* region, we detected more QTLs in IIt than in the whole-genome F_2 mapping population (3 vs 2; Table 3). Nevertheless, we found only a single QTL with a marginally significant LR score in IIs. The failure of QTL detection in IIs may be attributable to false positives in the other mapping populations. However, since the QTL was identified in both the whole-genome F_2 and IIt mapping populations, the *Ddc-Pcl* region effect appears to be a true positive. On the other hand, IIs and IIt should have greater detection power than the whole-genome F_2 mapping population because of their common genetic background and almost equal sample size (≈200 individuals). Hence, the low power of detection is not promising to explain the failure of QTL detection in IIs.

Another possibility is that a difference in marker cofactors fitted to control the genetic background in each analysis may explain the discrepancy in QTL detection among mapping populations (Dilda & Mackay, 2002). However, this was not supported either by the consistent results of the CIM and MIM analyses in the whole-genome F_2 and IIt mapping populations, except for one QTL (Table 3).

Although we could not rule out the above and other possibilities, an epistatic interaction between QTLs is a likely explanation for the failure of QTL detection in IIs. In fact, we detected significant epistatic interactions between QTLs in both the whole-genome F_2 and IIt mapping populations in the MIM analyses. These findings support the hypothesis that the effects of QTLs on the second chromosome depend on the genotypes of QTLs on the third chromosome.

The discrepancy in QTL mapping results lies not only in QTL identification but also in its estimated effect. A QTL was detected in the *vg-Pcl* region in both

https://doi.org/10.1017/S0016672306008111 Published online by Cambridge University Press

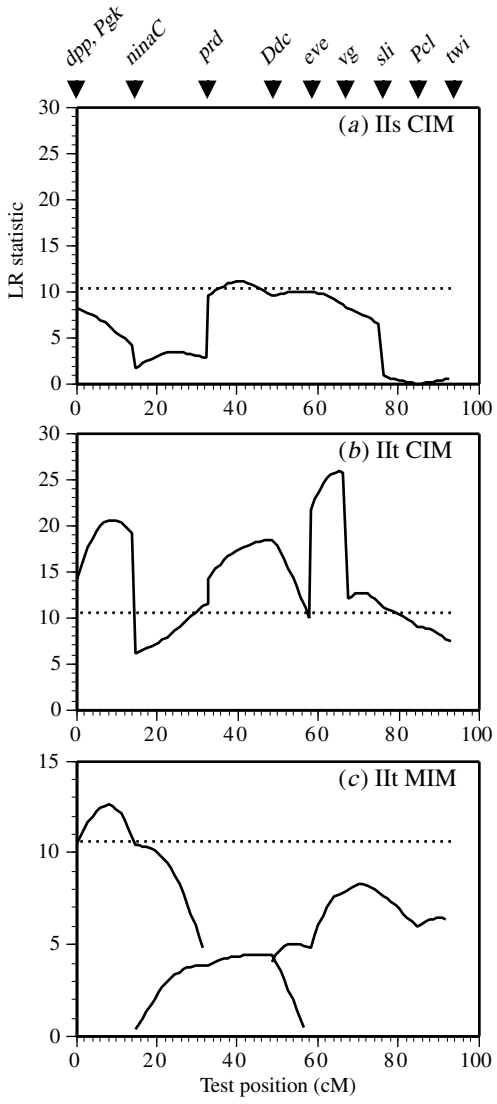

Fig. 4. QTL mapping results for two second-chromosome F_2 mapping populations (IIs and IIt). (*a*) CIM analysis for IIs. (*b*) CIM for IIt. (*c*) MIM under the information criterion of 2ln(ln(*n*)) for IIt. Likelihood ratio test statistic (LR; Zeng, 1994) is plotted at every 1 cM position. For CIM, the critical value estimated from the randomization procedure is 10·44 for IIs and 10·63 for IIt. MIM did not detect a QTL in IIs.

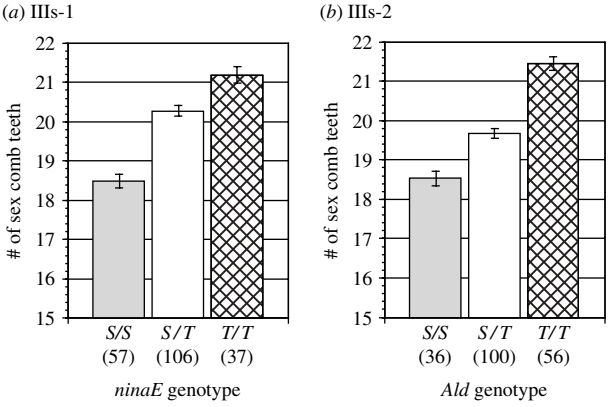

Fig. 5. Effects of two third-chromosome regions on the number of sex-comb teeth in a homozygous Sim-3 background. (*a*) *Antp-ninaE* region (IIIs-1). (*b*) *hb-Ef1α100E* region (IIIs-2). *ninaE* and *Ald* are chosen as markers for IIIs-1 and IIIs-2, respectively. The mean numbers of sex-comb teeth with their standard errors (the numbers of flies) are shown for three genotypes.

the whole-genome F_2 and IIt mapping populations and by both the CIM and MIM methods. However, their estimated additive effects differed greatly: the MIM estimates were −1 in the whole-genome F_2 mapping population, but 0·7 in IIt. In the whole-genome F_2 mapping population, the *vg-sli* QTL was involved in the epistatic interaction with the third-chromosome *hb-Ald* QTL. This interaction, in turn, seems to explain the discrepancy in the estimated additive effect between the whole-genome F_2 and IIt mapping populations.

In contrast to the second-chromosome QTLs, the regions of the three third-chromosome QTLs identified in the whole-genome F_2 also had significant effects in the Sim-3 background (IIIs-1 and -2). In addition to the locations, the estimated additive and dominance effects of these third-chromosome QTLs were very similar in the two genetic backgrounds (Table 3), meaning that the genetic effects of the third-chromosome QTLs are little affected by the genotypes of the second-chromosome QTLs. These results imply that the third-chromosome QTL(s) is epistatic to the second-chromosome QTL(s). From the epistatic relationships among QTLs, we might infer the order of gene action in the pathway with two kinds of information: the character of mutations (a loss of function or a gain of function) and the nature of the steps in the pathway (an obligatory sequence or a conditional one) (Wilkins, 1993). Although both of them are unknown, under a conditional sequence model as often assumed in many developmental pathways, the third-chromosome QTL(s) might act nearer to the final output, namely downstream of the second-chromosome QTL(s).

(ii) *Epistatic interactions between QTLs*

Epistatic interactions of quantitative variations have been uncovered in studies of cultivated plants and their wild relatives (Doebley *et al*., 1995; Lark *et al*., 1995; Eshed & Zamir, 1996), and in those of natural populations of *Drosophila melanogaster* (e.g., Shrimpton & Robertson, 1988; Long *et al*., 1995). However, the prevalence of such interactions has not been fully recognized (Tanksley, 1993; Lynch & Walsh, 1998). This is due to the relatively low power for detecting epistasis. With specialized populations, such as recombinant congenic strains and chromosome-substitution strains, we can overcome this problem by

https://doi.org/10.1017/S0016672306008111 Published online by Cambridge University Press

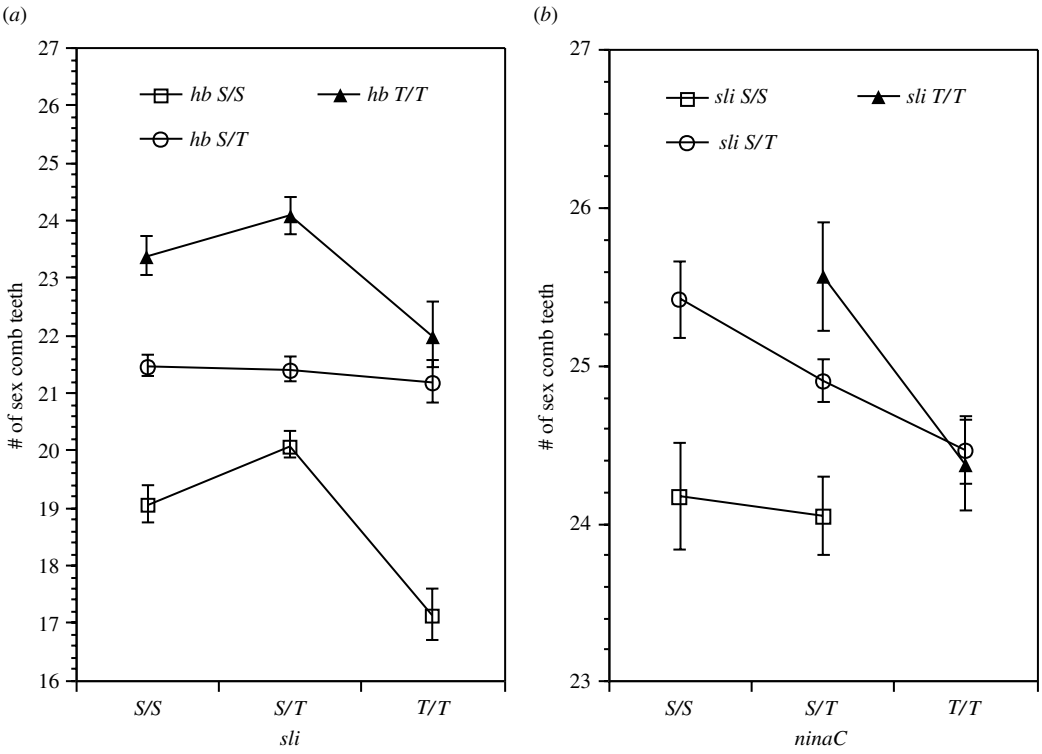

Fig. 6. Epistasis between QTLs on sex-comb tooth number. (*a*) Epistasis detected in the whole-genome F_2 mapping population. Mean tooth number is plotted against three *sli* genotypes for each *hb* genotype. (*b*) Epistasis detected in the IIt mapping population. Mean tooth number is plotted against three *ninaC* genotypes for each *sli* genotype. Standard error of the mean is overlaid.

reducing the genetic complexity and increasing the precision of phenotypic estimates for each genotype. For example, Fijneman *et al*. (1996) studied mouse lung tumours induced by *N*-ethyl-*N*-nitrosourea (ENU) treatment with recombinant congenic strains of two inbred mouse strains. They used a multiple-QTL-model mapping method and identified four interacting QTLs, where the main effects could not be determined due to partially counteracting interactions. Recently, Dilda & Mackay (2002) used six populations of recombinant isogenic strains to dissect genetically the intraspecific variation in bristle number in *Drosophila* and found many epistatic interactions.

As described in the previous section, the present analysis provided evidence for epistasis in the male sex-comb tooth number. Interspecific backcross analyses in both directions also reveal background-dependent QTL effects to some degree in this trait (True *et al*., 1997; Macdonald & Goldstein, 1999), but only one generation of backcrossing with typical sample sizes is not likely to be sufficient to uncover epistasis. Given the prevalence of epistasis in intra- and interspecific variation, the evolution of this trait should be understood in a multiple-locus context. Epistasis may, in turn, be useful for reconstructing the evolutionary history. If inflated phenotypic variability induced by epistasis contradicts the conservative nature of phenotypic evolution, we may be able to infer the order of changes during evolution, in which atypical phenotypes do not appear at any time.

(iii) *Comparison of intra- and interspecific variation*

The present results facilitate comparisons of the genetic architecture for intra- and interspecific variation for sex-comb tooth number. True *et al*. (1997) identified two third-chromosome QTLs responsible for the difference in sex-comb tooth number between *D. simulans* and *D. mauritiana*. One of these QTLs is found near *Antp*, and the other is located between *Ald* and *janA*. We identified QTLs in both regions. Because the only other QTL identified on the third chromosome is also close to *Ald*, the same genes may be responsible for both the variation within *D. simulans* and the observed differences between *D. simulans* and *D. mauritiana*.

All three third-chromosome QTLs identified in this study had large estimated additive effects (Table 3). Large effects of two regions (*Antp-ninaE* and *hb-Ef1α 100E*) were confirmed using congenic strains (Fig. 5). The homozygous effects of the QTLs identified in IIIs-1 (2 times the estimated additive effects = 2·7; see Table 3) and IIIs-2 (3·0) could explain 44 % and 48 %, respectively, of the difference between parental

https://doi.org/10.1017/S0016672306008111 Published online by Cambridge University Press

Table 4. *Summary of candidate genes*

Chromosome	QTL	Cytological location in *D. melanogaster*	Candidate genes
2	*Pgk-ninaC*	23A–27F	*Alp*, 24A1; *tkv*, 25D1–2
2	*prd-Ddc*; *Ddc-eve+eve-vg*	33C–49E	[*esc*], 33A2; *dac*, 36A1–2; *her*, 36A10; *sxc*, 41C1–2; *cos*, 43B1–2, *ptc*, 44D5–E1; *E(Pc)*, 47F13–14; *en*, 47F17–48A1; *Psc*, 49E6
2	*vg-sli+sli-Pcl*	49E–55B	*Psc*, 49E6; *cg*, 50E1; *Asx*, 51A4; *tra2*, 51B6; *Pcl*, 55B8; [*M(2)56F*], 56F5–15; [*mus209*], 56F11
3	*Antp-ninaE*	71D–84F+87F–93F	*brm*, 72C1; *tra*, 73A10; *Su(z)12*, 76D4; *skd*, 78A2–5; *Pc*, 78C6–7; AP–2, 78D8–E1; *vtd*, 80F; *corto*, 82E7; *noi*, 83B4; *gpp*, 83E6–7; *pb*, 84A5; *Scr*, 84A5; *Antp*, 84A6–B2; *Ta*, 84C1–2; *rn*, 84D3; *dsx*, 84E5–6; *Dipr*, 84F1–11; [*Vha55*], 87C2–3; [*Su(var)3–7*], 87E3; *trx*, 88B1; *put*, 88C9; *eff*, 88D2; *mor*, 89A11; *ss*, 89B14–15; *Trap80*, 90F6; *Dl*, 92A1–2
3	*hb-Ald*; *Mlc1-janA*	84F–85A+93F–100D	*Dipr*, 84F1–11; [*Scm*], 85E2; [*hyd*], 85E5; [*hth*], 86C1–3; *ash2*, 96A13; *Sce*, 98B1; *pygo*, 100C6; [*pho*], 102D6

Note that a large inversion (84F; 93F) is fixed between *D. melanogaster* and three species of the *simulans* clade (*simulans*, *mauritiana* and *sechellia*). Brackets indicate that the genes are very close to, though not within, the predicted interval.

strains. In the interspecific analyses (True *et al*., 1997), the QTL near *Antp* also had a large additive effect (54% the *simulans–mauritiana* difference).

A backcross analysis of *D. simulans* and *D. sechellia* led to the identification of one X and three second-chromosome QTLs (Macdonald & Goldstein, 1999), and the intervals of these second-chromosome QTLs overlapped those of the QTLs found in the present study. In this case, however, the coincidences of the QTL regions do not necessarily imply that the same genes are responsible for the intra- and interspecific variations, since the support intervals for the QTLs identified in this study cover large genomic regions (approximately 60% of the entire second chromosome). Macdonald & Goldstein (1999) also found one third-chromosome QTL in their reanalysis after factoring out variation in body size (more exactly, tibia length); however, we did not detect a QTL at this position.

In summary, the third chromosomal QTLs identified in this study were concordant with the locations of the QTLs responsible for the previously observed differences in sex-comb tooth number between *D. simulans* and *D. mauritiana* (True *et al*., 1997). Nuzhdin & Reiwitch (2000) have found a QTL responsible for the difference between two strains of *D. melanogaster* on the X chromosomal region, in which another interspecific QTL has been suggested by a multiple regression analysis (but not by a CIM analysis; True *et al*., 1997). However, whether the same genes are responsible for both intra- and interspecific variation remains unsolved in both cases. Furthermore, even if the same genes are involved, it is not clear whether the interspecific divergence is due to 'old' polymorphism within the ancestral population or to new mutations in the same gene. More work, including high-resolution mapping, is needed to resolve these questions.

(iv) *Candidate genes*

The present QTL mapping results of sex-comb tooth number variation are summarized in Table 4 with candidate genes for these QTLs identified via a search of FlyBase (FlyBase Consortium, 2003). The list of candidate genes includes sex determination genes (*dsx*, *tra*, *tra2*, *her*), selector genes (*en*) including the homeotic genes in the *Antennapedia complex* (*Antp*, *Scr*), and transcriptional regulators, in particular regulators for the homeotic genes (*Pc*, *Pcl*, *trx*).

Because of the consistent and large effects in different mapping populations, we focus on the third-chromosome QTLs and discuss their candidate genes. *Sex comb reduced* (*Scr*; 84A5) is a member of the *Hox* family of genes and a candidate for the *Antp-ninaE* QTL, which may be responsible for both the intra- and interspecific variation in number of sex-comb teeth. In fact, decreased SCR activity results in a reduction in the number or almost complete absence of sex-comb teeth (Lewis *et al*., 1980). This phenotype is generally interpreted to be a homeotic transformation of the first to the second thoracic identity. In this sense, it is of great interest to examine whether *Scr* contributes quantitatively to the variation in sex-comb tooth number without a segment identity transformation. The candidates also include the *Polycomb* and *trithorax* group protein genes such as *brahma* (*brm*, 72C1), *Su(z)* (76D4), *Polycomb* (*Pc*; 78C6–7),

https://doi.org/10.1017/S0016672306008111 Published online by Cambridge University Press

Sex comb on midleg (*Scm*, 85E2), *trithorax* (*trx*; 88B1), *moira* (*mor*; 89A11) and *absent, small, or homeotic discs 2* (*ash2*; 96A13), which are known to maintain spatial expression patterns of *Hox* genes.

On the other hand, mutants of *punt* (*put*; 88C9) and *Delta* (*Dl*; 92A1–2) manifest duplication or multiplication of the sex comb, which could be a patterning defect or a partial transformation of leg segments (Simin *et al*., 1998; Mishra *et al*., 2001). There is no evidence for a common genetic basis underlying both sex-comb tooth number and sex-comb number variation found in the *melanogaster* species group (Schawaroch, 2002; Kopp & True, 2002). This is an intriguing possibility worthy of further investigation.

The lack of sex-comb-specific markers has made analysis of sex-comb development itself difficult. Studies of natural variants and their interactions can complement mutation analyses and may improve our understanding of the formation of a morphological structure.

We would like to express our thanks to Y. Ishii for technical assistance, H. Kishino, T. Ohta, Z. Ze and R. Nakamichi for constructive and valuable comments on the interpretation of our results, and S. Wang for kindly correcting bugs in QTL software. We also thank Trudy Mackay and two anonymous reviewers for comments and suggestions on the manuscript. This study was supported in part by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (No. 12640604 to H.T.).

References

Basten, C. J., Weir, B. S. & Zeng, Z.-B. (2002). *QTL Cartographer: A Reference Manual and Tutorial for QTL Mapping*. Raleigh, NC: Department of Statistics, North Carolina State University.

Carson, H. L. (1975). The genetics of speciation at the diploid level. *American Naturalist* **109**, 83–92.

Churchill, G. A. & Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963–971.

Cook, R. M. (1977). Behavioral role of the sexcombs in *Drosophila melanogaster* and *Drosophila simulans*. *Behavior Genetics* **7**, 349–357.

Cornforth, T. E. & Long, A. D. (2003). Inferences regarding the numbers and locations of QTLs under multiple-QTL models using interval mapping and composite interval mapping. *Genetical Research* **82**, 139–149.

Coyne, J. A. (1985). Genetic studies of three sibling species of *Drosophila* with relationship to theories of speciation. *Genetical Research* **46**, 169–192.

Dilda, C. L. & Mackay, T. F. C. (2002). The genetic architecture of *Drosophila* sensory bristle number. *Genetics* **162**, 1655–1674.

Doebley, J., Stec, A. & Gustus, C. (1995). *teosinte branched1* and the origin of maize: evidence for epistasis and the evolution of dominance. *Genetics* **141**, 333–346.

Eshed, Y. & Zamir, D. (1996). Less-than-additive epistatic interactions of quantitative trait loci in tomato. *Genetics* **143**, 1807–1817.

Fijneman, R. J. A., de Vries, S. S., Jansen, R. C. & Demant, P. (1996). Complex interactions of new quantitative trait loci, *Sluc1*, *Sluc2*, *Sluc3*, and *Sluc4*, that influence the susceptibility to lung cancer in the mouse. *Nature Genetics* **14**, 465–467.

FlyBase Consortium (2003). The FlyBase database of the *Drosophila* genome projects and community literature. *Nucleic Acids Research* **31**, 172–175 (http://flybase.org/).

Foss, E., Lande, R., Stahl, F. W. & Steinberg, C. M. (1993). Chiasma interference as a function of genetic distance. *Genetics* **133**, 681–691.

Kao, C.-H., Zeng, Z.-B. & Teasdale, R. D. (1999). Multiple interval mapping for quantitative trait loci. *Genetics* **152**, 1203–1216.

Kopp, A. & True, J. R. (2002). Evolution of male sexual characters in the Oriental *Drosophila melanogaster* species group. *Evolution and Development* **4**, 278–291.

Kopp, A., Duncan, I. & Carroll, S. B. (2000). Genetic control and evolution of sexually dimorphic characters in *Drosophila*. *Nature* **408**, 553–559.

Kopp, A., Graze, R. M., Xu, S., Carroll, S. B. & Nuzhdin, S. V. (2003). Quantitative trait loci responsible for variation in sexually dimorphic traits in *Drosophila melanogaster*. *Genetics* **163**, 771–787.

Lander, E. S. & Botstein, D. (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185–199.

Lark, K. G., Chase, K., Adler, F., Mansur, L. M. & Orf, J. H. (1995). Interactions between quantitative trait loci in soybean in which trait variation at one locus is conditional upon a specific allele at another. *Proceedings of the National Academy of Sciences of the USA* **92**, 4656–4660.

Lewis, R. A., Kaufman, T. C., Denell, R. E. & Tallerico, P. (1980). Genetic analysis of the Antennapedia gene complex (ANT-C) and adjacent chromosomal regions of *Drosophila melanogaster*. I. Polytene chromosome segments 84B–D. *Genetics* **95**, 367–381.

Liu, J. J., Mercer, J. M., Stam, L. F., Gibson, G. C., Zeng, Z.-B. & Laurie, C. C. (1996). Genetic analysis of a morphological shape difference in the male genitalia of *Drosophila simulans* and *D. mauritiana*. *Genetics* **142**, 1129–1145.

Long, A. D., Mullaney, S. L., Reid, L. A., Fry, J. D., Langley, C. H. & Mackay, T. F. C. (1995). High resolution mapping of genetic factors affecting abdominal bristle number in *Drosophila melanogaster*. *Genetics* **139**, 1273–1291.

Lynch, M. & Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits*. Sunderland, MA: Sinauer Associates.

Macdonald, S. J. & Goldstein, D. B. (1999). A quantitative genetic analysis of male sexual traits distinguishing the sibling species *Drosophila simulans* and *D. sechellia*. *Genetics* **153**, 1683–1699.

Maekawa, B., Cole, T. G., Seip, R. L. & Bylund, D. (1995). Apolipoprotein E genotyping methods for the clinical laboratory. *Journal of Clinical Laboratory Analysis* **9**, 63–69.

Mayr, E. (1954). Change of genetic environment and evolution. In *Evolution as a Process* (ed. J. Huxley, A. C. Hardy & E. B. Ford). London: Allen & Unwin.

Mishra, A., Agrawal, N., Banerjee, S., Sardesai, D., Singh Dalal, J., Bhojwani, J. & Sinha, P. (2001) Spatial regulation of DELTA expression mediates NOTCH signalling for segmentation of *Drosophila* legs. *Mechanisms of Development* **105**, 115–127.

Nuzhdin, S. V. & Reiwitch, S. G. (2000). Are the same genes responsible for intra- and interspecific variability for sex comb tooth number in *Drosophila*? *Heredity* **84**, 97–102.

Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. & Sekiya, T. (1989). Detection of polymorphisms of human

DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proceedings of the National Academy of Sciences of the USA* **86**, 2766–2770.

Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1986). Analysis of enzymatically amplified β-globin and HLA-DQα DNA with allele-specific oligonucleotide probes. *Nature* **324**, 163–166.

Schawaroch, V. (2002). Phylogeny of a paradigm lineage: the *Drosophila melanogaster* species group (Diptera: Drosophilidae). *Biological Journal of the Linnean Society* **76**, 21–37.

Shrimpton, A. E. & Robertson, A. (1988). The isolation of polygenic factors controlling bristle score in *Drosophila melanogaster*. I. Allocation of third chromosome sternopleural bristle effects to chromosome sections. *Genetics* **118**, 437–443.

Simin, K., Bates, E. A., Horner, M. A. & Letsou, A. (1998). Genetic analysis of punt, a type II Dpp receptor that functions throughout the *Drosophila melanogaster* life cycle. *Genetics* **148**, 801–813.

Spieth, H. T. (1952). Mating behavior within the genus *Drosophila* (Diptera). *Bulletin of the American Museum of Natural History* **99**, 395–474.

Takano, T. S. (1998). Loss of notum macrochaetae as an interspecific hybrid anomaly between *Drosophila melanogaster* and *D. simulans*. *Genetics* **149**, 1435–1450.

Takano-Shimizu, T. (1999). Local recombination and mutation effects on molecular evolution in Drosophila. *Genetics* **153**, 1285–1296.

Takano-Shimizu, T. (2000). Genetic screens for factors involved in the notum bristle loss of interspecific hybrids between *Drosophila melanogaster* and *D. simulans*. *Genetics* **156**, 269–282.

Tanksley, S. D. (1993). Mapping polygenes. *Annual Review of Genetics* **27**, 205–233.

Templeton, A. R. (1980). The theory of speciation *via* the founder principle. *Genetics* **94**, 1011–1038.

True, J. R., Liu, J., Stam, L. F., Zeng, Z.-B. & Laurie, C. C. (1997). Quantitative genetic analysis of divergence in male secondary sexual traits between *Drosophila simulans* and *Drosophila mauritiana*. *Evolution* **51**, 816–832.

Wang, S., Basten, C. J. & Zeng, Z.-B. (2004). *Windows QTL Cartographer*, version 2.0. Raleigh, NC: Department of Statistics, North Carolina State University (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm).

Wilkins, A. S. (1993). *Genetic Analysis of Animal Development*, 2nd edn. New York: Wiley.

Zeng, Z.-B. (1994). Precision mapping of quantitative trait loci. *Genetics* **136**, 1457–1468.

Zeng, Z.-B., Liu, J., Stam, L. F., Kao, C.-H., Mercer, J. M. & Laurie, C. C. (2000). Genetic architecture of a morphological shape difference between two *Drosophila* species. *Genetics* **154**, 299–310.