Quantitative Trait Loci Responsible for Variation in Sexually Dimorphic Traits in *Drosophila melanogaster*

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ABSTRACT

To understand the mechanisms of morphological evolution and species divergence, it is essential to elucidate the genetic basis of variation in natural populations. Sexually dimorphic characters, which evolve rapidly both within and among species, present attractive models for addressing these questions. In this report, we map quantitative trait loci (QTL) responsible for variation in sexually dimorphic traits (abdominal pigmentation and the number of ventral abdominal bristles and sex comb teeth) in a natural population of *Drosophila melanogaster*. To capture the pattern of genetic variation present in the wild, a panel of recombinant inbred lines was created from two heterozygous flies taken directly from nature. High-resolution mapping was made possible by cytological markers at the average density of one per 2 cM. We have used a new Bayesian algorithm that allows QTL mapping based on all markers simultaneously. With this approach, we were able to detect small-effect QTL that were not evident in single-marker analyses. Our results show that at least for some sexually dimorphic traits, a small number of QTL account for the majority of genetic variation. The three strongest QTL account for >60% of variation in the number of ventral abdominal bristles. Strikingly, a single QTL accounts for almost 60% of variation in female abdominal pigmentation. This QTL maps to the chromosomal region that Robertson *et al.* have found to affect female abdominal pigmentation in other populations of *D. melanogaster*. Using quantitative complementation tests, we demonstrate that this QTL is allelic to the *bric a brac* gene, whose expression has previously been shown to correlate with interspecific differences in pigmentation. Multiple *bab* alleles that confer distinct phenotypes appear to segregate in natural populations at appreciable frequencies, suggesting that intraspecific and interspecific variation in abdominal pigmentation may share a similar genetic basis.

ONE of the greatest challenges in evolutionary biology is to understand the origin of interspecific differences and the relationship between variation in natural populations and species divergence (Lynch and Walsh 1998). To achieve this, it is necessary to characterize the patterns of genetic variation within and among species and to elucidate the evolutionary forces acting on this variation. Sexually dimorphic morphological traits can serve as a powerful paradigm for addressing these questions. In many groups of animals, secondary sexual characters can differ dramatically among closely related species, suggesting that these characters evolve at a higher rate than most other morphological traits (Carson 1978; Eberhard 1985; Andersson 1994; Civetta and Singh 1998; Kopp and True 2002). Theoretical models suggest that sexual dimorphism may evolve as a result of sexual selection, intra- and interspecific competition for resources, or a combination of these factors (Slatkin 1984). The role of sexual selection appears to be particularly important. Secondary sexual traits have been shown to affect mating preferences in a variety of animals (Andersson 1994; Gray and Cade 2000; Greene *et al.* 2000; Boughman 2001), and both theoretical and experimental studies indicate that sexual selection can drive rapid character divergence and speciation (Fisher 1958; Carson 1978; Lande 1981; Kirkpatrick 1982; Rice and Holland 1997; Holland and Rice 1998; Arnqvist *et al.* 2000; Gavrilets 2000; Hosken *et al.* 2001; Knowles and Markow 2001; Panhuis *et al.* 2001). Further, conflicting selective forces acting on males and females, and antagonistic allelic effects in the two sexes, can drive rapid molecular evolution (Swanson *et al.* 2001).

Interspecific differences in secondary sexual traits evolve as a result of divergent sexual selection on genetic variation present in natural populations (Gross 1985; Sappington and Taylor 1990; Gray and Cade 2000; Greene *et al.* 2000; Boughman 2001; Panhuis *et al.* 2001). However, the patterns of genetic variation for sexually dimorphic characters within species, and the mechanisms that maintain this variation, are poorly understood (see Lynch and Walsh 1998, for review). On the basis of studies in Drosophila, a substantial proportion of intraspecific differences appear to be controlled by quantitative trait loci (QTL) with sex-specific or sex-

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limited effects. For instance, the effects of natural QTL alleles on the duration of life, the number of bristles, and reproductive traits are more frequently sex specific than shared across sexes (Nuzhdin *et al.* 1997; Gurganus *et al.* 1998; Wayne *et al.* 2001). Similarly, Lyman *et al.* (1996) have detected significant genotype-by-sex interactions in examining the effects of *P*-element-induced mutations, while Long *et al.* (1998) have identified nucleotide polymorphisms with sex-specific effects in candidate genes associated with variation in bristle number. Recent work suggests that sexual selection maintains large amounts of variation in genes that affect sexually dimorphic traits (Chippindale *et al.* 2001).

Further progress in understanding the mechanisms of morphological evolution can best be achieved by identifying the genes responsible for intra- and interspecific differences and characterizing variation in these genes within and among species. In Drosophila, sexually dimorphic development is controlled by a regulatory cascade that is initiated by the *Sex lethal* (*Sxl*) gene and results in the production of sex-specific isoforms of the *doublesex* (*dsx*) transcription factor (Burtis and Baker 1989; McKeown 1992; Cline and Meyer 1996). In males, the male-specific isoform (DsxM) promotes the development of male phenotypes and prevents the development of female phenotypes, while the female-specific protein (DsxF) performs the reciprocal function in females (Baker and Ridge 1980; Burtis 1993). In the absence of either protein, or when both protein isoforms are coexpressed, phenotypically intermediate animals develop (Baker and Ridge 1980).

In contrast to our good understanding of sex determination in Drosophila, very little is known about the genetic control of sex-specific differentiation of particular morphological structures. The sex of each somatic cell is determined autonomously, suggesting that the Dsx transcription factor functions by activating or repressing the expression of different target genes in different developing tissues. The only known direct target genes of *dsx* are the *yolk protein* (*yp*) genes, which serve as a paradigm for sex-specific gene regulation in Drosophila (Burtis *et al.* 1991; Coschigano and Wensink 1993). In the absence of either Dsx isoform, the *yp* genes are expressed at a level intermediate between normal male and female expression. The DsxM protein represses this basal transcription, while DsxF increases it severalfold (Coschigano and Wensink 1993). It is possible that other genes involved in sex-specific differentiation in Drosophila are regulated in a similar fashion. Recently, expression of several important regulatory genes in sexually dimorphic tissues was shown to be regulated by *dsx*. These genes include both transcription factors, such as *bric a brac* (*bab*; Kopp *et al.* 2000) and *dachshund* (*dac*; Keisman and Baker 2001), and intercellular signals, such as *wingless* (*wg*) and *decapentaplegic* (*dpp*; Keisman and Baker 2001; Keisman *et al.* 2001; Sanchez and Guerrero 2001; Sanchez *et al.* 2001). However, it remains to be determined whether *dsx* regulates any of these genes directly.

In this report, we combine genome-wide QTL mapping with a candidate gene approach in an attempt to identify genes responsible for the variation in sexually dimorphic traits in a natural population. *Drosophila melanogaster* owes its name to the male-specific dark pigmentation of the last two abdominal segments (Figure 1). This pattern is of recent evolutionary origin and is present in only a subset of Drosophila species. Male-specific pigmentation, as well as bristle and trichome formation, is controlled by the *bab* gene, which in turn is jointly regulated by *dsx* and by the homeotic (HOX) gene *Abdominal-B* (*AbdB*; Kopp *et al.* 2000). In females, *bab* expression in the abdomen is required to prevent the development of male-specific pigmentation. In males, *bab* is repressed by *AbdB* in posterior abdominal segments, allowing dark pigmentation to develop, but suppressing bristles and trichomes. In other Drosophila species, the pattern of *bab* expression in the pupal abdomen correlates with the presence or absence of sexually dimorphic pigmentation, suggesting that changes in *bab* regulation have played an important role in the evolution of this trait (Kopp *et al.* 2000).

Females in natural populations of *D. melanogaster* show extensive variation in the pigmentation of posterior abdominal segments (Robertson and Louw 1966; Robertson *et al.* 1977). Robertson *et al.* (1977) have found that a large portion of this variation maps to a locus they named *female abdominal pattern* (*fap*). In this report, we show that a single QTL located in the same chromosomal region as *fap* accounts for ~60% of variation in female abdominal pigmentation in recombinant inbred lines derived from a single natural population. The QTL affects neither abdominal bristles and trichomes controlled by the same pathway nor sex comb tooth number, on which the effect of *fap* was seen by Robertson *et al.* (1977). Using quantitative complementation tests, we demonstrate that this QTL is allelic to *bab*, suggesting that intraspecific and interspecific variation in sexually dimorphic pigmentation may share a similar genetic basis. We also find that a small number of genes account for the majority of variation in the number of abdominal bristles, although the identity of these genes remains unknown.

MATERIALS AND METHODS

Recombinant inbred lines: A panel of 144 recombinant inbred lines (RILs) was generated by crossing a single virgin female from the F_1 progeny of a fertilized female caught in the wild (Winters, California) to a single male from the F_1 progeny of a different fertilized female caught at the same location. Chromosomes of the parental flies were allowed to recombine for one generation, and recombinant F_2 genomes were isogenized by 25 generations of full-sib inbreeding. Since the parental individuals were heterozygous, up to four different alleles may be segregating at each locus.

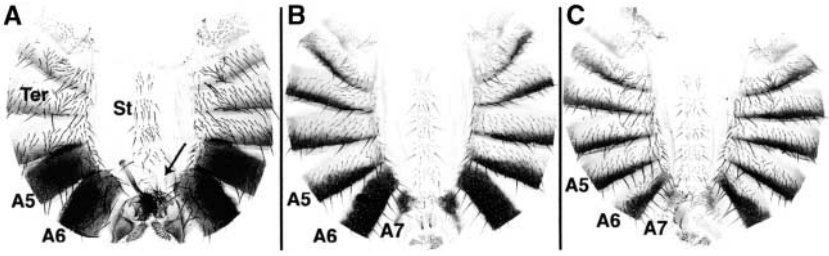

FIGURE 1.—Abdominal pigmentation in *D. melanogaster*. Ter, tergite; St, sternite; A5–A7, abdominal segments 5–7, respectively. (A) Male: note the solid black pigmentation of A5 and A6. A7 is rudimentary and lacks a tergite. (B) A dark female, similar to the darkest phenotype seen in our sample. Note the complete pigmentation of A6. (C) A lighter female, similar to the lightest phenotype seen in our sample. Note the partial pigmentation of A6.

Molecular markers: As markers, we have used positions of transposable elements that are highly heterogeneous in natural populations (see CHARLESWORTH and LANGLEY 1989, for review). Elements of the *roo* family are present in ~50 copies per gamete at random (at the cytological level of resolution) chromosomal positions. Positions of the *roo* elements were determined by *in situ* hybridization to polytene salivary gland chromosomes, using a biotinylated DNA probe (SHRIMPTON *et al.* 1986). For use as probe, a plasmid carrying a full-length *roo* element was labeled with bio-7-dATP (Bethesda Research Laboratories, Gaithersburg, MD) by nick translation. Hybridization was detected using the Elite Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and visualized with diaminobenzidine. Slides were photographed on a Zeiss Axioplan 2 microscope using a high-resolution CCD camera (total magnification of 800× including an Optovar device) connected to a Flashpoint frame grabber. Element locations were determined from the images at the level of cytological bands on the standard Bridges' map of *D. melanogaster* polytene chromosomes (LEFEVRE 1976).

We genotyped five individuals per line. The marker was recorded as present if detected in all larvae, absent if not detected in any larva, and segregating otherwise. Initial linkage of markers on the four (three) homologous chromosomes per autosome (X chromosome) was inferred from residual linkage disequilibrium in the population of RILs (Proc CORR; SAS INSTITUTE 1989). Since the markers are very dense (~1 per 2 cM), we experienced no difficulty in reconstructing the initial marker arrangement, which was further tested for consistency with the pattern of allele segregation in 12 F_1 larvae from the cross of two parental flies. Several *roo* elements either were uninformative (present in more than one complementation group) or gave a hybridization signal that was too weak to be scored reliably. Such elements were dropped from further analysis, leaving 152 markers that segregated between parental chromosomes (Figure 2). All chromosomes were homosequential, with the exception of an inversion on 3R that contains ~7% of the physical genome (~89EF; 96A). Genotype information may be requested from S. Nuzhdin.

Phenotypic traits: The female abdomen of *D. melanogaster* is composed of seven segments (A1–A7; Figure 1). Segments A2–A7 contain ventral cuticular plates (sternites) that carry a number of mechanosensory bristles. The male abdomen consists of only six segments (A1–A6); the A7 is rudimentary, while the A6 sternite lacks bristles. The dorsal cuticular plates (tergites) of the last two abdominal segments (A5 and A6) are completely melanized in the male. In females, the pigmentation of A5, A6, and A7 tergites varies extensively (Figure 1, B and C). The abdominal traits analyzed in this report include the number of sternite bristles on A5–A7 in the female and A5 in the male and the extent of pigmentation of A5–A7 tergites in females. We also scored the number of sex comb bristles in males, as described by NUZHDIN and REIWITCH (2000). All 144 RILs were scored for female A6 pigmentation traits. The sex comb was scored in 138 lines, A5 pigmentation in 83 lines, and A7 pigmentation in 64 lines. Five females and 5 males from each line were scored for abdominal traits, and 10 different males were scored for the sex comb.

To score the abdominal traits, flies were fixed in a 3:1 ethanol/glycerol mixture, and the abdomens were removed and examined under a dissecting microscope. The extent of pigmentation was quantified as follows (see Figure 3). The pigment in A5 and A6 is distributed in a band that begins at the posterior edge of the tergite and extends a variable distance to the anterior. This band is typically wider at the dorsal midline and narrower at the lateral edges of the tergite. We measured the width (*i.e.*, the anterior-posterior extent) of the pigment band at the dorsal midline and at the lateral edge of the tergite. Each was measured as a percentage of the total width of the tergite at the corresponding location, in increments of 10%, using a reticulated eyepiece. From these, the total pigmented tergite area can be expressed as follows (Figure 2). Assume that the total width of the tergite at the dorsal midline is X, its width at the lateral edge is Z, and the total length of the half-tergite, from the lateral edge to the dorsal midline, is Y. The shape of the tergite is largely invariable, so we assume $Z = cX$. The tergite area is then $S = (X + cX)/2 \times Y$. Now, assume that A is the width of the pigment band at the dorsal midline, as a fraction of X, and B is its width at the lateral edge, as a fraction of Z. Pigmented area is then $s = (AX + BcX)/2 \times Y$. The parameter we are interested in is the relative extent of pigmentation $s/S = (A + Bc)/(1 + c)$. We estimated c to be 0.75, so that $s/S = (A + 0.75B)/1.75$. While this method of estimation is somewhat imprecise, it allows relatively rapid scoring of large numbers of individuals. The shape of the pigmented area of the A7 tergite is highly irregular and varies among lines, so it could not be measured in the same fashion as A5 and A6. Instead, the relative extent of A7 pigmentation was estimated roughly by eye in increments of 10%.

Analysis of variance: We used Kolmogorov-Smirnov and Shapiro-Wilkinson tests to verify the normality of the traits (Proc UNIVARIATE; SAS INSTITUTE 1989). For normally distributed traits (bristles and sex combs), variance was analyzed with the procedures GLM and VARCOMP. One-way analysis of variance with the line effect random was employed for all traits except A5 bristles, where the fixed effect of sex and random effect of sex-by-line interaction were included. Genetic correlations were computed from the variance components (Proc VARCOMP) as $\sigma_{L12}/(\sigma_{L1} \times \sigma_{L2})$, where σ_{L12} is the covariance between traits among lines from the joint analysis, and $\sigma_{L1} = \sqrt{\sigma^2_{L1}}$ and $\sigma_{L2} = \sqrt{\sigma^2_{L2}}$, where σ^2_{L1} and σ^2_{L2} are the variances among lines for traits 1 and 2, respectively (ROBERTSON 1959).

For pigmentation, significance of the line component of variance was inferred with the nonparametric Kruskal-Wallis test (Proc NPAR1WAY). For traits with nonnormal distribution, we computed Spearman's rank order correlations (Proc CORR).

Single-marker analysis: For each trait, we first tested whether the genes contained within the inversion segregating among

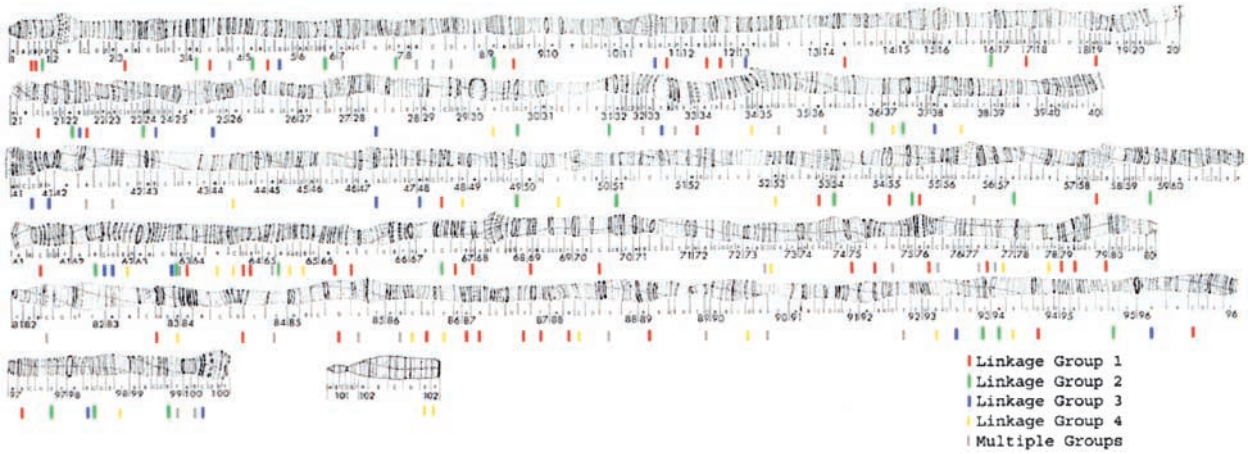

FIGURE 2.—Initial linkage of marker alleles on four chromosomes. Markers are 1CD, 1E, 3A, 3D, 4A, 4C, 4E, 5A, 5C, 5D, 6E, 7A, 7F, 8B, 8C, 8D, 8E, 9A, 9C, 11C, 11F, 12C, 12F, 13A, 14B, 16F, 17C, 17E, 19A, 19B, 21C, 21D, 22A, 22B, 22C, 23A, 24CD, 25D, 28C, 30B, 30D, 31F, 32F, 33B, 33D, 35CD, 36B, 38A, 38CD, 40AC, 41CE, 41F, 42B, 42D, 44C, 47B, 48A, 48DE, 48F, 49B, 49F, 50C, 51A, 53E, 54A, 54D, 55A, 55C, 55D, 56E, 57B, 57E, 57F, 58D, 59D, 60E, 61C, 62B, 62CD, 63E, 63F, 64D, 64E, 65AB, 66A, 66B, 67C, 67D, 67F, 69A, 69D, 70C, 73C, 73D, 75A, 75C, 75D, 76B, 76C, 77B, 77E, 78E, 79A, 79D, 79F, 80AC, 82C, 82F-83A, 83D, 84DE, 84F, 85D, 85E, 86B, 86C, 86D, 86E, 87A, 87B, 87E, 87F, 88B, 88C, 88E, 89AB, 89DE, 89EF, 90EF, 91D, 92E, 93B, 93D, 93F, 94A, 94B, 94D, 94F, 95E, 96A, 96C, 97B, 97C, 98B, 98C, 98E, 99E, 99F, 100B, 100C, 102E, and 102F.

the RILs had a significant effect. The inversion affected A5, A6, and A7 bristles of females ($P < 0.0001$, $P = 0.0020$, $P < 0.0001$ correspondingly), as well as all pigmentation traits (all $P < 0.0001$ except for lateral pigmentation on A6, $P = 0.0009$, and A7 pigment area, $P = 0.018$). Sex comb tooth number was also affected by the inversion ($P = 0.0003$). The effect of inversion in all subsequent analyses was accounted for by including it in the model as a fixed-effect cofactor.

For each of the marker loci, the significance of the difference in the mean trait values was tested between two groups of lines, one with the marker allele containing the *roo* insert and the other marker allele having no *roo* insert (Proc GLM). An empirical distribution of the test statistics under the null hypothesis of no association between any of the markers and trait values was obtained by randomly permuting the trait data 1000 times and calculating the most significant probability for marker-trait associations across all markers for each permutation. Statistics from the original data that were exceeded <50 times by those from the permutation are significant at $P = 0.05$ (DOERGE and CHURCHILL 1996).

Mixed-model analysis: With our crossing design, a *roo* insert is present on one out of four (three for the X) parental recombining chromosomes. The RILs that carry a *roo* insert at a marker locus share one putative QTL allele (assuming no recombination between the QTL and the marker). However, the effects of up to three different alleles (two for the X) may be mixed in the group of RILs that lack a *roo* insert at that locus. Since the existing QTL mapping programs were developed to deal with recombination between two rather than four parental chromosomes, we were unable to use them for multiple-marker analyses. Instead, we adopted the new mixed-model methodology in which the marker effects under investigation are treated as random effects, whereas other effects that are not of our interest (such as classifiable environmental effects) are treated as fixed. When treated as random effects, all markers can be simultaneously included in the model without concern about the loss of degrees of freedom. The genetic effects of markers can then be estimated using the method of best linear unbiased prediction (HENDERSON 1975) as conditioned expectations given the phenotypic data and the variance components. A brief explanation of the technique is presented below, while a more detailed treatment can be found in the accompanying article (Xu 2003, this issue).

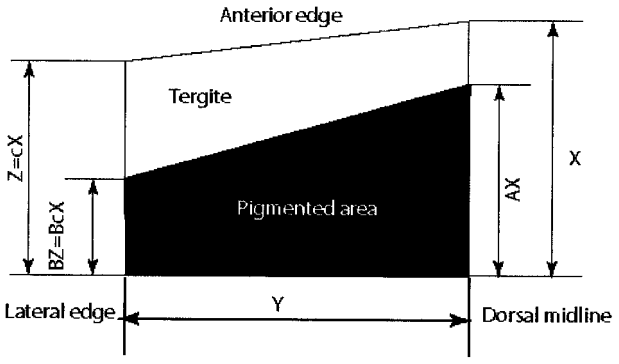

FIGURE 3.—Pigmentation measurements. Shown is a schematic representation of the left hemitergite from the lateral edge to the dorsal midline. Anterior is up. See text for further explanations.

Let y_j be the phenotypic value of individual j for $j = 1, \ldots, n$, where n is the total number of individuals in the mapping population. The mixed-linear model describing y_j is

$$y_j = \sum_{i=1}^{p} x_{ij} b_i + \sum_{k=1}^{m} z_{kj} u_k + e_j, \qquad (1)$$

where b_i for i $= 1, \ldots, p$ denotes the ith fixed effect; x_{ij} is the corresponding indicator for the presence of b_i; u_k denotes the effect of marker k for $k = 1, \ldots, m$; z_{kj} is the corresponding indicator for the presence of u_k; and e_j is the random environmental effect assuming normal distribution with mean of zero and variance of σ_e^2. The kth marker effect u_k is assumed to be sampled from a normal distribution with mean of zero and variance of σ_k^2. Two alleles are assumed to be sampled for each locus, u_k represents the allelic difference between the two allelic values at marker k, and the estimate of σ_k^2 is actually obtained by $\sigma_k^2 = u_k^2$. The overall genetic variance in the mapping population is $\sigma_G^2 = \Sigma_{k=1}^{m} \mathrm{var}(z_k)\sigma_k^2$, where $\mathrm{var}(z_k) = p_k(1 -$

p_k) and p_k is the allelic frequency of marker k. The total phenotypic variance is defined as $\sigma_P^2 = \sigma_G^2 + \sigma_e^2$. The proportion of the phenotypic variance explained by marker k is $h_k^2 = p_k(1 - p_k)\sigma_k^2/\sigma_P^2$.

Each trait was analyzed separately. To define significance thresholds for simultaneous tests of all marker effects, u_k, for $k = 1, \ldots, m$, the data were permuted 1000 times, and each permuted data set was analyzed using the same procedure. The actual estimated u_k was compared with the interval between 2.5 and 97.5 percentiles of the permuted sample u_k. If the estimated u_k is beyond the interval, the effect is declared as significant at the 5% level. If the estimate is beyond the interval between 0.5 and 99.5 percentiles, significance is declared at the 1% level.

Complementation test: In the traditional test for noncomplementation, we infer that mutations m and m^* are alleles of the same locus if the phenotype of m/m^* is qualitatively different from $m/+$ and $m^*/+$ (where + is the wild-type allele). Complementation tests have also been extended to alleles with quantitative effects on the phenotype (Mackay and Fry 1996). In this case, noncomplementation between a QTL and a known gene is detected in the analysis of variance as a significant interaction term between two QTL alleles (QTL_1 and QTL_2) and the mutant and wild-type alleles of the suspected gene (m and +). The weakness of this approach is that interactions may occur not only between alleles of the same locus but also between different loci. Therefore, to have any confidence in the results of quantitative complementation tests, genetic backgrounds of the QTL alleles must be either identical or randomized. We followed the latter approach by testing 10 RILs for each of the two QTL alleles. Thus, the only consistent difference between the two sets of lines was at the QTL, while the rest of the genome was being sampled in a random and unbiased way. Each RIL was tested over bab^{An07}, a null allele of *bab* (Couderc *et al.* 2002).

RESULTS

Trait variation: The goal of this work is to understand the genetic architecture of variation for sexually dimorphic traits in natural populations. Existing panels of recombinant inbred lines originated from parental stocks that had been kept in the laboratory for hundreds of generations (Nuzhdin *et al.* 1997) and often underwent artificial selection for morphological or life-history traits (Long *et al.* 1995; Curtsinger *et al.* 1998; Weber *et al.* 1999). To capture the pattern of genetic variation present in the wild, we created a new panel of RILs from a pair of heterozygous flies taken directly from nature.

For each trait, we find a significant genetic component of variation. The means (standard deviations) for bristle numbers on the male A5 and female A5, A6, and A7 sternites were 17.59 (1.77), 21.12 (2.56), 19.76 (2.44), and 9.62 (1.49), respectively (Figure 4A). The mean and standard deviation for the number of sex comb bristles on both legs was 20.55 (1.23; Figure 4B). Distributions did not deviate from normality except for A5 bristles in males (Kolmogorov-Smirnov test for nonnormality, $P = 0.013$) and females (Shapiro-Wilkinson test, $P = 0.0033$). For these traits, the line components of variance were significant with the nonparametric Kruskal-Wallis test ($\chi^2_{141} = 370.5$, $P < 0.0001$ in males; $\chi^2_{141} = 437.58$, $P < 0.0001$ in females). The line component of variance was also highly significant for normally distributed A6 bristles ($F_{141,141} = 7.13$, $P < 0.0001$), A7 bristles ($F_{141,141} = 4.42$, $P < 0.0001$), and the sex comb ($F_{136,136} = 7.61$, $P < 0.0001$). For A5 bristles, the line, sex, and line-by-sex interaction components of variance were all significant ($F_{141,141} = 4.54$, $P < 0.0001$; $F_{1,141} = 502.30$, $P < 0.0001$; $F_{141,1127} = 2.11$, $P < 0.0001$).

The pigmentation of A5 and A6 tergites in females was described by three parameters: the width of the pigment band at the dorsal midline and at the lateral edge of the tergite and the relative pigmented area calculated from these two measurements (Figure 4C; see MATERIALS AND METHODS). The medial and lateral width of the pigment band in A6 had the means (standard deviations) of 96.96 (5.80) and 82.06 (16.64), respectively. Distributions were strongly and significantly nonnormal for both traits (Kolmogorov-Smirnov test, $P < 0.01$) and could not be normalized by any of the standard transformations (not shown). This could be due to the segregation of a major-effect QTL at the tip of 3L (see below). For this reason, nontransformed data were used for all pigmentation traits (medial and lateral width and relative area for A5 and A6 and approximate area for A7). The line component of variance was significant ($P < 0.0001$) for all seven traits: medial and lateral width of the pigment band and relative pigmented area for A5 and A6 and approximate pigmented area for A7 (Kruskal-Wallis test: $\chi^2_{141} = 532.05$, 629.96, and 630.62, respectively, for A6; $\chi^2_{83} = 360.68$, 299.80, and 351.99 for A5; and $\chi^2_{62} = 281.77$ for A7).

Genetic correlation between traits: Strong genetic correlation between two quantitative traits may suggest that these traits share a similar genetic and developmental basis, as was predicted for pigmentation and abdominal bristles affected by a single pathway, and for the sex comb *fap* effect, which was previously observed by Robertson *et al.* (1977). We therefore investigated whether the various sexually dimorphic traits were correlated with each other. The results are shown in Table 1.

All seven pigmentation traits were strongly and significantly correlated with each other (Spearman correlation coefficients of 0.56–0.84; $P < 0.0001$ in each case), likely reflecting a common genetic control. The line-by-trait interaction component was significant for every comparison ($P < 0.0001$), indicating that genetic correlations between these traits are significantly less than one. The numbers of abdominal bristles on A5–A7 in females and A5 in males were also strongly and significantly correlated with each other (genetic correlations of 0.29–0.93; $P < 0.0001$ in each case). Line-by-trait interaction was significant ($P < 0.0001$) for all comparisons except between A5 and A6 bristles in females. For these two traits, there is no reason to suspect that genetic control differs between segments. In contrast, we find no significant correlation between pigmentation and

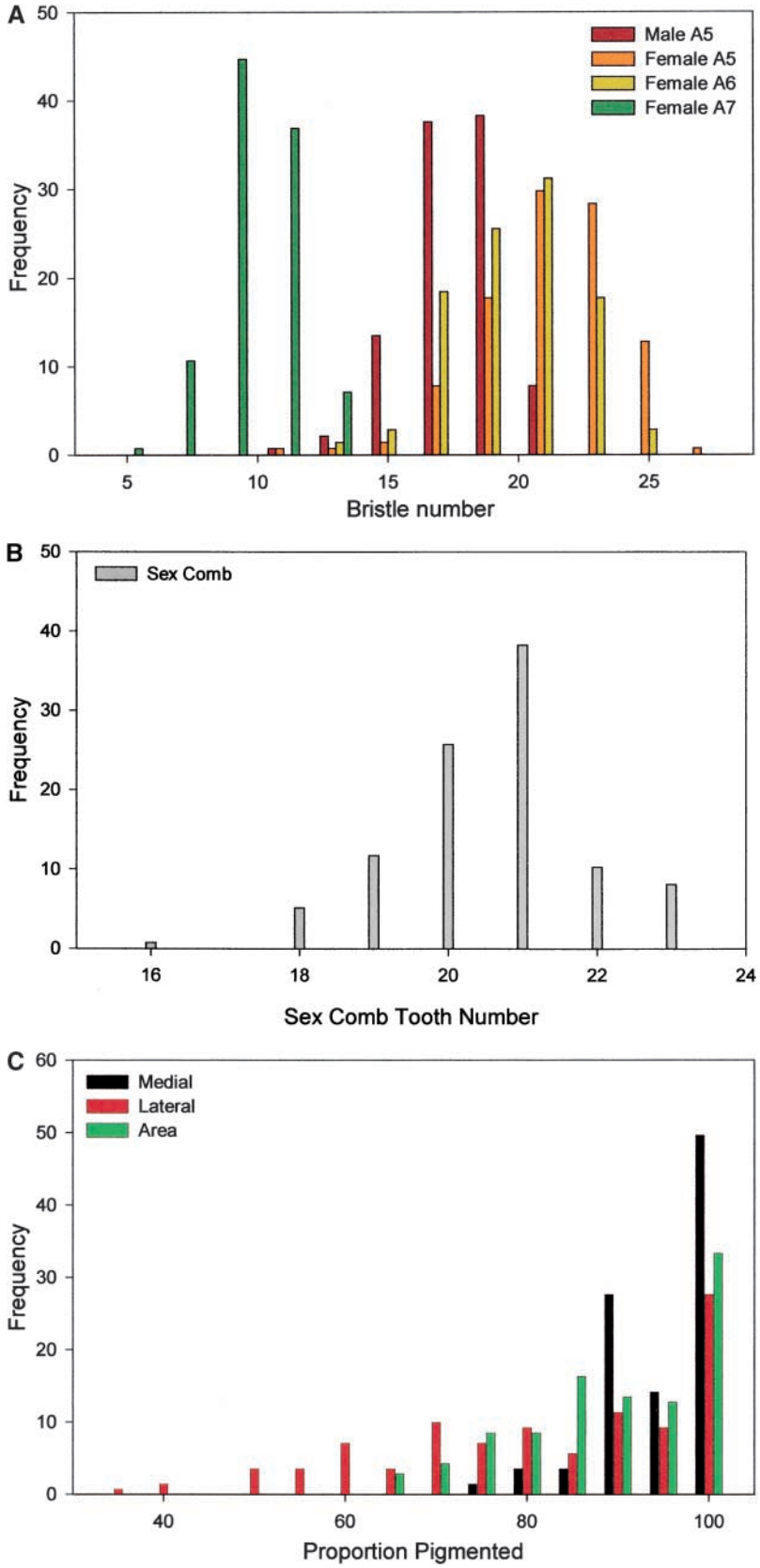

FIGURE 4.—Distribution of phenotypic values among RILs. (A) Sternite bristle number. (B) Sex comb tooth number. (C) A6 pigmentation.

bristle traits (Table 1), suggesting that different genetic factors contribute to natural variation in these traits.

The number of sex comb teeth was significantly, but weakly, correlated with the number of A5–A7 bristles in females (P = 0.0072, 0.021, 0.0035) and was not significantly correlated with the number of A5 bristles

TABLE 1

Genetic correlations between traits and sexes

Tr	A5, f	A6, f	A7, f	Pl6	Pm6	Pa6	Pl5	Pm5	Pa5	Pa7	A5, m	Sc
A5, f	=0.52	=0.93*	=0.46*	ns	ns	ns	ns	ns	ns	ns	=0.69*	=0.016
	<0.0001	<0.0001	<0.0001								<0.0001	=0.0072
		ns	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
A6, f		=0.55	=0.50	ns	ns	ns	ns	ns	ns	ns	=0.66*	=0.021
		<0.0001	<0.0001								<0.0001	=0.021
			<0.0001	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
A7, f			=0.41	ns	ns	ns	ns	ns	ns	ns	=0.29*	=0.052
			<0.0001								<0.0001	=0.0035
				<0.0001	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Pl6	*ns*	*ns*	*ns*	=0.69	=0.66*		=0.44*	=0.27*	=0.46*	=0.27	ns	=0.052
				<0.0001	<0.0001		=0.0080	<0.0001	<0.0001	=0.94		=0.0451
					<0.0001		*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Pm6	*ns*	*ns*	*ns*	=0.81*	=0.84		=0.46*	=0.31*	=0.48*	=0.45*	ns	=0.076
				<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001		=0.30
							<0.0001	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Pa6	*ns*	*ns*	*ns*			=0.85	=0.51*	=0.33*	=0.52*	=0.43	ns	=0.075
						<0.0001	<0.0001	<0.0001	<0.0001	=0.1175		=0.16
							<0.0001	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Pl5	*ns*	*ns*	*ns*	*=0.63**	*=0.58**	*=0.61**	=0.83	=0.44		=0.22	ns	ns
				<0.0001	*<0.0001*	*<0.0001*	<0.0001	<0.0001		=0.0008	*<0.0001*	*<0.0001*
								<0.0001		*<0.0001*		
Pm5	*ns*	*ns*	*ns*	*=0.56**	*=0.56**	*=0.57**	*=0.58**	=0.68		=0.36	ns	ns
				<0.0001	*<0.0001*	*<0.0001*	*<0.0001*	<0.0001		=0.084		
										<0.0001	*<0.0001*	*<0.0001*
Pa5	*ns*	*ns*	*ns*	*=0.67**	*=0.63**	*=0.66**			=0.83	=0.33	ns	ns
				<0.0001	*<0.0001*	*<0.0001*			<0.0001	=0.0078	*<0.0001*	*<0.0001*
										<0.0001		
Pa7	*ns*	*ns*	*ns*	*=0.66**	*=0.84**	*=0.82**	*=0.62**	*=0.65**	*=0.70**	=0.90	ns	ns
				<0.0001	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	<0.0001		
											<0.0001	*<0.0001*
A5, m				*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	0.44	=0.0075
											<0.0001	=0.1482
												<0.0001
Sc				*=0.19*	*=0.25*	*=0.25*	*ns*	*ns*	*ns*	*ns*		0.39
				=0.032	*=0.0031*	*=0.0041*						<0.0001

Pl, Pm, Pa: pigmentation lateral, medial, area. A, number of abdominal bristles; 5–7, abdominal segments 5 through 7, respectively; f, female; m, male. Line components of variance and their significances are plotted on the diagonal. Above the diagonal (roman type) are genetic correlations between traits, significance of their difference from zero (underlined if significant), and significance of the line-by-trait component of variance (italicized if significant). Below the diagonal (italic type) are Spearman rank-order correlations for the traits with nonnormal distributions.

TABLE 2

QTL positions and effects of their alleles (single-marker analysis): bristle number

Marker/trait	A5, f	A6, f	A7, f	A5, m
Permutation significance ($\times 10^5$)	38	42	40	31
22B	*−1.68* 143	*−1.32* 1001	*−0.58* 18117	*−1.14* 31
	−0.24 0.55	*−0.36* 0.52	0.01 0.97	*0.28* 0.36
22C	*2.44* 1.8	*2.10* 9.4	*1.14* 371	*1.59* <1
	1.22 0.0097	*1.33* 0.034	*1.32* 0.036	*0.7* 0.057
47E	*−1.43* 297	*−1.64* 25	*−0.76* 1073	*−0.97* 216
	−0.65 0.034	*−0.89* 0.030	*−1.26* 0.0023	*−0.61* 0.012
50C	*−3.32* 49	*−2.72* 274	*−2.16* 5.5	*−1.5* 1318
	−1.74 0.0034	*−3.46* <0.0001	*−3.20* <0.0001	*−2.19* <0.0001

In the first row, the 51st highest experiment-wise significance from 1000 permuted trait data. On the first line within cells in other rows, the difference of the trait value between RILs with different marker alleles (italicized) and its significance (underlined if exceeding the experiment-wise threshold), from single-marker analysis, are shown; on the second line within cells, marker effects and their significances estimated from the model with multiple markers included are shown.

in males ($P = 0.15$). The number of sex comb teeth was also weakly correlated with the dorsal and lateral width of the pigment band and the relative pigmented area of A6 in females (Spearman correlation coefficients 0.19–0.25; $P = 0.032$, 0.0031, 0.0041, respectively).

Single-marker analysis: We have identified markers that cosegregated with trait values for the sex comb, all bristle traits, and A6 pigmentation. No significant associations were found for A5 and A7 pigmentation, possibly due to the low number of lines scored (or, in the case of A7, to imprecise phenotypic measurements). A single marker at 67D was associated with variation in the number of sex comb teeth. The difference between groups of RILs with different marker alleles at this locus was 0.89 teeth, and the segregation of these alleles accounted for 20% of the line component of variance (Proc VARCOMP). For abdominal bristles and A6 pigmentation, the positions and effects of significant markers are listed in Tables 2 and 3, respectively.

The number of sternite bristles in females was associated with markers at 22C for A5, 22C and 47E for A6, and 50C for A7. The number of A5 bristles in males cosegregated with markers at 22B and 22C (Table 2). Since genetic correlations between all bristle traits are high and positive (Table 1), we included all markers that had a significant association with at least one trait into a multiple-regression model and fitted this model for every trait. The marker at 22B was not significant for any of the traits, while the other three markers were significant for every trait. The joint segregation of the markers at 22C, 47E, and 50C accounted for 53% of the line component of variance in the number of A5 bristles in males and for 67, 65, and 65% of variance in A5, A6, and A7 bristles in females (Proc VARCOMP).

The lateral width of the A6 pigment band was associated with the markers at 61C and 62B and the medial width with these same markers plus 20 other markers of the third chromosome. The pigmented area was affected by the segregation of the markers at 61C, 62B, and by 13 out of the above 20 markers (Table 3). Notably, the marker at 61C had a very large individual effect ($P < 10^{-21}$), and its associations with the other markers could account for their significance. We therefore reanalyzed the effects of all significant markers after including the 61C marker as a cofactor. The marker 62B retained significance (Table 2), but the effects of 14 markers out of 20 were no longer significant. Adding the marker 62B as a cofactor did not affect the significance of the remaining markers. These markers (67D–75A) are in strong linkage disequilibrium (data not shown) and may represent a single QTL. To check this, we reanalyzed the data after including the most significant of them (75A) as a cofactor. In this analysis, the markers at 61C, 62B, and 75A gave the only significant effects for all traits (except for 75A, which was not significant for the lateral width of the pigment band). The joint segregation of these markers accounted for 41, 81, and 75% of variation in the lateral and medial width of the pigment band and in the pigmented area, respectively. Of this 33, 62, and 59% were accounted for by 61C and 62B alone. Note that *roo* hybridization at 61C, 62B, 62C, and 62E marks four different parental chromosomes (Figure 2). Since QTL alleles linked to *roo* at 61C and 62B are associated with significantly increased pigmentation for the former and decreased for the latter in comparison with alleles linked to *roo* at 62C and 62E (Figure 5), at least three alleles with different effects on pigmentation should be segregating in the region 61–62. No linkage disequilibrium was found between any of the 61; 62 markers and the inversion (3R) 89EF; 96A, suggesting that the large effects of alternative alleles were not due to epistatic interactions with inversion haplotypes.

Mixed-model analysis: Since most traits are affected

TABLE 3

QTL positions and effects of their alleles (single-marker analysis): pigmentation

Marker/Trait	Pl6	Pm6	Pa6
Permutation significance ($\times 10^5$)	26	37	33
61C	*−5.16* 5	*−23.59* <1	*−13.52* <1
	−2.431 0.014	*−15.40* <0.0001	*−8.52* <0.0001
62B	*4.81* 16/0.021	*18.66* <1/0.0014	*11.00* <1/0.0016
	2.55 0.034	*8.24* 0.0012	*5.03* 0.0015
63E	363	<1[a]	<1
63F	512	<1	<1
64D	10191	<1	<1
64E	842	<1	<1
65AB	39852	<1	26
66A	26952	<1	30
66B	30485	<1	28
67D	6088	<1/0.0072[b]/0.0076	4/0.047/0.049
67F	13981	<1	13
69A	4407	<1/0.016/0.014	16/0.043/0.040
70C	1337	<1/0.0059/0.0063	14/0.012/0.014
73C	1161	<1/0.0074/0.036	29/0.014
75A	*4.05* 5262	*17.49* <1/0.0033/0.0008	*9.97* 4/0.017/0.00478
	1.33 0.23	*8.42* 0.0008	*4.39* 0.0048
75C	7976	<1/0.0050/0.0012	13/0.038/0.012
76B	14464	13	81
79B	15299	11	75
79C	18338	11	85
83D	5344	7	37
84DE	16882	9	97
94D	3508	26	70

See Table 2 for details.

[a] The effect of a marker was not included if significance of this marker was not retained in the model including markers at 61C, 62B, and 75A as cofactors (see RESULTS for explanations).

[b] Significance of the marker effect when the markers 61C, 62B + 61C, and 75A were included as cofactors. A marker was retested with more cofactors only if it was significant.

by multiple segregating QTL, models allowing for simultaneous effects of multiple markers are preferable. Since with our design, up to four alleles per QTL might segregate among RILs, we were not able to use software designed for crosses of isogenic lines. Instead, we applied Bayesian analyses fitting the model for trait variation with all the markers at once. The effect of each marker on each trait is shown in Figure 6 for bristles and the sex comb and in Figure 7 for pigmentation. Figures 6 and 7 also show the 5 and 1% significance thresholds for marker effects obtained by permutation of the data sets (see MATERIALS AND METHODS). Markers exceeding the 5% threshold (declared suggestive) and the 1% threshold (declared significant) are listed in Tables 4, 5, and 6.

For bristles, the markers found by single-marker analysis (SMA) are also significant (22C and 50C) or suggestive (47E) in mixed-model analysis (MMA). Additionally, MMA detects 1 other significant marker (76B) and 13 more markers suggestive for one or more traits (Table 4). Similarly for pigmentation, the markers significant in SMA (61C, 62B, and 75A) are also significant in MMA. Seven significant (62EF, 63E, 83F–84A, 84F, 87F, 90EF, and 98B) and 9 suggestive markers are also found for pigmentation of A5 and A7, for which no QTL were detected with SMA. For the sex comb, the marker at 67D (significant in SMA) is suggestive in MMA, and 8 other suggestive markers are observed. Overall, MMA supports inferences from SMA and adds more putative QTL with weak-effect alleles.

Complementation tests: Consistent with the earlier results of ROBERTSON *et al.* (1977), we find that segregation of two markers at the tip of 3L (61C and 62B) accounts for most of the genetic variation in A6 pigmentation, for instance, 58.9% of pigment area. A strong candidate for this QTL is the *bab* gene, which is located in 61F and is known to be involved in the development of abdominal pigmentation (KOPP *et al.* 2000). We therefore tested our QTL alleles for complementation with a null allele of *bab*, bab^{Ar07}. Two subsets of 10 lines each were sampled: one with *roo* present at 61C and absent at 62B (QTL_1) and the other with no *roo* at 61C and *roo* present at 62B (QTL_2). Each line was crossed to the stock that carried bab^{Ar07} over the TM3 balancer chromosome,

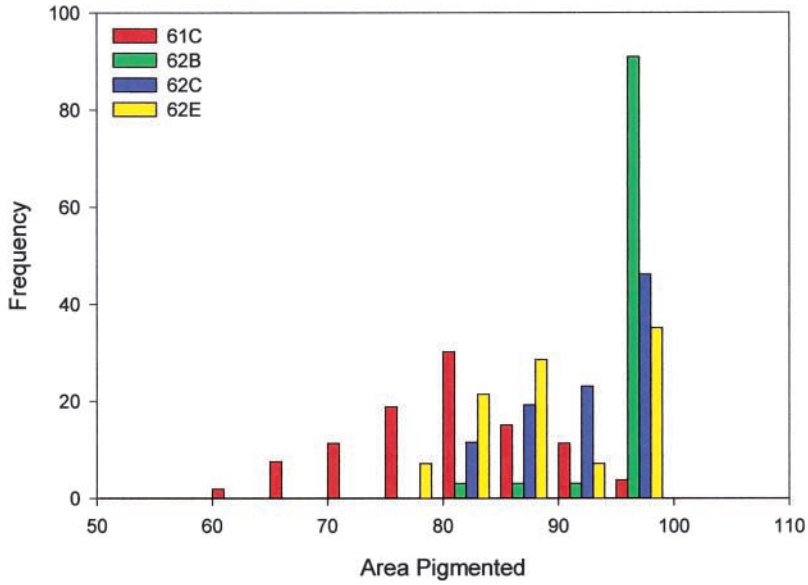

FIGURE 5.—Distribution of A6 pigmentation area values in RILs that carry different *roo* insertions (proxy for QTL alleles) in the 61; 62 chromosomal region.

and the heterozygous F_1 females (QTL_1/bab^{Ar07}, QTL_2/bab^{Ar07}, QTL_1/TM3, and QTL_2/TM3) were scored for A5 pigment area (*bab* loss-of-function mutations have a strong dominant phenotype, so that A6 is always completely pigmented). Since the analysis was performed in the F_1, hybrid dysgenesis could not be a confounding factor. The line component of variance was significant, pointing to a partial dominance of the QTL alleles ($F_{19,18} = 2.67$, $P = 0.021$). The effect of the *bab* allele (bab^{Ar07} *vs.* TM3; *i.e.*, wild type for pigmentation) was also significant ($F_{1,18} = 323.00$, $P < 0.0001$). Importantly, the line-by-*bab*-allele interaction component was highly significant ($F_{18,148} = 6.03$, $P < 0.0001$). To test whether the significance of the interaction term is explained by the QTL alleles, we repeated the test after pooling lines within marker classes. Again, the QTL allele ($P < 0.0001$), *bab* allele ($P < 0.0001$), and interaction ($P = 0.0002$) effects were all highly significant. Similarly, all these terms were significant when we included in the analyses the pigmentation scores for flies homozygous at QTL_1 and QTL_2 (Figure 8). Since 7 of the lines used in complementation tests carried inversion, we tested whether this could explain the above significant interactions by including inversion as a cofactor. The QTL allele-by-*bab*-allele interaction term was significant ($P = 0.0038$), but inversion by *bab* was not ($P = 0.15$), rejecting this conjecture. We conclude that QTL_1 and QTL_2 are alleles of the *bab* locus.

The *bab* locus contains two closely related, tandemly duplicated genes, *bab1* and *bab2* (COUDERC *et al.* 2002). Both genes contribute to the control of sexually dimorphic pigmentation, and their regulation appears to evolve in parallel (KOPP *et al.* 2000; COUDERC *et al.* 2002). Since the bab^{Ar07} mutation inactivates both *bab1* and *bab2*, the QTL alleles present in the wild could in principle be alleles of either or both genes.

DISCUSSION

We have identified QTL responsible for intraspecific variation in several sexually dimorphic morphological traits, including female abdominal pigmentation and the number of ventral abdominal bristles in males and females. The large number of RILs and the dense spacing of cytogenetic markers used in our study allowed us to map the QTL with high resolution, opening the way for candidate gene analysis. With the help of quantitative complementation tests, we identified *bab* as the locus responsible for most of the variation in sexually dimorphic pigmentation but not in bristles. Our results support the earlier findings of ROBERTSON *et al.* (1977), who also mapped a major-effect gene affecting female abdominal pigmentation (*fap*) to the region where we now know *bab* to be located (3–0.5). There is little doubt that *fap* and *bab* are the same gene.

For some of the traits, a small number of genes accounts for an unexpectedly large proportion of genetic variation among the RILs analyzed in this study. In particular, the three strongest QTL account for >60% of variation in the number of ventral abdominal bristles, and *bab* alone accounts for ~60% of variation in female pigmentation. Since the flies used to establish the RILs were taken directly from nature and did not undergo any inbreeding or artificial selection, our results suggest that QTL of large effect are present, and possibly common, in natural populations. At the same time, it is important to remember that these results are based on only four haploid genomes sampled from a single population. The generality of our results can be assessed only by conducting similar genetic studies in other, geographically isolated populations.

Quantitative genetics of sexually dimorphic phenotypes: As a group, the pigmentation traits considered in our study appear to share a common genetic basis.

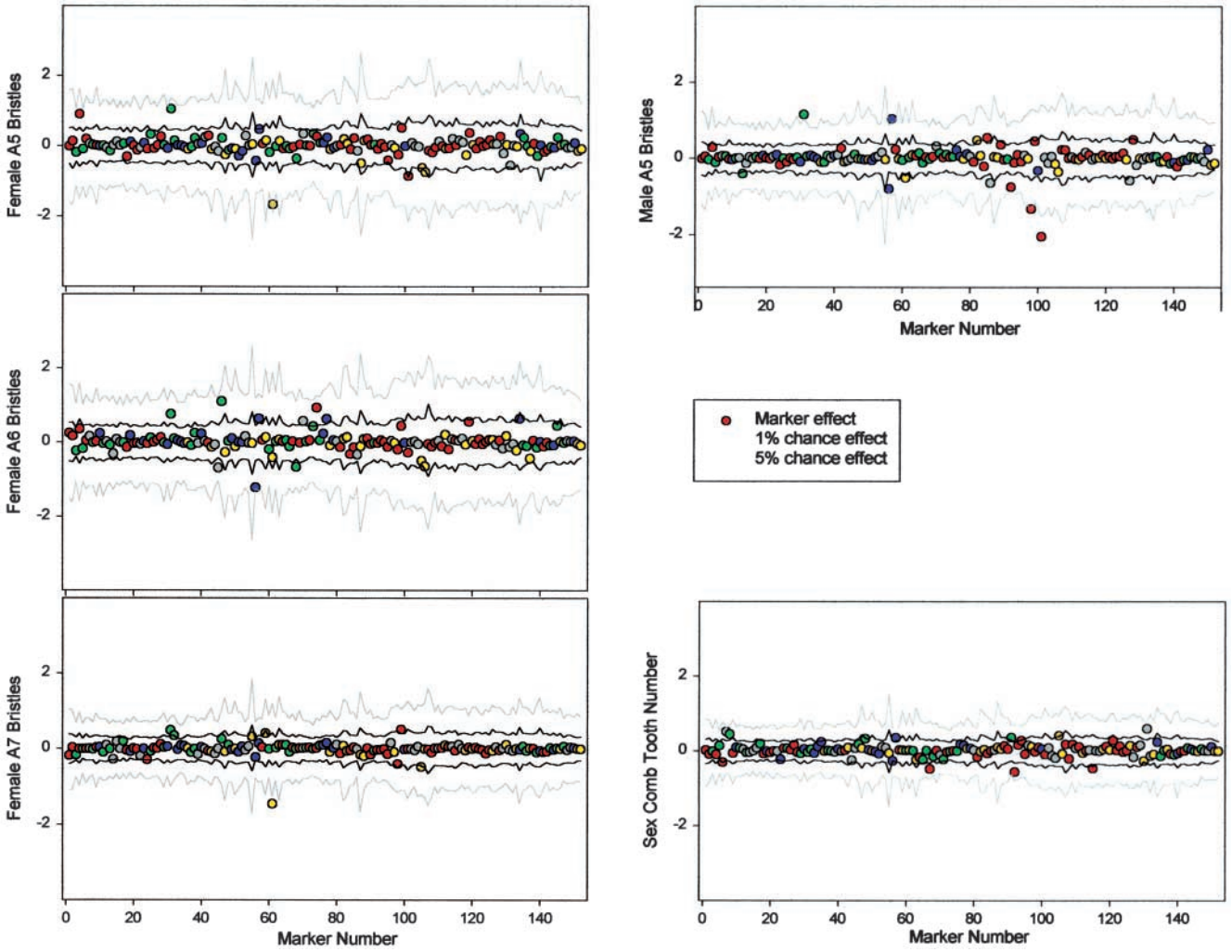

FIGURE 6.—MMA estimates for the effects of markers on abdominal bristles and sex comb teeth. The jagged gray and black lines denote significance thresholds (1 and 5%, respectively) obtained from 1000 permutations. Note that significance thresholds vary with chromosomal location. Marker values are shown as colored circles, with the four colors denoting the original linkage groups described in Figure 2. A marker is declared significant if it falls outside the 1% (gray) threshold and suggestive if it falls between the 5 and 1% thresholds.

We find a strong positive genetic correlation between the pigmentation of adjacent abdominal segments, as well as between the medial and lateral extent of the pigment bands. Moreover, the two large-effect QTL for A6 pigmentation detected by SMA were also found to affect A5 and A7 pigmentation in the more sensitive multiple-marker analysis. Remarkably, these two QTL together account for 75% of the variation in the A6 pigment area among our RILs; of these, *bab* alone accounts for 59%. Additional QTL of small effect were also identified by MMA, suggesting that the phenotypes of *bab* alleles are modified by other loci. *A priori*, these loci may include genes involved in sex determination (*e.g.*, *dsx*), regional patterning (*e.g.*, the HOX gene *Abd-B*; note that this gene is covered by inversion that has significant effect on pigmentation), or in the differentiation of pigment-producing cells (*e.g.*, components of the melanin synthesis pathway).

The numbers of sternite bristles on A5–A7 in the female, and A5 in the male, are also highly positively correlated among themselves and are affected by the same QTL. Three regions identified in SMA had similar effects on all bristle traits, with the magnitude of effects ranging from 0.6 to 3.5 bristles. Interestingly, these estimates obtained for random natural alleles are similar to those for the alleles responsible for selection response (LONG *et al.* 1995; NUZHDIN *et al.* 1999) or fixed between old laboratory lines (GURGANUS *et al.* 1998; NUZHDIN *et al.* 1998). Two of these three QTL have been mapped before (21E and 57D in GURGANUS *et al.* 1998). The line-by-trait interaction term was not significantly different from zero for A5 and A6 bristles in females, but was greater than zero for A7 bristles in females and A5 bristles in males. Accordingly, we find perfect genetic correlation between A5 and A6 bristles in females, whereas genetic correlations in all other comparisons

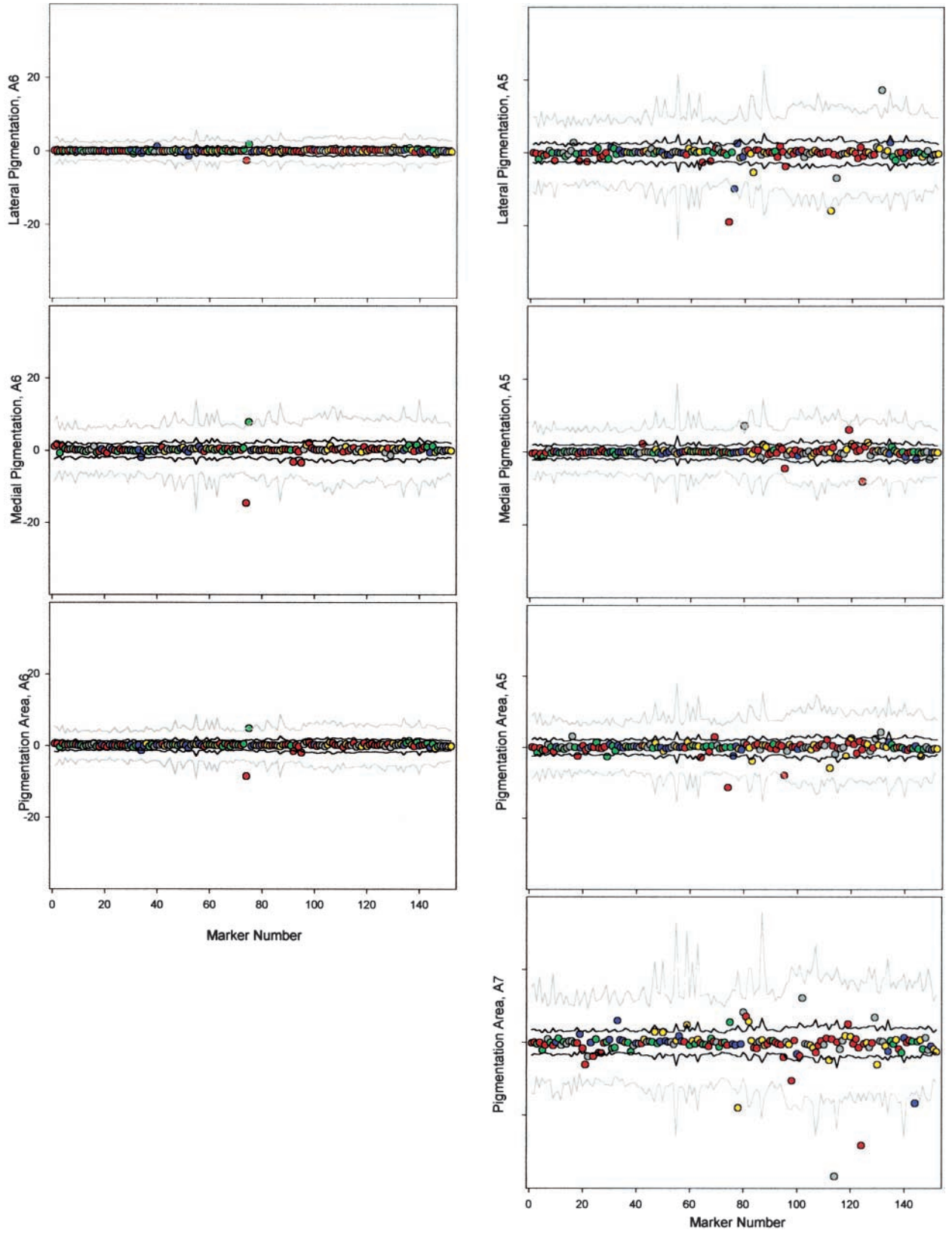

FIGURE 7.—MMA analysis of pigmentation. See Figure 6 for explanation.

TABLE 4

Effects of markers significant from multiple-marker analysis: bristles

Marker/trait	A5, m	A5, f	A6, f	A7, f
3A	*0.29*/0.44	*0.90*/0.51	*0.35*/0.62	*−0.00*/−0.38
22C	*1.15*/1.04	*1.04*/0.45	*0.76*/0.50	*0.49*/0.35
36AB	*0.09*/0.36	*−0.16*/−0.46	*−0.69*/−0.42	*−0.00*/−0.32
36F	*0.11*/0.39	*0.23*/0.56	*1.10*/0.57	*0.28*/0.40
47E	*−0.80*/−0.35	*−0.43*/−0.50	*−1.22*/−0.50	*−0.23*/−0.31
48A	*1.03*/0.96	*0.47*/0.49	*0.64*/0.53	*0.14*/0.35
50C	*−0.51*/−0.45	*−1.66*/−0.58	*−0.41*/−0.71	*−1.46*/−1.26
64B	*0.48*/0.48	*0.20*/0.70	*0.10*/0.68	*0.07*/0.48
64E	*0.55*/0.40	*0.22*/0.59	*−0.14*/−0.57	*0.04*/0.36
65AB	*−0.64*/−0.39	*−0.14*/−0.47	*−0.33*/−0.51	*−0.00*/−0.32
67D	*−0.75*/−0.42	*−0.09*/−0.58	*−0.02*/−0.54	*−0.04*/−0.37
75A	*−1.33*/−0.57	*−0.25*/−0.66	*−0.19*/−0.61	*−0.41*/−0.51
76B	*−2.05*/−1.51	*−0.86*/−0.89	*−0.28*/−0.73	*−0.01*/−0.56
77E	*−0.14*/−0.51	*−0.62*/−0.63	*−0.50*/−0.60	*−0.48*/−0.45
78E	*−0.35*/−0.52	*−0.75*/−0.51	*−0.65*/−0.71	*−0.00*/−0.49
88E	*−0.58*/−0.47	*0.05*/0.63	*−0.06*/−0.67	*0.04*/0.48

Listed are estimated effect (italicized; and underlined if significant) for markers significant in MMA for at least one trait (marker is underlined if significant in SMA)/effect expected by chance (underlined if significant at 1% level).

are significantly different from one. This suggests that female A7 and male A5 bristles are affected by additional segment- and sex-specific modifiers, as observed previously (see Mackay 1995, 1996 for review).

We find no significant correlation between pigmentation and bristle traits, and the two groups of characters appear to be controlled by nonoverlapping sets of QTL. Mutations in many regulatory genes, including *bab*, *Abd-B*, and *dsx*, qualitatively transform both pigmentation and bristle patterns (Baker and Ridge 1980; Duncan 1987; Kopp *et al.* 2000). There might be multiple reasons why natural quantitative variation does not express similar patterns of pleiotropy. First, gene expression is often controlled by multiple independent, cell-type-specific regulatory elements. For instance, *bab* expression is excluded from bristle-forming cells during late pupal stages (Kopp *et al.* 2000; A. Kopp, unpublished results). Second, different traits can have different sensitivity to the levels of gene expression. In particular, a twofold reduction in *bab* dosage has a strong effect on pigmentation, but little or no effect on bristle number (Kopp *et al.* 2000; A. Kopp, unpublished results).

TABLE 5

Effects of markers significant from multiple-marker analysis: sex comb

Marker/trait	Sex comb
4E	*0.51*/0.27
5A	*0.44*/0.28
47E	*−0.26*/−0.26
48A	*0.35*/0.28
67C	*0.36*/0.29
67D	*−0.56*/−0.32
85D	*−0.47*/−0.41
90EF	*0.60*/0.30

See Table 4 legend for details.

It is important to understand how multiple alleles of large phenotypic effect, such as those at the *bab* locus, can be maintained in a single population. Extensive variation in female abdominal pigmentation is present in many different populations of *D. melanogaster* from several continents (Robertson and Louw 1966; Robertson *et al.* 1977), as well as in other species of the *melanogaster* complex (our unpublished observations). Robertson *et al.* have mapped a large proportion of this variation to what we now suspect to be the *bab* locus and showed that multiple phenotypically distinguishable alleles of this locus segregated in various populations. In our work, three out of four *bab* alleles sampled from nature had a major effect on pigmentation. In principle, the genetic variation we observe could be neutral and governed strictly by genetic drift; it could be deleterious and maintained by mutation-selection balance, or it could be maintained by some form of balancing selection. The presence of multiple, major-effect *bab* alleles at appreciable frequencies in many geographically isolated populations would suggest that balancing selection may be the most likely explanation. However, further phenotypic and molecular studies are clearly necessary to elucidate the evolutionary forces maintaining variation at the *bab* locus.

The pattern of variation in *bab* could also be influenced by its intralocus duplication. The duplicate genes, *bab1* and *bab2,* are functionally similar, so that the pigmentation phenotype is determined by the combined

TABLE 6

Effects of markers significant from multiple-marker analysis: pigmentation

Marker/trait	Pl6	Pm6	Pa6	Pl5	Pm5	Pa5	Pa7
8C	*0.13*/0.99	*1.10*/1.97	*0.58*/1.46	*2.77*/2.63	*0.19*/2.20	*3.04*/2.21	*0.27*/3.08
12C	*−0.31*/−0.92	*−0.82*/−1.98	*−0.51*/−1.30	*−2.46*/−2.31	*−0.18*/−1.90	*−0.70*/−2.21	*−6.16*/−2.77
33B	*1.19*/0.96	*0.13*/1.95	*0.14*/1.50	*0.14*/2.35	*−0.47*/−1.86	*0.11*/2.08	*−0.27*/−3.29
41F	*−1.42*/−0.99	*−0.50*/−2.06	*−0.81*/−1.42	*−0.59*/−2.63	*0.16*/2.06	*−0.27*/−2.04	*0.05*/2.77
61C	*−2.63*/−2.73	*−14.64*/−6.85	*−8.55*/−4.40f	*−18.98*/−8.40	*−0.17*/−1.86	*−11.34*/−7.53	*−0.82*/−2.99
62B	*1.80*/0.90	*7.81*/6.60	*4.78*/4.40	*0.15*/2.56	*−0.35*/−2.13	*0.06*/1.86	*5.49*/3.23
62EF	*−0.10*/−1.09	*0.11*/2.83	*−0.02*/−1.83	*−1.38*/−3.32	*−0.07*/−2.55	*−0.41*/−2.71	*−17.97*/−14.06
63E	*0.28*/0.81	*0.07*/2.18	*0.13*/1.36	*0.44*/2.33	*7.18*/1.73	*1.33*/2.14	*8.27*/2.88
63F	*0.06*/0.93	*0.20*/2.08	*0.18*/1.46	*0.71*/2.47	*0.18*/2.19	*0.72*/2.07	*7.07*/3.19
64B	*−0.22*/−1.30	*0.26*/2.92	*0.09*/1.95	*−0.23*/−3.58	*0.15*/2.92	*−0.14*/−3.48	*5.67*/4.51
64C	*−0.54*/−1.27	*0.01*/3.07	*−0.08*/−1.75	*−5.36*/−3.15	*−0.52*/−2.89	*−3.85*/−3.28	*0.45*/4.59
67D[a]	*−0.16*/−1.05	*−3.25*/−2.29	*−1.62*/−1.33	*−1.18*/−2.83	*−0.28*/−1.76	*−0.57*/−2.10	*−0.03*/−3.24
70C[a]	*−0.58*/−0.86	*−3.43*/−1.99	*−1.99*/−1.51	*−3.76*/−2.90	*−4.51*/−2.12	*−7.90*/−7.74	*−4.13*/−3.21
75A[a]	*0.39*/1.35	*2.09*/2.73	*1.08*/1.83	*−0.26*/−3.90	*−0.58*/−2.67	*0.09*/3.30	*−10.56*/−4.65
76C	*0.12*/1.17	*0.12*/2.62	*0.14*/1.84	*−1.19*/−3.78	*−0.44*/−2.71	*−0.22*/−2.65	*12.16*/4.74
83F-84A	*0.08*/1.18	*1.00*/2.56	*0.37*/1.73	*15.94*/14.34	*−0.06*/−2.09	*−5.83*/−2.74	*−4.94*/−3.79
84F	*0.03*/1.11	*0.41*/2.63	*0.16*/1.86	*−6.96*/−3.51	*−1.49*/−2.48	*−1.93*/−2.62	*−36.76*/−15.73
86D	*0.52*/1.12	*0.94*/2.70	*0.72*/1.61	*0.50*/3.23	*6.08*/2.40	*1.34*/2.97	*4.99*/3.77
87F	*0.20*/1.17	*0.24*/2.77	*0.06*/1.59	*−0.94*/−2.86	*−8.14*/−2.51	*−0.64*/−2.52	*−28.27*/−16.21
89EF	*−0.11*/−1.27	*−1.37*/−2.59	*−0.48*/−1.72	*1.58*/3.78	*0.09*/2.49	*2.41*/3.12	*6.85*/4.57
90D	*0.88*/1.05	*0.58*/2.45	*0.64*/1.55	*1.20*/2.70	*0.43*/2.05	*0.58*/2.29	*−6.14*/−3.50
90EF	*0.17*/1.01	*−0.03*/−2.33	*0.04*/1.68	*17.21*/9.41	*0.20*/2.37	*4.22*/2.45	*0.32*/3.93
98B	*−0.33*/−1.07	*−0.61*/−2.53	*−0.48*/−1.73	*0.35*/3.28	*−2.06*/−2.34	*0.35*/3.27	*−16.77*/−14.29

See Table 4 legend for details.

[a] Each of these markers was individually significant in SMA, but likely depicting a single QTL; see RESULTS for more explanations.

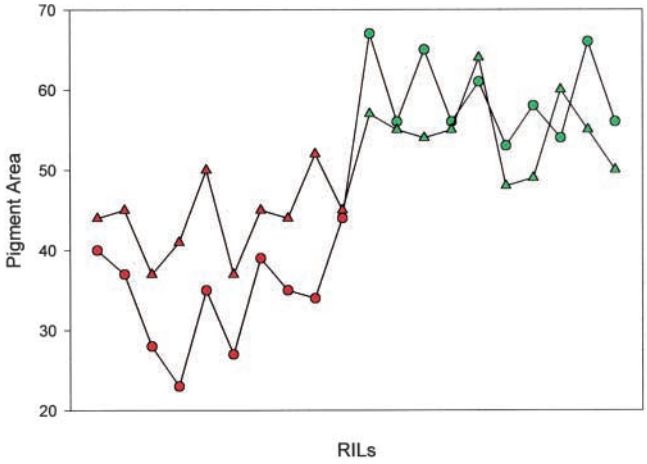

FIGURE 8.—Quantitative complementation tests. A5 pigmentation area scores in homozygous RILs are depicted as circles (red for 61C-present and green for 61C-absent) and in $bab^{Ar0}7$/RIL heterozygotes as triangles.

dosage of the two gene products (KOPP *et al.* 2000). Both theoretical and empirical work suggests that the redundancy of function following gene duplication can accelerate DNA sequence evolution and the acquisition of new functions and expression domains by the duplicate genes (WANG *et al.* 1996; WAGNER 1999; MASSINGHAM *et al.* 2001). Indeed, the two *bab* duplicates appear to be in the early stages of regulatory and functional divergence (COUDERC *et al.* 2002). This tendency may increase the amount of genetic variation in natural populations.

Do interspecific differences evolve by fixation of genetic variants segregating within populations?: Abdominal pigmentation varies both within and between species in close relatives of *D. melanogaster*, presenting an attractive model to study the links between variation in natural populations and species divergence. Sexually dimorphic pigmentation has evolved recently in Drosophila and is mostly confined to the *melanogaster* species group; in most other species, females and males are pigmented identically (BOCK and WHEELER 1972; KOPP *et al.* 2000). Within the *melanogaster* group, male-limited pigmentation of A5 and A6 is fixed in the "Oriental" lineage that includes *D. melanogaster*, whereas members of the more distantly related *ananassae* and *montium* subgroups can differ in both male and female pigmentation (KOPP *et al.* 2000). At the same time, several species show intraspecific variation for abdominal pigmentation in either males or females. For example, some species in the *ananassae* subgroup have distinct allopatric subspecies in which males either have or lack the dark A5/A6 pigmentation (BOCK 1971). Female variation can be either continuous, as in *D. melanogaster* and its close relatives *D. simulans* and *D. mauritiana* (ROBERTSON *et al.* 1977 and our observations), or discrete, as in several members of the *montium* species subgroup (OHNISHI and WATANABE 1985). Importantly, these intraspecific variants recapitulate the phenotypes that distinguish different species. So, does this intraspecific variation serve as material for species divergence?

To answer this question, we must first understand the genetic basis of pigmentation differences both within and among species. In *D. melanogaster*, the *bab* gene acts as a repressor of abdominal pigmentation and is required to prevent the development of male-specific pigmentation in females. In males, *bab* is repressed in A5 and A6 by *Abd-B*, allowing the pigmentation to develop (KOPP *et al.* 2000). Comparative analysis has shown that the pattern of *bab* expression correlates with the presence of sexually dimorphic pigmentation. In Drosophila species that show male-specific pigmentation of A5 and A6, *bab* is repressed or downregulated in these segments in males, but not in females. However, in sexually monomorphic species, *bab* expression is identical in both sexes and in all abdominal segments (KOPP *et al.* 2000). These observations suggest that changes in *bab* regulation have played an important role in the origin and evolution of sexually dimorphic pigmentation.

Our results, as well as the earlier findings of ROBERTSON *et al.* (1977), suggest that *bab* is also responsible for a large portion of intraspecific variation in sexually dimorphic pigmentation in *D. melanogaster*. We find that most of this variation can be attributed to a QTL located at the tip of 3L—the region known to include *bab* (61F1–3). Moreover, quantitative complementation tests showed a significant interaction between different alleles at this QTL and a loss-of-function allele of *bab*. Thus, in this case, intraspecific and interspecific differences may share a similar genetic basis. However, two caveats must be considered in interpreting these results. First, there is a formal possibility that a different locus, closely linked to *bab* and strongly interacting with it, is responsible for the observed phenotypes. This scenario, while unlikely, cannot be ruled out with the data in hand. Second, if these variants are indeed alleles of *bab*, we do not know whether they represent structural or regulatory differences. Further studies will be required to identify the sequence polymorphisms responsible for phenotypic differences within and among species.

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