Genet. Res., Camb. (2002), **79**, *pp.* 65–74. *With 6 figures.* © 2002 Cambridge University Press
DOI: 10.1017/S0016672301005419 Printed in the United Kingdom

An introgression approach to mapping differences in mating success and sperm competitive ability in *Drosophila simulans* and *D. sechellia*

ALBERTO CIVETTA[1,2]*, HEIDI M. WALDRIP-DAIL[1] AND ANDREW G. CLARK[1]
[1] *Department of Biology, Pennsylvania State University, University Park, PA 16802, USA*
[2] *Department of Biology, University of Winnipeg, Winnipeg, Manitoba, Canada R3B 2E9*

(*Received 1 May 2001 and in revised form 20 August 2001*)

Summary

The progeny of *Drosophila* females doubly-mated to males from the same and a closely related species are mostly sired by conspecific males. We examined the genetic basis for conspecific mating preference and sperm precedence by using 186 *Drosophila* lines in which random chromosomal fragments of *D. sechellia* were introgressed into *D. simulans*. Sperm competition was measured for each of these lines by crossing *ebony D. simulans* female with *ebony D. simulans* males followed by wild-type males from the introgressed lines. Variation in sperm competition (proportion of progeny sired by the second male), mating discrimination (proportion of introgressed males that failed to remate), and male fecundity (proportion of progeny sired by introgressed males) were scored. The introgressed lines exhibited highly significant heterogeneity in the three phenotypes scored, motivating an analysis to locate quantitative trait loci (QTLs) responsible for the differences. Applying composite interval mapping, we found eight QTLs that explain a significant level of variation among introgressed lines in the phenotypes scored. Cytological position overlapped among some QTLs suggesting possible pleiotropic effects. Analysis of the joint effects of *simulans*/*sechellia* genetic composition at different QTLs and markers suggests that complex interactions among alleles are partially responsible for interspecific differences in sexual traits.

1. Introduction

The three species of the *Drosophila simulans* clade have a common ancestor only about 0.5–1 Mya (Lemeunier *et al.*, 1986; Hey & Kliman, 1993). The morphological traits that exhibit the greatest divergence among these species are in the genitalia, and proteins whose primary sequences have diverged the most are those expressed in their gonads (Thomas & Singh, 1992; Civetta & Singh, 1995, 1998). Despite this high level of divergence of reproductive traits, the species can hybridize and produce viable and fertile female hybrids in laboratory conditions. However, the male hybrids are completely sterile due to either pre- or post-meiotic failure during spermatogenesis (Kulathinal & Singh, 1998). It seems that traits linked to male reproduction are highly divergent even among closely related species, and this divergence may act as a partial barrier to interspecific hybridization.

The production of interspecific sterile or inviable hybrids can be costly for both parents' fitness, and species have evolved means to avoid interspecific mating through mate recognition and/or differences in mate competitive ability (Jallon & David, 1987; Coyne *et al.*, 1994; Price & Boake, 1995; Boake & Poulsen, 1997). In nature, *Drosophila* females are usually multiply inseminated by conspecific males (Cobbs, 1977; Harshman & Clark, 1998; Imhof *et al.*, 1998), and it is possible that maintenance of accessory gland secretions and sperm from conspecific males in female sperm storage organs (Civetta, 1999; Neubaum & Wolfner, 1999) may serve as an effective barrier to interbreeding among related species. Despite such barriers, hybridization can happen both in nature and under laboratory conditions (Bock, 1984).

* Corresponding author. Tel: +1 (204) 786 9436. Fax: +1 (204) 774 4134. e-mail: a.civetta@uwinnipeg.ca
*Proof to be sent to: Alberto Civetta, Department of Biology, University of Winnipeg, 515 Portage Ave, Winnipeg, Manitoba, Canada R3B 2E9

How do species remain distinct despite their potential ability to hybridize and produce viable and at least partially fertile hybrids? Studies in a wide variety of organisms have shown that when females mate with a conspecific and a heterospecific male, most progeny are sired by the conspecific male (Hewitt *et al*., 1989; Bella *et al*., 1992; Gregory & Howard, 1994; Wade *et al*., 1994; Price, 1997). This phenomenon is known as conspecific sperm precedence.

Whether conspecific sperm precedence is important in maintaining species identity depends on the rate of interspecific matings and the rate at which females mate with multiple males. In cages containing equal proportions of the two sister species of ground crickets *Allonemobius fasciatus* and *A. socius*, an average 40% heterospecific matings were recorded. However, hybrids were rare, ranging from none to 6% (Howard *et al*., 1998). Laboratory cage experiments with *D. melanogaster* females and mixtures of *D. melanogaster* and *D. simulans* males resulted in an average 24% of females producing hybrid progeny. Almost 70% of the hybrids produced by these females were the result of double mating, and in all cases it involved a heterospecific mating followed by a homospecific mating (Jamart *et al*., 1995). The fact that most of the progeny produced by a doubly-mated female are sired by the conspecific male shows that if a female mates with a heterospecific male she can overcome the negative effects of hybridizing by rapid remating with a conspecific male. These observations suggest that once mating recognition barriers have been overcome, post-mating barriers might be important in holding closely related species apart.

In *Drosophila* there is a clear-cut difference in the results obtained from sperm competition experiments in which females are consecutively mated to two males from the same or different species. In experiments using conspecific males, the second male usually fathers most of the progeny (Clark *et al*., 1995). However, in double-mating experiments where females are successively crossed to a conspecific and a heterospecific male, females preferentially use conspecific sperm regardless of the order of mating (Price, 1997).

When *D. simulans* females are mated to males of the same species followed by *D. sechellia* males, only about 10% of the progeny are sired by the *D. sechellia* male, whereas the conspecific male sires about 90% of the progeny when he is last to mate (Price, 1997). This strong difference in sperm precedence suggests that it might be possible to identify the genetic basis for the factors that allow females to discriminate between conspecific and heterospecific sperm. Here we have constructed a low-resolution map of these factors by using a set of homozygous introgression lines whose genome is derived mostly from *D. simulans* with small segments from *D. sechellia*.

2. Materials and methods

(i) *Generation of* simulans/sechellia *introgressed (IG) lines*

The introgressed (*IG*) lines were generated by crossing *D. simulans* females from the 'sim2' line (Winters, CA) to *D. sechellia* males (Drosophila Species Stock center, 14021-0248·4), backcrossing the F1 female progeny to *D. simulans* twice, and sib-mating the resulting progeny (Dermitzakis *et al*., 2000). The progeny produced at the end of these two generations of backcrossing had an expected average of 1/8 of their autosomal genome from *D. sechellia* and 7/8 from *D. simulans*. Because recombination is expected to occur at random throughout the crossing scheme, different flies will have different insertions of the *D. sechellia* genome. Two hundred and twenty-one independent lines were established by single-pair sib-mating for 14 generations rendering independent homozygous lines.

(ii) *Scoring phenotypic differences*

We used males from the *IG* lines to test their sperm competitive ability in a double-mating experiment. Virgin 4- to 6-day-old *D. simulans* females homozygous for the recessive mutation *ebony* were mated to same-aged *D. simulans ebony* males *en masse* for 2 h. Females were then aspirated into single vials (vial 1) and 2 days later were offered two males from an IG_i line ($i = 1$–186) for the second mating. After 8 h, males were removed and females transferred to a second vial (vial 2) and 4 days later into vial 3. Progeny from all three vials were scored for body colour phenotype on the seventeenth day after oviposition began. To guarantee that females had mated with the tester *ebony* male and the male from the wild-type IG_i line, only sets of three vials that yielded both *ebony* and wild-type progeny were scored. The time *IG* males were allowed to stay with *D. simulans ebony* females was decided after trial observations of mating among already-mated *ebony* females and males from 27 different *IG* lines. We wanted to maximize the number of second matings and minimize possible multiple mating with the second male. In an 8-h period we observed only 4 females out of a sample of approximately 500 that engaged in mating more than once.

The fraction of all progeny in vials 2 and 3 that were sired by the second male was designated as the statistic *P2* (Boorman & Parker, 1976). We scored fecundity as the total count of progeny produced by each female summed over her three oviposition vials and mating discrimination as the proportion of IG_i males that failed to mate with an already-mated *D. simulans* female. Double-mated females produce large numbers of progeny and numbers below 20 are rare.

https://doi.org/10.1017/S0016672301005419 Published online by Cambridge University Press

Table 1. *Molecular markers scored for the first* (*a*), *second* (*b*) *and third* (*c*) *chromosomes*

(*a*)

Gene	Cytological position	Genetic position (*mel*)	Genetic position (*sim*/*sec*)	PCR primers	Differences scored
white	3C2	1–1·5	1–3·6	cacatacacagatttattgagccc acacacacttttatactctctccg	1
Cdk7	4F1–2	1–11	1–8·9	tatttcgctaacaaaccggc aacgcgatcacaaacatcaa	1
dec-1	7C4–5	1–20·7	1–14·3	atccaatgatgatgcagc caaaggcaatggacagag	2
sev	10A2–4	1–33·4	1–23·7	catcttaatgaggataaatttgttat aagcgacaagtttcaattaac	1
sog	13E3–8	1–53	1–42·7	gatccttggcggcaggggagcgaa tatgcaactccttgcacaa	1
Sh	16E4–F1	1–57·6	1–49	caagagatcccgagagagaga acgtgtgcgtgttgtttctc	1
shakB	19E3	1–64	1–57·9	gtggaaatggcagaggagag gttgttcatttgtttagcgg	1

(*b*)

Gene	Cytological position	Genetic position (*mel*)	Genetic position (*sim*/*sec*)	PCR primers	Differences scored
aop	22C3–D1	2–12	2–13	taatggggaatgggtgaatg gccgtgctcttttctcttacg	1
Acp26Ab	26A5	2–18	2–22	atgaactacttcgcggtg atagggttctcaacatgc	3
ninaC	27F5–6	2–22	2–28·5	tttgtcaatctctcacagcagg gccccgagtacatttattcaagc	1
da	31D11–E1	2–41·3	2–47·8	tgcccagcatcacatgatac ggtttttatggaagagaggg	1
Su(*h*)	35B10–C1	2–50·5	2–64	aacggctcacccctcgatcc tacttctccatggcgtcccg	1
cad	38E5–6	2–54	2–71·9	tcgggctcggaaatctctag aagtatggcggcttcgatgg	1
mam	50C23–D3	2–70·3	2–100·5	ggcggcctaccagttttcca cctgttgctcccaggtttgc	1
Amy-d	53F13–54A2	2–77·9	2–115·3	tacgtggatgtgatcttcaa gatgacctcctggacgat	2
AC004365	58A4–B1	2–107·6	2–146·5	gctttatcaatgcagcctcc ggccccaatatgtcctcgcc	1

(*c*)

Gene	Cytological position	Genetic position (*mel*)	Genetic position (*sim*/*sec*)	PCR primers	Differences scored
Cdc37	62B4	3–5	3–0·2	tatcctatgcaaacacaggcc ggccataactgaaaagctatgc	1
ple	65C3	3–18	3–20·3	ttctgagaggggctttta gaggctaacaaatgaag	1
Lanb2	67B10	3–28	3–30·8	cgtaggaaggaaagaaatcgg aatttgcagttgataggcagc	1
Cat	75D7–E1	3–47	3–71·7	ttcgacggatcagacttggtttttggc gcgttcgcctttcttagtcaatttcgg	2
AC001655	84C1–4	3–48	3–134·6	atgctacactcaaacaaatggg ttggaaccaaggatcttactgc	1
cpo	90C10–D1	3–62	3–97	tcgcacgagtccaactcc acggagtccatgctctgc	2
pnt	94E11–F1	3–79	3–141·5	caataacaattgccacacgg aattggtgatgcggatgg	1

The forward PCR primer is listed first followed by the reverse primer, both written in the 5′–3′ direction. Differences scored are: 1, microsatellite repeat; 2, insertion/deletion; 3, restriction site.

https://doi.org/10.1017/S0016672301005419 Published online by Cambridge University Press

We considered females producing fewer than 20 flies (fecundity < 20) to be aberrant due possibly to injury or infection, so they were eliminated from the analysis.

(iii) *Molecular markers*

We combined information on the phenotypic scores obtained from different *IG* males and their genetic make-up in order to establish possible gene–trait associations. We scored the parental species, *D. simulans* and *D. sechellia*, and the *IG* lines for 23 different molecular markers spread across the three major chromosomes of *Drosophila* (Table 1). For each marker we scored the parental species lines for differences in insertion/deletions that result in changes in size of a PCR product or gain/loss of restriction sites that result in a restriction length polymorphism. PCR amplifications were performed at 53 °C or 55 °C and $MgCl_2$ concentration of 1·25 or 2·5 mM.

We currently have information available on the genetic map position of genes in *D. melanogaster* through Flybase (http://flybase.bio.indiana.edu). However, the genetic map positions in *D. melanogaster* are not necessarily the same as in *D. simulans* and *D. sechellia*. We inferred the map position of our markers in the *simulans*/*sechellia IG* lines by using their relative order in *D. melanogaster*, and their actual genetic map position was estimated from their relative position to any pair of flanking markers previously mapped in *D. simulans*/*D. mauritiana* hybrids (Zeng *et al.*, 2000) (Table 1). This map proved to be compatible with that obtained from the segregation of markers among the IG lines.

(iv) *QTL mapping*

Test of association between molecular marker information and the phenotypic score (*P2*, fecundity, mating discrimination) were performed using QTL Cartographer (version 1.13) (Basten *et al.*, 1999). A composite interval mapping method of analysis (Zeng, 1994) was applied to our dataset. A forward/backwards stepwise regression method was used to decide on the number of background markers to be used in composite mapping. The significance of quantitative trait loci (QTLs) was tested by estimating a likelihood ratio (LR) score, defined as the ratio of the likelihood of having a locus for the quantitative trait to the likelihood of not having a QTL based on the molecular marker configuration. We plotted the LR as a function of map position and tested the significance of the LR score by a permutation test that randomly shuffles the observed phenotypic scores over the genotypes, generating a sample with the original marker information but with phenotypic values randomly assigned to them (Doerge & Churchill, 1996). Significance of our observed LR scores was tested both at the experimentwise level (comparing the observed values with the highest LR score obtained from the permutation) and comparisonwise level (comparing the observed values with the permuted datasets at each position).

3. RESULTS

(i) *Phenotypic variation*

Average *P2* scores were obtained for males from 186 different introgressed lines in counts of 267578 progeny from 2756 doubly-mated females. Angular transformed values of *P2* (*TP2*) adequately fitted normality and homoscedasticity assumption, and were used for analysis of variance. The average *P2* value for all *IG* lines was 0·74 and there was significant variation across lines in their ability to outcompete *D. simulans ebony* males (Fig. 1) ($F_{(185;2565)} = 4{\cdot}82$; $P < 0{\cdot}001$). Significant heterogeneity was also found for fecundity and

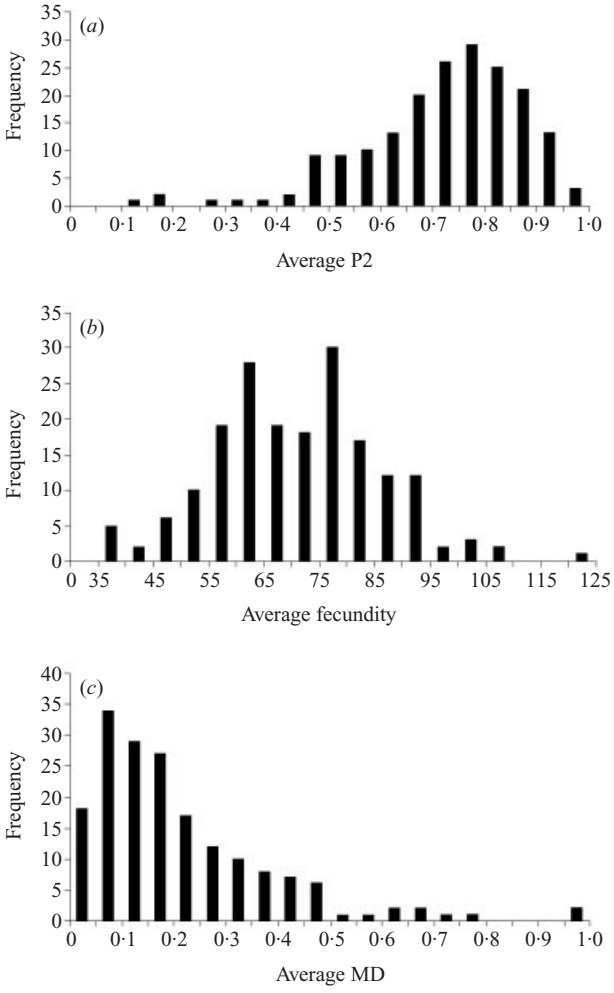

Fig. 1. Distribution of (*a*) average second male paternity success (*P2*), (*b*) average fecundity and (*c*) mating discrimination (MD) among different *IG* lines.

https://doi.org/10.1017/S0016672301005419 Published online by Cambridge University Press

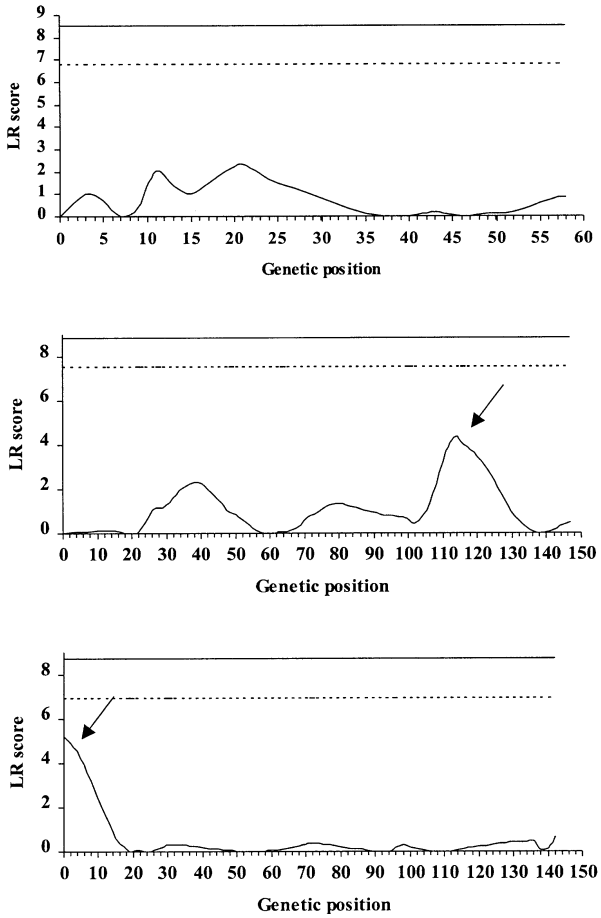

Fig. 2. QTLs for interspecific differences in second-male paternity success (*P2*) were found on the second and third chromosomes (top, middle and bottom panels are for first, second and third chromosomes respectively). Arrows point at comparisonwise significant QTL positions. Thresholds for marginally significant ($P < 0{\cdot}1$) and significant ($P < 0{\cdot}05$) experimentwise QTLs are denoted by a dotted and a continuous line respectively.

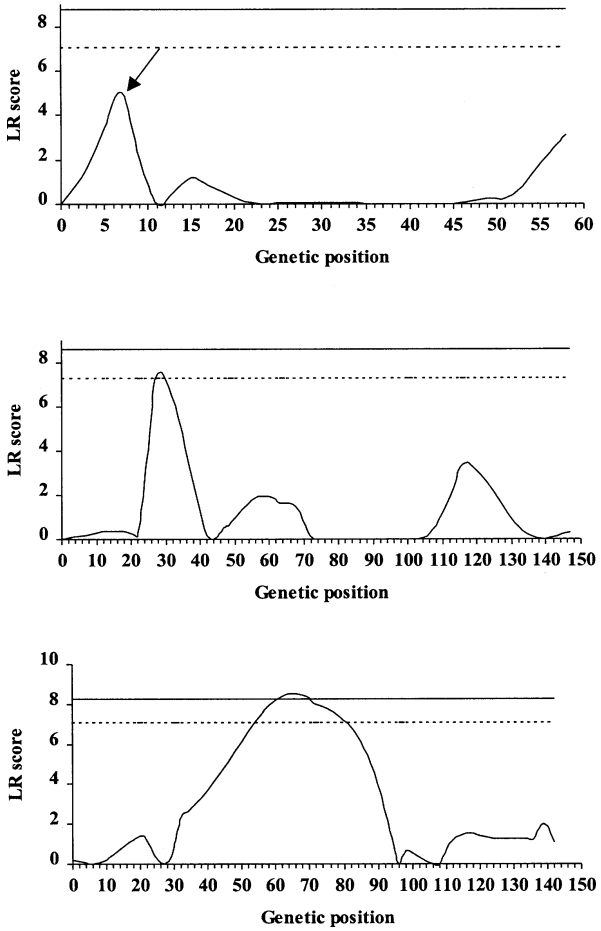

Fig. 3. Chromosomal location of QTLs explaining interspecific differences in male fecundity. Top, middle and bottom panels are for first, second and third chromosomes respectively. Comparisonwise and experimentwise QTLs are indicated as in Fig. 2.

mating discrimination ($F_{(185;2570)} = 8{\cdot}72$; $P < 0{\cdot}001$ and $F_{(187;3254)} = 4{\cdot}29$; $P < 0{\cdot}001$ respectively) (Fig. 1).

(ii) *QTL mapping*

We detected a total of eight QTLs explaining variation in the three phenotypes analysed. Only three of eight showed significance at the experimentwise level and they explained variation in either fecundity or second-male mating discrimination. For differences in second-male paternity success we detected two significant QTLs with observed LR scores in the 5% tail of the distribution of scores obtained from the permutated datasets at the QTL position (comparisonwise test).

Fig. 2 shows the mapping result for interspecific variation in *P2*. A comparisonwise significant LR score was obtained on the second chromosome between markers *mam* and *Amy* (genetic interval 114 to 120) and another on the third chromosome close to marker *Cdc37* (genetic interval 0 to 6).

The analysis of interspecific differences in male fecundity produced a significant experimentwise QTL on the third chromosome, between marker *lamb2* and *cathpo* in the genetic interval 58 to 71 (Fig. 3). A marginally significant experimentwise QTL was detected between markers *Acp26* and *ninaC* (genetic position 27 to 33) on the second chromosome (Fig. 3). Finally, a comparisonwise significant QTL peak was detected close to the centromere of the X chromosome between markers *white* and *Cdk7* (genetic position 5 to 8; Fig. 3).

A marginally significant experimentwise QTL was found to explain differences in *D. simulans* females' mating discrimination of *IG* line males. The interval mapped in the first chromosome between markers *Cdk7* and *dec-1* (genetic map position 8 to 13; Fig. 4). Another two comparisonwise significant QTLs were found on the second and the third chromosome. The second chromosome QTL mapped between markers *mam* and *Amy* (genetic position 112 to 129) and the

https://doi.org/10.1017/S0016672301005419 Published online by Cambridge University Press

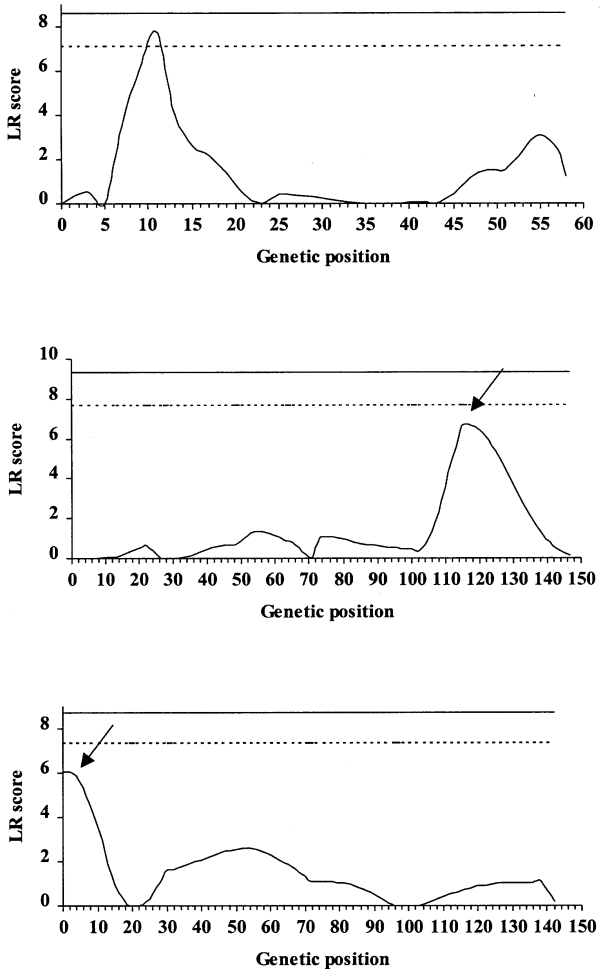

Fig. 4. QTLs explaining interspecific differences in mating discrimination. Top, middle and bottom panels are for first, second and third chromosomes respectively. Comparisonwise and experimentwise QTLs are indicated as in Fig. 2.

third chromosome QTL was found very close to the centromere between markers *Cdc37* and *ple* (3-0 to 3-6 genetic map position; Fig. 4).

Due to the small number of QTLs detected it is not possible to establish whether there is any statistically significant directionality on the effect of allele replacements for each trait analysed. However, an interesting observation is that for all traits analysed, *D. sechellia* alleles at QTL positions on the first and second chromosome increase the average phenotypic score of the introgressed lines whereas third chromosome QTLs harbouring *D. sechellia* alleles result in lower phenotypic scores.

(iii) *Pleiotropic effects and genetic clustering*

There is some physical overlap of the location of the QTLs explaining variation among *IG* lines in different traits. For example, the X chromosome QTL that explains interspecific variation in fecundity maps in the interval 4A–4E which partially overlaps with the X chromosome QTL interval explaining variation in second-male mating discrimination (4D–6A). There is also an overlap between the second chromosome QTL responsible for differences in second-male paternity success among *IG* lines (52D–53E) and the one explaining differences in second-male mating discrimination (52D–57A), as well as for those found in the third chromosome (positions 61F–62C and 61F–63B respectively).

A non-significant correlation was found between fecundity and second-male paternity success ($r = -0{\cdot}0235$, $P = 0{\cdot}222$), but a negative and significant correlation was observed between mating discrimination indices and both second-male paternity

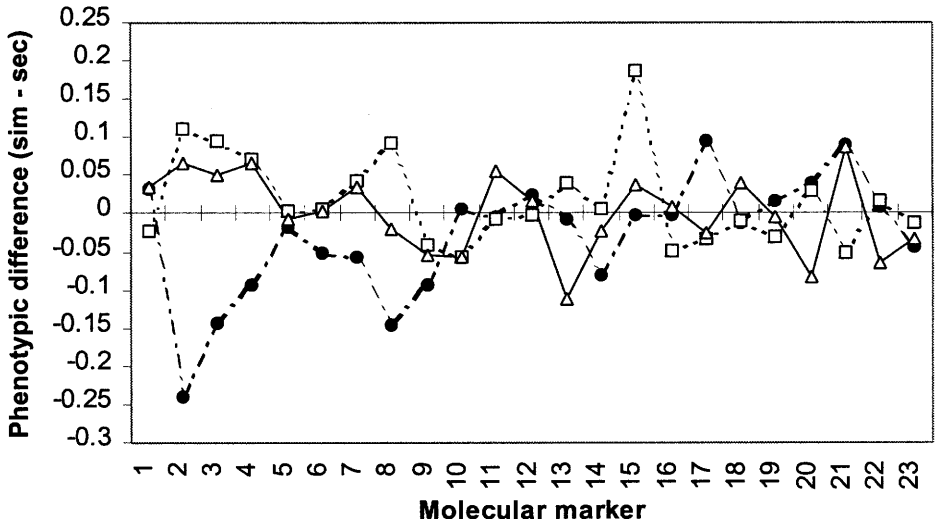

Fig. 5. Average differences in phenotypic value among flies carrying a *D. simulans* or *D. sechellia* allele at different molecular markers. The numbering of markers is in the same order as in Table 1 (markers 1 to 7 are X chromosome linked, 8 to 16 are second chromosome markers and 17 to 23 are third chromosome linked). Squares, *P2*; triangles, fecundity; filled circles, mating discrimination.

https://doi.org/10.1017/S0016672301005419 Published online by Cambridge University Press

success and fecundity ($r = -0{\cdot}4$, $P < 0{\cdot}01$; $r = -0{\cdot}33$, $P < 0{\cdot}01$, respectively). This result suggests possible pleiotropic effects of one major gene or group of genes as responsible for some of the variation among *IG* lines in second-male mating discrimination, fecundity and second-male paternity success.

Phenotypic variation in a given trait can be caused by complex interactions among many loci, as appears to be the case for interspecific hybrid sterility (Cabot *et al*., 1994; Davis *et al*., 1994; Palopoli & Wu, 1994; Perez & Wu, 1995). Under such a scenario, it may be more informative to search for any degree of clustering of the phenotypic values based on clustering of the genotypes, similar to cladistic analysis of single gene variation (Templeton & Sing, 1993). Fig. 5 shows differential phenotypic values for each molecular marker scored. An antagonistic pattern appears between the phenotypic effect of the allele origins on the first versus third chromosome in mating discrimination. *D. sechellia* alleles on the first chromosome (1 to 7) result in higher mating discriminatory indices than *D. simulans* alleles, while third chromosome *D. sechellia* alleles (markers 17 to 23) make flies less discriminatory than do *D. simulans* alleles. The patterns of clustering for fecundity and *P2* are more erratic, except that *D. simulans* alleles for markers on the first chromosome confer higher fecundity and *P2* values but less mating discriminatory scores than *D. sechellia* alleles (Fig. 5).

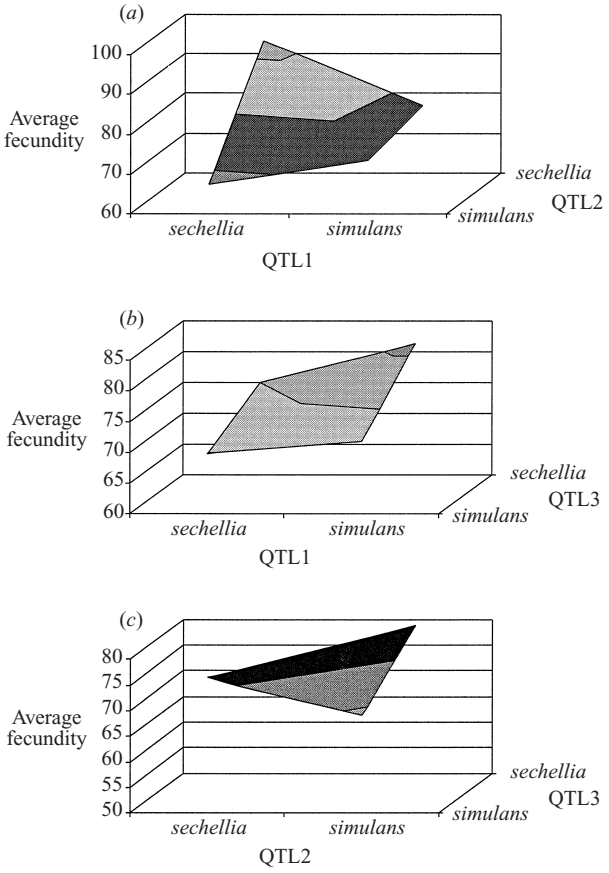

Fig. 6. Combined effects on average fecundity of *D. simulans* and *D. sechellia* allele identities at the three QTL positions detected in this study. The number of the QTL refers to chromosomes 1, 2 and 3 respectively.

(iv) *QTL interactions and their effects on phenotypic value*

We have been able to detect some genetic intervals that harbour genes with effects on phenotypic variation in male fecundity, male mating discrimination and second-male sperm competitive ability. The next question is whether these QTLs interact with each other to produce a measurable phenotypic effect. The introgression of *D. sechellia* chromosomal fragments into the *D. simulans* genome is expected to disrupt the phenotypic values of the lines tested against fully *D. simulans* lines. Then, the simplest scenario will be that of a constant increase in phenotypic value among lines with an increased proportion of *D. simulans* genome. Based on the scoring of molecular markers, it is possible to establish what proportion of the overall genome of the different *IG* lines is from one species or the other and then test for correlation between the overall genetic composition of the different *IG* lines and their phenotypic scores. We found no correlation among *IG* lines between the proportion of molecular markers being from a given species and their fecundity, *P2*, or second-male mating discrimination score ($r = -0{\cdot}032$, $P = 0{\cdot}674$; $r = -0{\cdot}033$, $P = 0{\cdot}667$; and $r = -0{\cdot}063$, $P = 0{\cdot}409$, respectively). This result suggests a lack of directionality on the effect of genes affecting fecundity, male mating discrimination and *P2*. The lack of consistent directionality in allelic effects is also seen when pairs of significant QTLs affecting one particular trait are analysed separately. For example, the second and third chromosome QTLs detected for variation in male fecundity show an increasing effect on male average fecundity when they harbour a *D. sechellia* allele, whereas the first chromosome QTL shows the reverse effect (Fig. 6), and the best combination of alleles between pairs of QTLs affecting male fecundity is not necessarily the one predicted by the direction of each QTL allelic effect (Fig. 6*a*), suggesting complex interactions among genes.

4. DISCUSSION

Our results show extensive variation in second-male paternity success, fecundity and mating discrimination among introgressed lines with different *D. simulans*/*D. sechellia* genetic composition, and particular fragments of chromosomal segments (QTLs) appear to be

https://doi.org/10.1017/S0016672301005419 Published online by Cambridge University Press

responsible for the observed phenotypic variation. The QTLs we have detected might be a conservative estimate of the real number of QTLs affecting phenotypic variation in the traits scored, as recent studies attempting to map QTLs have shown that under different environmental conditions different genetic variants may become major players explaining phenotypic variation. A well-studied example is differences in lifespan among recombinant inbred lines of *D. melanogaster* (Nuzdhin *et al*., 1997). A total of six QTLs have been found to affect lifespan under different conditions such as sex, heat shock, growing temperature, feeding and crowding (Nuzdhin *et al*., 1997; Vieira *et al*., 2000; Leips & Mackay, 2000). It is possible that other QTLs might also affect variation in the traits surveyed in our study depending on the environmental conditions under which the phenotypes are tested.

It could be argued that a non-uniform distribution of introgressed *D. sechellia* genome fragments into a *D. simulans* background could affect the number and distribution of QTLs detected in this study, with QTLs tending to appear in areas of either low or high introgression. In fact, True *et al*. (1996) found that a significantly lower number *of D. mauritiana* segments introgressed into the second chromosome of *D. simulans* when compared with other chromosomes. In our study, the distribution of introgressed *D. sechellia* segments, as can be evaluated from the proportion of *D. sechellia* alleles at each marker, is not uniform. The lowest proportion of introgressed *D. sechellia* segments is 0·6% for marker 13 on the second chromosome and the highest is 54% for marker 6 on the first chromosome, with the average across chromosomes being 15%. However, this non-uniform distribution does not seem to affect the location of QTLs detected in this study since they can be found at positions where markers show as little as 2% of *D. sechellia* alleles to as much as 34%.

(i) *Pleiotropy among QTLs*

The occurrence of pleiotropy among loci affecting different traits is suggested by the co-localization of an X chromosome QTL explaining interspecific variation in male fecundity and male mating discrimination and a second and a third chromosome QTL explaining interspecific differences in second-male paternity success and male mating discrimination. Due to the level of resolution of our map, the concordance between positions could be coincidental. However, the traits for which overlaps in the physical location of QTLs were detected are also the ones showing significant correlations among themselves. The significant negative correlations show that genes making males more prone to discrimination by *D. simulans* females might also affect the males' mating ability by rendering them less able to outcompete sperm stored from previous mating to *D. simulans* males, and also less successful at increasing the female's fecundity. Therefore, the phenotypic effects caused by same QTLs in different traits show no tradeoff, but rather they influence phenotypic differences among lines in the same direction (relative to fitness).

The co-localization of QTLs might be due to the evolution of linkage disequilibrium between genes affecting different characters analysed. If so, the co-localization of QTLs for traits that are phenotypically correlated is suggestive since models of sexual selection predict that linkage would evolve between genes determining female choice (mating discrimination) and male characters (fecundity, sperm competition). The correlation result is similar within species, where males from different chromosome extracted lines of *D. melanogaster* that are less discriminated by already-mated females are also better at outcompeting resident sperm from a previous mating (Clark *et al*., 1995).

(ii) *Epistasis among QTLs*

Quantitative genetic studies try to identify major genes responsible for phenotypic variation, but as the number of genes with small effect and complex interactions increases, it becomes more difficult to identify single locus effects. A clear example of complex epistatic interactions affecting interspecific differences comes from studies attempting to map genes responsible for interspecific hybrid male sterility. Wu and collaborators have established through detail genetic analysis of even small introgressions of a chromosomal segment from *D. sechellia* or *D. mauritiana* into *D. simulans* that a single X chromosome genetic factor is not sufficient to confer full hybrid male sterility (Cabot *et al*., 1994; Palopoli & Wu, 1994; Perez & Wu, 1995) and similarly second and third chromosome homozygous introgressions into *D. simulans* genome are simultaneously required for hybrid female sterility (Davis *et al*., 1994). These studies have clearly shown that many genes with complex epistatic interactions determine the genetic basis of interspecific hybrid sterility between sibling species.

Our analysis of allelic effects of different QTLs shows that it is not possible to detect a clear directionality of combinations of alleles from different QTLs based on their origin (*D. simulans* vs *D. sechellia*), and so we can conclude that allelic variants from different loci show complex epistatic interactions in their effect on the phenotype. Therefore, although single loci can be mapped as genetic factors explaining the bulk of interspecific differences in these traits, this is far from implying that interspecific differences are the result of single locus systems, but rather it seems likely that complex genetic interactions may be part of

the genetic basis of interspecific differences in traits of reproduction.

(iii) *Finer mapping and candidate genes*

Given the level of mapping resolution in this study, the cytological regions covered by the QTLs are quite wide and several genes might be responsible for differences in the phenotypes measured. For example, the experimentwise QTL found on the third chromosome as responsible for interspecific differences in male fecundity covers the *D. melanogaster* cytological region 69D–77A, where several male sterility genes (*ms*), recessive lethal genes (*l*), and two accessory gland proteins, *Acp70A* and *Acp76A*, have been mapped. Any one of these could serve as a candidate gene(s) to explain the differences scored in male fecundity.

In order to resolve the mapped QTLs to a more manageable number of candidate genes, fine mapping by surveying additional molecular markers will be needed. Once candidate genes can be identified, finer molecular quantitative trait nucleotide (QTN) mapping and association studies (Long & Langley, 1999) could be used to verify the role of different candidate genes and to establish how gene replacements are responsible for interspecific variation in traits affecting reproductive success.

We thank Bridget Todd, Pam Gehris, Bonnie Sceurman, Keith Alcorn, Heather Surkala and Jessica Rosevear for help in scoring flies. This work was funded by grant DEB-9527952 from the National Science Foundation to A.G.C.

References

Basten, C. J., Weir, B. S. & Zeng, Z.-B. (1999). *QTL Cartographer*, version 1.13. Raleigh, NC: Department of Statistics, North Carolina State University.

Bella, J. L., Butlin, R. K., Ferris, C. & Hewitt, G. M. (1992). Asymmetrical homogamy and unequal sex ratio from reciprocal mating-order crosses between *Chorthippus parallelus* subspecies. *Heredity* **68**, 345–352.

Boake, C. R. B. & Poulsen, T. (1997). Correlates versus predictors of courtship success: courtship song in *Drosophila sylvestris* and *D. heteroneura*. *Animal Behaviour* **54**, 699–704.

Bock, I. R. (1984). Interspecific hybridization in the genus Drosophila. *Evolutionary Biology* **18**, 41–70.

Boorman, E. & Parker, G. A. (1976). Sperm (ejaculate) competition in *D. melanogaster* and the reproductive value of females to males in relation to female egg and mating status. *Ecological Entomology* **1**, 145–155.

Cabot, E. L., Davis, A. W., Johnson, N. A. & Wu, C.-I. (1994). Genetics of reproductive isolation in the *Drosophila simulans* clade: complex epistasis underlying hybrid male sterility. *Genetics* **137**, 175–189.

Civetta, A. (1999). Direct visualization of sperm competition and sperm storage in *Drosophila*. *Current Biology* **9**, 841–844.

Civetta, A. & Singh, R. S. (1995). High divergence of reproductive tract proteins and their association with postzygotic reproductive isolation in *Drosophila melanogaster* and *Drosophila virilis* group species. *Journal of Molecular Evolution* **41**, 1085–1095.

Civetta, A. & Singh, R. S. (1998). Sex and speciation: genetic architecture and evolutionary potential of sexual versus non-sexual traits in the sibling species of the *Drosophila melanogaster* complex. *Evolution* **52**, 1080–1092.

Clark, A. G., Aguadé, M., Prout, T., Harshman, L. G. & Langley, C. H. (1995). Variation in sperm precedence and its association with accessory gland protein loci in *Drosophila melanogaster*. *Genetics* **139**, 189–201.

Cobbs, G. (1977). Multiple insemination and male sexual selection in natural populations of *Drosophila pseudoobscura*. *American Naturalist* **111**, 641–656.

Coyne, J. A., Crittenden, A. P. & Mah, K. (1994). Genetics of pheromonal differences contributing to reproductive isolation in *Drosophila*. *Science* **265**, 1461–1464.

Davis, A. W., Noonburg, E. G. & Wu, C.-I. (1994). Evidence of complex genic interactions between conspecific chromosomes underlying hybrid female sterility in the *Drosophila simulans* clade. *Genetics* **137**, 191–199.

Dermitzakis, E. T., Masly, J. P., Waldrip, H. M. & Clark, A. G. (2000). Non-Mendelian segregation of sex chromosomes in heterospecific *Drosophila* males. *Genetics* **154**, 687–694.

Doerge, R. W. & Churchill, G. A. (1996). Permutation tests for multiple loci affecting a quantitative character. *Genetics* **142**, 285–294.

Farris, J. S. (1970). Methods for computing Wagner trees. *Systematic Zoology* **19**, 83–92.

Gregory, P. G. & Howard, D. J. (1994). A postinsemination barrier to fertilization isolates two closely related ground crickets. *Evolution* **48**, 705–710.

Harshman, L. G. & Clark, A. G. (1998). Inference of sperm competition from broods of field-caught *Drosophila*. *Evolution* **52**, 1334–1341.

Hewitt, G. M., Mason, P. & Nichols, R. A. (1989). Sperm precedence and homogamy across a hybrid zone in the alpine grasshopper *Podisma pedestris*. *Heredity* **62**, 343–353.

Hey, J. & Kliman, R. M. (1993). Population genetics and phylogenetics of DNA sequence variation at multiple loci within the *Drosophila melanogaster* species complex. *Molecular Biology and Evolution* **10**, 804–822.

Howard, D. J., Gregory, P. G., Chu, J. J. & Cain, M. L. (1998). Conspecific sperm precedence is an effective barrier to hybridization between closely related species. *Evolution* **52**, 511–516.

Imhof, M., Harr, B., Brem, G. & Schlötterer, C. (1998). Multiple mating in wild *Drosophila melanogaster* revisited by microsatellite analysis. *Molecular Ecology* **7**, 915–917.

Jallon, J.-M. & David, J. R. (1987). Variations in cuticular hydrocarbons among the eight species of the *Drosophila melanogaster* subgroup. *Evolution* **4**, 294–302.

Jamart, J. A., Casares, P., Carracedo, M. C. & Piñeiro, R. (1995). Consequences of homo- and heterospecific rapid remating on the fitness of *Drosophila melanogaster* females. *Journal of Insect Physiology* **41**, 1019–1026.

Kulathinal, R. & Singh, R. S. (1998). Cytological characterization of premeiotic versus postmeiotic defects producing hybrid male sterility among sibling species of the *Drosophila melanogaster* complex. *Evolution* **52**, 1067–1079.

Leips, J. & Mackay, T. F. (2000). Quantitative trait loci for life span in *Drosophila melanogaster*: interactions with genetic background and larval density. *Genetics* **155**, 1773–1788.

Lemeunier, F., David, J. R., Tsacas, L. & Ashburner, M. (1986). The *melanogaster* species group. In *Genetics and Biology of Drosophila*, vol. 3E (ed. M. Ashburner, H. L. Carson & J. N. Thompson), pp. 147–256. London: Academic Press.

Long, A. D. & Langley, C. H. (1999). The power association studies to detect the contribution of candidate genetic loci to variation in complex traits. *Genome Research* **9**, 720–731.

Neubaum, D. M. & Wolfner, M. F. (1999). Wise, winsome, or weird? Mechanisms of sperm storage in female animals. *Current Topics in Developmental Biology* **41**, 67–97.

Nuzdhin, S. V., Pasyukova, E. G., Dilda, C. L. & Zeng, Z.-B. (1997). Sex-specific quantitative trait loci affecting longevity in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA* **94**, 9734–9739.

Palopoli, M. F. & Wu, C.-I. (1994). Genetics of hybrid male sterility between *Drosophila* sibling species: a complex web of epistasis is revealed in interspecific studies. *Genetics* **138**, 329–341.

Perez, D. E. & Wu, C.-I. (1995). Further characterization of the Odysseus locus of hybrid sterility in *Drosophila*: one gene is not enough. *Genetics* **140**, 201–206.

Price, C. S. C. (1997). Conspecific sperm precedence in *Drosophila*. *Nature* **388**, 663–666.

Price, D. K. & Boake, C. R. B. (1995). Behavioral reproductive isolation in *Drosophila silvestris*, *D. heteroneura*, and their F_1 hybrids (diptera: Drosphilidae). *Journal of Insect Behaviour* **8**, 595–616.

Templeton, A. R. & Sing, C. F. (1993). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. *Genetics* **134**, 659–669.

Thomas, S. & Singh, R. S. (1992). A comprehensive study of genic variation in natural populations of *Drosophila melanogaster*. VII. Varying rates of genic divergence as revealed by two-dimensional electrophoresis. *Molecular Biology and Evolution* **9**, 507–525.

True, J. R., Weir, B. S. & Laurie, C. C. (1996). A genome-wide survey of hybrid incompatibility factors by the introgression of marked segments of *Drosophila mauritiana* chromosomes into *Drosophila simulans*. *Genetics* **142**, 819–837.

Vieira, C., Pasyukova, E. G., Zeng, Z.-B., Hacket, J. B., Lyman, R. F. & Mackay, T. F. C. (2000). Genotype–environment interaction for quantitative trait loci affecting life span in *Drosophila melanogaster*. *Genetics* **154**, 213–227.

Wade, M. J., Patterson, H., Chang, M. W. & Johnson, N. (1994). Postcopulatory, prezygotic isolation in flour beetles. *Heredity* **72**, 163–167.

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.