Genetic Analysis of a Morphological Shape Difference in the Male Genitalia of *Drosophila simulans* and *D. mauritiana*

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ABSTRACT

Two closely related species of Drosophila, *D. simulans* and *D. mauritiana*, differ markedly in morphology of the posterior lobe of the male genital arch. Both size and shape aspects of lobe variation can be quantified by a morphometric descriptor based on elliptical Fourier and principal components analyses. The genetic architecture of this quantitative trait (PC1) was investigated by hybridizing inbred lines to produce two backcross populations of ~200 individuals each, which were analyzed jointly by a composite interval mapping procedure with the aid of 18 marker loci. The parental lines show a large difference in PC1 (30.4 environmental standard deviations), and the markers account for >80% of the phenotypic variation in backcross populations. Eight of 15 intervals analyzed show convincing evidence of quantitative trait loci (QTL), and the range of estimated QTL effects is 5.7–15.9% of the parental difference (1.7–4.8 environmental standard deviations). These estimates may represent the joint effects of multiple QTL within a single interval (which averaged 23 cM in length). Although there is some evidence of partial dominance of *mauritiana* alleles and for epistasis, the pattern of inheritance is largely additive.

THE genetic basis of morphological diversity in extant organisms and in the fossil record has been a controversial issue in evolutionary theory ever since DARWIN (1859) suggested that dramatic differences in form and function could result from the gradual accumulation of small individual differences through the process of natural selection. The most recent framework for this controversy is an attempt to explain the "punctuated equilibrium" pattern of variation in the fossil record, in which most evolutionary change appears to occur in relatively rapid bursts separated by long periods of stasis (ELDREDGE and GOULD 1972). Some explanations suggest that the periods of rapid change coincide with speciation events that involve "genetic revolutions" in small populations (as originally described by MAYR 1954), and that large populations are resistant to genetic change (GOULD and ELDREDGE 1977). In addition, GOLDSCHMIDT's (1940) notion of the saltational origin of adaptations through macromutation has been revived as a possible contribution to the process of rapid divergence (STANLEY 1979; GOULD 1980).

Although the extreme form of saltational evolution advocated by GOLDSCHMIDT has essentially no supporting evidence (CHARLESWORTH *et al.* 1982; MAYNARD SMITH 1983), the relative importance of major and minor gene effects in morphological evolution remains an important unresolved issue. Several lines of reasoning suggest that mutations with large effects may play a lesser role than polygenes with small individual effects (LANDE 1983). (1) Spontaneous or induced mutations with major phenotypic effects on morphology usually have deleterious pleiotropic effects. (2) Mutations with small effect occur more frequently than those with large effect. (3) Mutations with small effect may have a greater probability of improving adaptation (as originally argued by FISHER). (4) Population genetic models show that strong selection over many generations is required for the fixation of major effect mutations because of negative pleiotropy. However, minor effect mutations may also have deleterious pleiotropic effects that are more difficult to detect (ORR and COYNE 1992); FISHER's mechanical arguments about the probability of improving adaptation with mutational effects of different magnitude are not entirely convincing, and the deleterious pleiotropic effects of major mutations can be ameliorated by modifier genes (MAYNARD SMITH 1983). Clearly, empirical data are needed to settle this issue.

Throughout this century there have been many studies of the genetic basis of morphological (and other trait) differences between species, natural populations and domesticated strains of plants and animals (see reviews by LANDE 1981a; MAYNARD SMITH 1983; GOTTLIEB 1984; ORR and COYNE 1992; TANKSLEY 1993). Although some traits, such as color patterns in Lepidopterans, show evidence of segregation of factors with qualitatively distinct effects on the phenotype, most traits show more or less continuous variation in F_2 or

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backcross populations, which indicates polygenic inheritance. Most genetic analyses of such quantitative traits have utilized either a biometrical procedure (WRIGHT 1968; LANDE 1981a; ZENG 1992) or the marker linkage approach pioneered by SAX (1923). Both procedures provide minimal estimates of the number of genetic factors contributing to the trait difference but neither provides reliable estimates of the relative effects of different loci. The biometrical method estimates an effective number of factors with equal magnitudes of effect (LANDE 1981a), and the marker linkage approach confounds magnitude of effect with map distance between the marker and the quantitative trait locus (ZENG 1994). Applications of these methods suggest that many traits are polygenic (*e.g.*, LANDE 1981a; COYNE 1983, 1984, 1989), but there is little power in either method to distinguish different types of genetic architecture (ZENG *et al.* 1990; ZENG 1994).

In recent years, two new developments have greatly improved the methodology for detecting, mapping and estimating the effects of quantitative trait loci (QTL). One is the availability of molecular marker technologies that are widely applicable to a variety of organisms so that high density genetic maps can be constructed (TANKSLEY 1993). The other is a statistical approach called interval mapping, which localizes QTL on the genetic map and provides unbiased estimates of their effects (LANDER and BOTSTEIN 1989). TANKSLEY (1993) has summarized the results of interval mapping studies of a number of quantitative trait differences in domesticated plants that show that QTL vary widely in the magnitude of their effects. In several cases, a large proportion of the variation can be explained by segregation in a few relatively small regions that may contain a single QTL or clusters of closely linked factors. So far, interval mapping studies have been restricted mainly to agricultural crop plants. However, one recent study provides a high resolution analysis of the factors causing a bristle number difference between high and low strains of *Drosophila melanogaster* produced by artificial selection (LONG *et al.* 1995). This study revealed several factors with relatively large effects, each of which corresponds in map position to a putative candidate gene for which classical mutants affecting bristle number are known. Another study investigated the genetic basis of interspecific differences in floral characters of Mimulus that appear to act as isolating mechanisms and found some genes that individually account for >25% of the variance in a particular trait (BRADSHAW *et al.* 1995). More analyses of trait differences between natural species are needed to provide inferences about the types of genetic architecture created by natural and sexual selection.

Here we present an interval mapping study of quantitative traits that describe the size and shape of a morphological structure, the posterior lobe of the male genital arch of Drosophila. In *D. melanogaster* and its closest relatives, the male genital arch is a bilaterally symmetric, horseshoe-shaped structure that has three cuticular projections on each side: the posterior lobe (also known as the epandrium), the clasper and the lateral plate (see ASHBURNER 1989a, Figure 37.2). The posterior lobe, a relatively flat structure that is well described in two dimensions, varies dramatically in size and shape among species within the *melanogaster* subgroup of Drosophila (Figure 1).

The genetic analysis presented here involves crosses between *D. simulans* and *mauritiana,* which (together with their sibling species *D. sechellia*) constitute the *simulans* clade. These three species are very closely related to each other and to *D. melanogaster* (see review by ASHBURNER 1989a). *D. mauritiana* is endemic to the island of Mauritius, *sechellia* is endemic to a few of the Seychelle islands, while *simulans* and *melanogaster* are essentially cosmopolitan, but allopatric with *mauritiana* and *sechellia* (LACHAISE *et al.* 1988). The *simulans* clade species readily hybridize with each other (in at least one direction) and produce fertile female but sterile male hybrids. Hybridization between *simulans* clade members and *melanogaster* is also possible, but both male and female hybrids are sterile. It is estimated that *melanogaster* diverged from the ancestor of the *simulans* clade ~2.5–3.4 million years ago, while *simulans* and *mauritiana* diverged from one another ~0.6–0.9 million years ago (HEY and KLIMAN 1993). The *simulans* clade members have homosequential polytene chromosomes but differ from *melanogaster* by one large paracentric inversion on 3R and a few other very small rearrangements (LEMEUNIER and ASHBURNER 1976).

The size of the posterior lobe (measured as area) has been analyzed previously using the traditional marker linkage approach in backcross hybrids involving all three pairs of species in the *simulans* clade. In the *mauritiana/simulans* pair, all five markers used (one per chromosome arm) were associated with significant differences in area (COYNE 1983). In the *sechellia/simulans* pair, three of the five markers (one on each chromosome) showed a significant effect (COYNE and KREITMAN 1986) and in the *mauritiana/sechellia* pair, two of three markers (one on each autosome) showed significant effects (COYNE *et al.* 1991). The mean difference between marker genotypes differs among marker locations in these experiments, suggesting variation in the magnitude of QTL effects on different chromosomes. However, as noted above, such differences reflect both QTL effects and the genetic distance between QTL and the markers. Therefore, more work is needed to determine the number of QTL and the distribution of their effects on this character.

The study reported here is an investigation of genetic architecture by interval mapping of QTL with respect to a series of molecular markers segregating in backcross populations from hybrids between *D. mauritiana* and *D. simulans.* The traits analyzed are measures of both size and shape of the posterior lobe. We have a special inter-

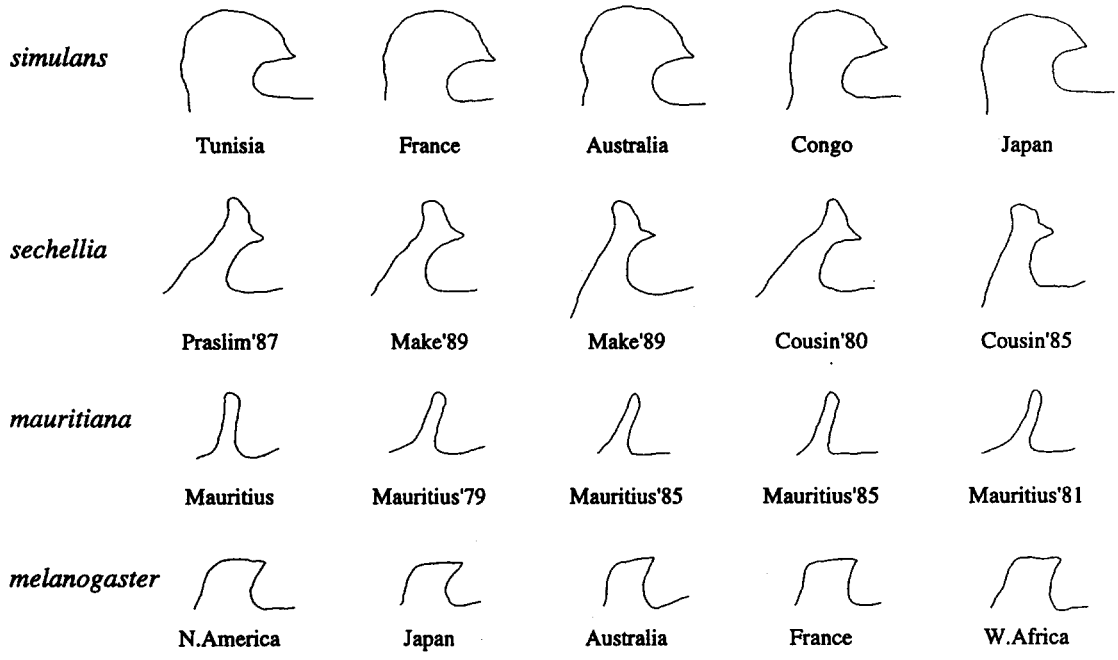

FIGURE 1.—Posterior lobe outlines from a sample of five isofemale lines from each of four Drosophila species. Additional information about the lines is provided in Table 1.

est in the shape of this structure, since very little is known about the genetic control of morphological shape variation, in spite of the fact that it constitutes a key feature of morphological diversity in many structures and organisms (see LAWRENCE 1992, p. 152). Although shape is often difficult to quantify, we show that the morphometric technique of elliptical Fourier analysis (KUHL and GIARDINA 1982) provides a very useful description of the shape variation in the backcross populations analyzed here.

MATERIALS AND METHODS

Drosophila stocks: Several isofemale lines of each of four species (*D. melanogaster, simulans, mauritiana, sechellia*) were collected at various times and places and maintained in the laboratory for several years before the present experiment (see Table 1). In addition, w^- mutant stocks of *D. mauritiana* and *simulans* were obtained from J. A. COYNE. Two inbred strains (Rob A JJ and 13w JJ) were used in the backcross experiment. Each was derived by 20 generations of full sib mating and then selected for homozygosity at each of 17 molecular marker loci, as described below. The original stocks from which the inbred strains were derived are "mau 2" in Table 1 (Rob A JJ) and a w^- strained called "13w" provided by J. A. COYNE (13w JJ).

Experimental designs: Experiment I is a survey of variation among five isofemale lines each of *melanogaster, simulans, mauritiana* and *sechellia.* A single cross of five males and five females was set up simultaneously for each of the 20 lines, and two male progeny per cross were collected for morphological analysis.

Experiment II is a more detailed survey of variation among isofemale, w^- mutant and inbred lines of *simulans* and *mauritiana* (eight lines per species). For each of the 16 lines, three vials (each with five males and five females) were set up simultaneously and two male progeny per vial were collected for morphological analysis.

Experiment III is a backcross analysis for QTL interval mapping. Females from the inbred *simulans* line 13w JJ were crossed to males from the inbred *mauritiana* line Rob A JJ to produce F_1 females, which were backcrossed to males of each parental line. The following crosses were set up simultaneously: 40 vials each of F_1 females × *mauritiana* males and F_1 females × *simulans* males, five vials each of *mauritiana* × *mauritiana, simulans* × *simulans,* and *simulans* females × *mauritiana* males. The vials each contained five female and five to 10 male parents and five male progeny were collected from each vial for morphological and (for backcross flies) DNA analysis. This provides a total of 200 males from each backcross and 25 males from each parental line and the F_1.

All crosses were made on standard cornmeal-molasses medium at 25°.

Morphological data acquisition: The genital arch and one foreleg were dissected from each male, mounted on a slide in Hoyer's medium and incubated at 66° overnight. The posterior lobe is a cuticular projection from the genital arch that can be described in two dimensions when flattened slightly by a coverslip. The lobe outline was traced by hand on paper (using a compound microscope with camera lucida) and then artificially closed by drawing a baseline that coincides with the relatively flat region extending from the lobe to the lateral plate of the genital arch (compare Figures 1 and 2). [See Figure 1 of COYNE (1983) for photographs of the genital arch with its lateral plate and posterior lobe projections.] The outlines were digitized at pixel level resolution using a video camera and the automatic tracing feature of JAVA 1.4 software (Jandel Scientific, Inc.). The number of (x, y) coordinate pairs collected was in the range of 600–1000, depending on outline size. Length of the foreleg tibia was measured with a camera lucida and stage micrometer. Tibia length is assumed to provide a measure of overall body size, since leg segment length is significantly correlated with body mass in male *D. melanogaster* (CATCHPOLE 1994).

Morphometric analysis: Because the posterior lobe has no reliable landmarks, a Fourier series representation of the outline was chosen as the morphometric descriptor. An elliptical

TABLE 1

Isofemale lines of Drosophila used in the study

Stock no.	Collection location	Collection date	Provided by
D. melanogaster			
mel 1	North Carolina, USA	1982	C. LAURIE
mel 2	Kochi, Japan	1982	T. MUKAI
mel 3	Victoria, Australia	1982	R. SINGH
mel 4	Vienville, France	1978	R. SINGH
mel 5	Benin, West Africa	1978	R. SINGH
D. simulans			
sim 1	South France	1983	R. SINGH
sim 2	Tunisia	1983	R. SINGH
sim 3	Brazzaville, Congo	1983	R. SINGH
sim 4	Palmers, Australia	1986	J. BARKER
sim 5	Munakata, Japan	1986	T. YAMAZAKI
sim 6	Capetown, S. Africa	1983	R. SINGH
sim 7	North Carolina, USA	1984	C. LAURIE
D. mauritiana			
mau 1	Mauritius	—	J. DAVID
mau 2	Mauritius	1979	H. ROBERTSON
mau 3	Mauritius	1985	D. HICKEY
mau 4	Mauritius	1985	D. HICKEY
mau 5	Mauritius	1981	J. COYNE
mau 6	Mauritius	—	J. DAVID
mau 7	Mauritius	1981	J. COYNE
D. sechellia			
sech 1	Praslim	1987	G. SIMMONS
sech 2	Make	1989	M. KREITMAN
sech 3	Make	1989	M. KREITMAN
sech 4	Cousin Island	1980	J. COYNE
sech 5	Cousin Island	1985	J. COYNE

Fourier analysis was used because the strongly recurved tip of some lobes can produce multivalued functions that cannot be handled simply in nonparametric Fourier analysis. A similar approach has been used to describe the shape variation in mussel shells (FERSON *et al.* 1985). Fourier coefficients were computed separately from a parametric representation of the x and y coordinates, which are regarded as functions of the arc length. Outlines were reconstructed from Fourier coefficients a_i, b_i, c_i and d_i using the following equations, where s is arclength standardized to the interval 2π ($0 \leq s \leq 2\pi$) and n is the number of harmonics:

$$x(s) = a_0 + \sum_{i=1}^{n} (a_i \cos(is) + b_i \sin(is)),$$

$$y(s) = c_0 + \sum_{i=1}^{n} (c_i \cos(is) + d_i \sin(is)).$$

Thus, each outline is represented by a $4n$ component vector of coefficients $[a_1, \ldots, a_n, b_1, \ldots, b_n, c_1, \ldots, c_n, d_1, \ldots, d_n]$. Zero order harmonics were not included since they contain only location information. The KUHL and GIARDINA (1982) algorithm was used because of its ability to handle nonuniformly spaced data points. It was implemented with a C program interfaced to Mathematica 2.2.2 (WOLFRAM 1991) using MathLink.

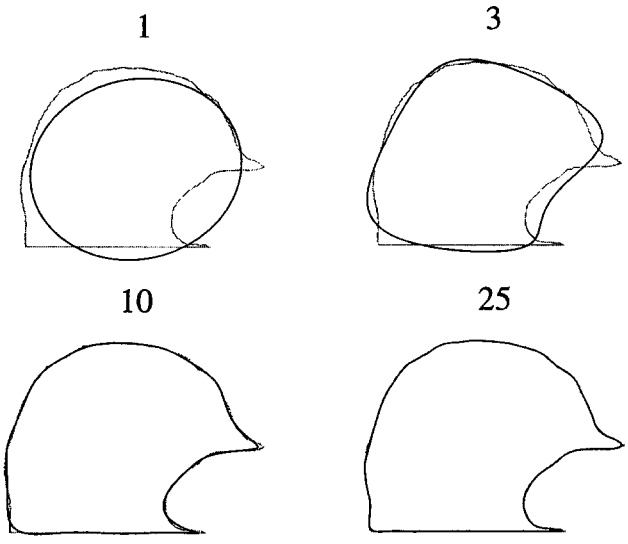

FIGURE 2.—The effect of harmonic number on the accuracy of reconstruction of a posterior lobe outline by elliptical Fourier analysis.

Each outline was placed in a standard configuration so that the Fourier coefficients are independent of location, orientation and handedness. The origin of the coordinate system was placed at the centroid of the outline, which was calculated using a trapezoidal-rule integration routine. Outlines from the left side of the fly were reflected in the y-axis to produce outlines of the same handedness from both sides. All outlines were oriented so that their baselines are parallel. Size was taken to be the area of the polygon formed by joining the points in the coordinate list with line segments. In some cases, size was removed by dividing each coordinate by the square root of the polygon area (giving every outline an area of 1). These calculations, as well as outline reconstructions from Fourier coefficients and coordinate lists, were performed in Mathematica (WOLFRAM 1991).

The elliptical Fourier procedure provides a descriptor that can reproduce the original outline to any desired degree of precision, depending on how many harmonics are used. Figure 2 shows the effect of harmonic number and demonstrates that even 10 harmonics gives an excellent fit, while with 25 harmonics the Fourier reconstruction and the original outline coincide with a high degree of accuracy. For all the analyses presented here, 25 harmonics were used.

Molecular marker development: A total of 18 markers were used in the backcross experiment (Figure 3), one visible mutant (w) and 17 molecular markers, which were developed in the following three steps. (1) Primer pairs for PCR were designed to occur in exonic sequences flanking a small intron [on the basis of *melanogaster* sequence information in Flybase (1994)]. (2) Fragments were amplified from both the inbred *mauritiana* and *simulans* strains. (3) Fragments were sequenced and analyzed for restriction site differences, which were verified by enzymatic digestion. Table 2 summarizes the marker locations, primer sequences, PCR conditions, restriction enzymes and gel conditions for resolving fragment size differences. The uncut fragment sizes range from 200 to 520 bases in length. The cytological position of each marker locus inferred from its position in *melanogaster* was verified for 12 of the markers by *in situ* hybridization according to LIM (1993).

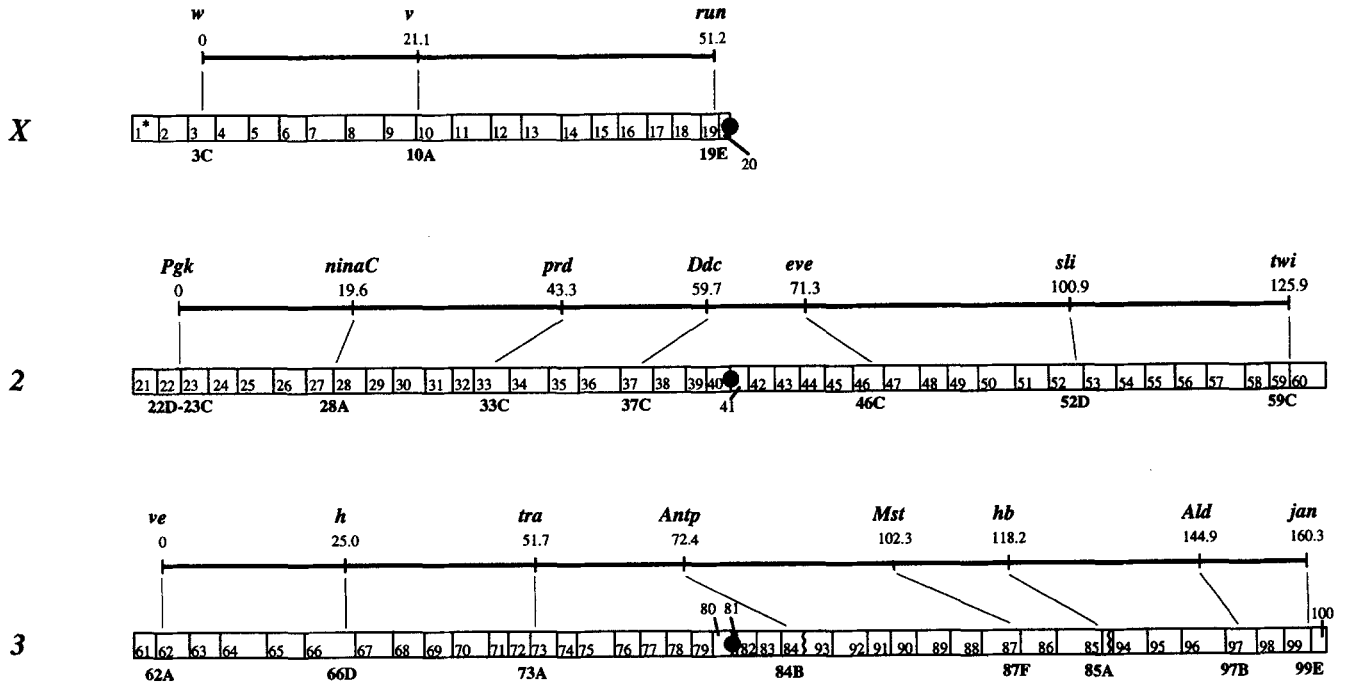

FIGURE 3.—Cytogenetic maps of the molecular markers used in composite interval mapping of QTL. The numbered blocks represent the numbered sections of the polytene chromosomes. The cytological localization of the marker is given below the chromosome. The genetic map drawn above the chromosome was inferred from the progeny of F_1 hybrid females produced by crossing *D. simulans* females to *mauritiana* males.

To develop strains that were homozygous for different alleles at all 17 marker loci, a series of single pair matings from the inbred line of each species were set up. After progeny production, the marker genotypes of parents were determined and new lines were established from those pairs for which both parents were homozygous for the greatest number of markers. This procedure was repeated one more time to obtain lines homozygous for all 17 markers.

DNA analysis: The genomic DNA of a single fly was extracted by minor modifications of protocol 48 in ASHBURNER (1989b). The genotype at each marker locus was determined by the following procedure: (1) set up PCR reaction with 1-μl template DNA, 0.5 μl primers (100 ng/μl) and 11 μl reaction mix (Stratagene, Inc.), (2) PCR for 40 cycles according to the temperature program in Table 2, (3) digest 5 μl of PCR product plus 0.5 μl of control plasmid DNA (1 μg/μl) (to verify activity of restriction enzyme) with the enzyme designated in Table 2 with buffer and concentration recommended by manufacturer, (4) analyze fragment sizes on either 2% agarose or 4% high resolution MetaPhor™ agarose (FMC BioProducts), as shown in Table 2.

Genetic map construction: The segregation ratio for each locus and each backcross was analyzed for deviation from the expected 1:1 by a χ^2 test. Four markers in the *mauritiana* backcross and five in the *simulans* backcross showed significant deviations, presumably due to viability differences. In cases where two adjacent markers both showed a significant deviation, the corrected recombination fraction of BAILEY (1961, p. 53) was used. The recombination fractions between adjacent markers were tested for heterogeneity between the two backcrosses (by χ^2) and two intervals showed a significant difference, which is probably also due to differential viability. The recombination fractions were converted to map distance using KOSAMBI's (1944) mapping function and the map distances for each interval were averaged over backcrosses to give the values used in the QTL analysis. All pairwise map distances are consistent with the order of markers established from their cytological positions.

Statistical analyses: The 100 Fourier coefficients for each individual (four for each of 25 harmonics) were treated as variables in a principal components analysis, which was performed on covariance matrices rather than correlation matrices to minimize the undue influence of small outline irregularities and measurement error that can arise with standardized variables (ROHLF and ARCHIE 1984). In experiment III, this analysis was performed on the pooled data from both parents, the F_1 and both backcrosses. The principal components analysis, as well as other standard procedures such as linear regression and analysis of variance, were performed with programs of the SAS statistical package (SAS Institute, Inc.).

Experiment II was analyzed with the GLM procedure of SAS. The posterior lobe variables from each species were analyzed separately using sources: strains within species, vials within strains and flies within vials. The strain effect was tested with the mean square error for vials. Tibia lengths from both species were analyzed together by adding species as a source in the model. The species effect was tested with the mean square error for strains within species. A combined analysis of lobe variables was not done because of heterogeneity of variances between species.

In experiment III, means of F_1 and backcross populations were compared with expected values (linear combinations of parental and/or F_1 values) with an approximate t-test that allows for unequal variances (STEEL and TORRIE 1980, p. 106).

Composite interval mapping of QTL: The composite interval mapping procedures of ZENG (1994) and JIANG and ZENG (1995) were modified to combine data from two backcrosses in a joint QTL analysis.

The model: The statistical model for QTL analysis is

$$y_{ij} = \mu_i + b_i^* x_{ij}^* + \Sigma\, b_{ik}\, x_{ijk} + e_{ij},$$

where i indexes the two backcrosses ($i = 1, 2$), k indexes the

TABLE 2

Summary of the molecular markers

Marker	Cytological position	Primers	PCR condition[a]	Enzyme	Gel condition[b]
v	10A	TGTCCCTATGCAGGAAACGG TGAACAGATGCTCATCGTGC	A	*Taq*I	I
run	19E	AGTGCATACCGAGAATCCGC ATTGATGGCGATTGCGGAGG	A	*Bsi*EI	I
Pgk	22D-23C	GGCAAGCGGGTGTTGATGCG TTGGCAGGATCGGCCTTGAC	B	*Alu*I	I
ninaC	28A	GAAGTCCATCTTCCAGGTCC TATGGGCACTGGCAGTGGTC	C	*Ava*II	I
prd	33C1-2	GATGCAAGGTGAGTGTCTATCG GCCATGGGATACACGTAGCT	D	*Msp*I	II
Ddc	37C	TGGCTGACGAGAAGAAGAAC CAAGATTCCGGAAGACGACG	A	*Csp*45I	I
eve	46C	TTGTGGACCTCTTGGCCACC AACTCCTTCTCCAAGCGACC	E	*Dra*I	I
sli	52D	TTACCAGCTTTAAGGGCTGC CATTTGTTCTCCAGGCAAGG	F	*Bfr*I	I
twi	59C	TCCCTGCAGCAGATCATCCC ATCATCCGAGCTGAGCATGC	A	*Apo*I	I
ve	62A	GAGAACCCAACGCAGAATGT ATATCCTCCGACTCCGGAAG	G	*Pst*I	I
h	66D	ACTCAAGACTCTGATTCTGG TGTCTTCTCCAGAATGTCGG	A	*Mse*I	II
tra	73A	GAACAAGCGAGAGGGATAGC CTTTGGCGGTGGATTATACC	A	*Bbv*I	I
Antp	84B	ACGGACGTTGGAGTTCCCGA ACATGCCCATGTTGTGATGG	E	*Mse*I	II
Mst	87F	TCCTTTGCCTCTTCAGTCCG TCCACAGGCATAGCATGGTC	F	*Msp*I	I
hb	85A3-B1	TCTGCCCATCTAATCCCTTG CTGCGTCGAGTTTTTTCCTC	H	*Bsi*YI	II
Ald	97A-B	ATGGGCCCTCACCTTCTC GTGGTCATCCACATGCAAAG	D	*Xmn*I	II
jan	99E	CGCATTGAACACAATCCCGA CTCATCGGAGATTTCGATCG	A	*Hind*III	I

[a] A, 94° 1 min/55° 30 sec/72° 30 sec; B, 94° 1 min/57° 1 min/72° 2 min; C, 94° 1 min/55° 30 sec/72° 2 min; D, 94° 1 min/55° 30 sec/72° 1 min; E, 94° 1 min/60° 30 sec/72° 30 sec; F, 94° 1 min/50° 30 sec/72° 30 sec; G, 94° 1 min/53° 30 sec/72° 1 min; H, 94° 1 min/53° 1 min/72° 1 min.

[b] I, 2% agarose gel; II, 4% high-resolution Metaphor agarose gel (FMC BioProducts).

markers ($k = 1, 2, \ldots m$), j indexes individuals within a backcross ($j = 1, 2, \ldots n_i$), y_{ij} = phenotypic value of individual j from backcross i, μ_i = mean phenotypic value in backcross i, b_i^* = effect of a putative QTL in backcross i, x_{ij}^* = QTL indicator variable [which has value 0 or 1 with probability depending on the genotype of markers flanking the interval containing the putative QTL (see Table 1 of ZENG 1994)], b_{ik} = partial regression coefficient of y_{ij} on x_{ijk}, x_{ijk} = indicator variable of individual j of backcross i for marker k (which takes a value of 0 or 1 depending on the genotype of marker k), e_{ij} = residual effect of individual j of backcross i, and Σ is the summation over all markers except those flanking the interval containing the putative QTL (model I of ZENG 1994). Summation over all markers is appropriate in this experiment because all markers are widely separated.

Likelihood and hypothesis testing: Under the assumption that the e_{ij} are independent and normally distributed with mean zero and variance σ_i^2, the likelihood function of the data is

$$L = \prod_{i=1}^{2} \prod_{j=1}^{n_i} [p_{ij} f_1(y_{ij}) + (1 - p_{ij}) f_0(y_{ij})],$$

where $p_{ij} = \text{Prob}(x_{ij}^* = 1)$, $f_1(y_{ij})$ and $f_0(y_{ij})$ represent normal density functions of y_{ij} with means $\mu_i + b_i^* + \Sigma_k b_{ik} x_{ijk}$ and $\mu i + \Sigma_k b_{ik} x_{ijk}$, respectively, and variance σ_i^2. Hypotheses were tested by calculating a likelihood ratio test statistic,

$$\text{LOD} = \log_{10}(L_1/L_0),$$

where L_0 is the maximum likelihood value under the null hypothesis H_0 and L_1 is the maximum likelihood value under the alternative hypothesis H_1.

The following hypotheses were tested: (1) separate mapping for each backcross (H_0: $b_i^* = 0$; H_1: $b_i^* \neq 0$), (2) joint mapping for both backcrosses (H_0: $b_1^* = 0$ and $b_2^* = 0$; H_1: $b_1^* \neq 0$ and $b_2^* \neq 0$), and (3) testing for QTL × backcross interaction (H_0: $b_1^* = b_2^*$; H_1: $b_1^* \neq b_2^*$).

Critical values for LOD scores: When mapping QTL, it is important to choose appropriate critical values for different tests. In the above tests, each test statistic at each fixed genomic position is asymptotically χ^2 distributed with degree of freedom being the number of parameters tested. However, because multiple tests (in multiple genomic positions) are

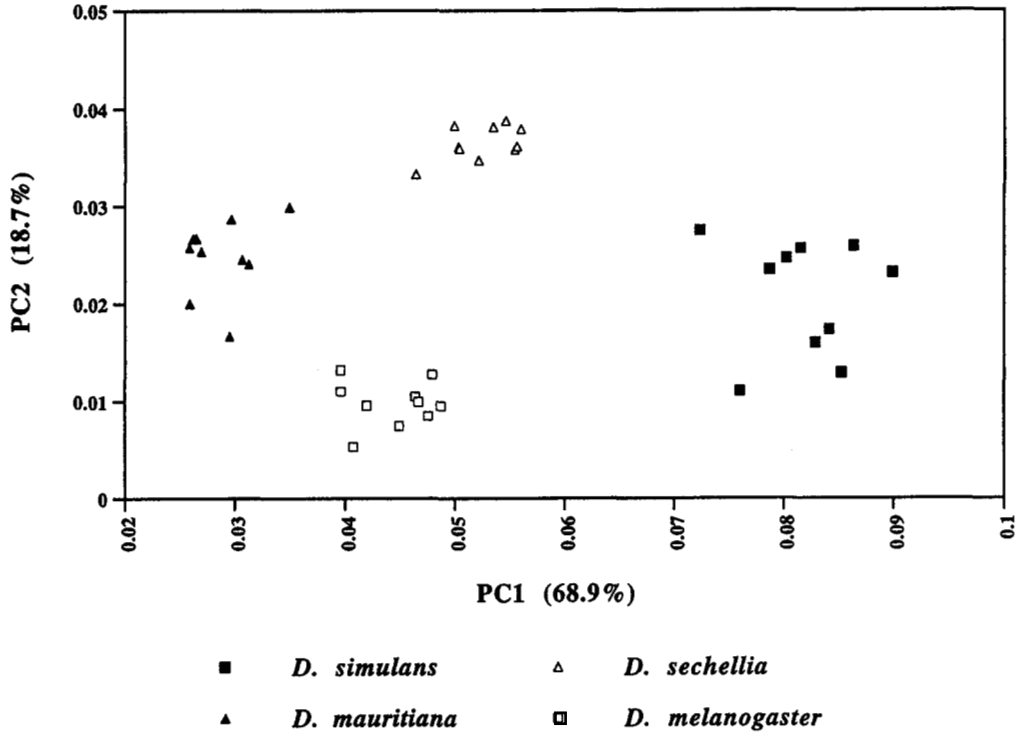

FIGURE 4.—A plot of the first two principal components of the Fourier coefficients from posterior lobe outlines. Each point represents a single lobe. There are two individuals from each of five strains of each of four species. The percentage of variation in the Fourier coefficients accounted for by each principal component is given in parentheses.

performed to search for QTL, the significance level for each test has to be adjusted. ZENG (1994) showed by simulation that, provided the sample size is large and the number of markers is not too great, the $100\alpha\%$ critical value of the likelihood ratio test statistic [LR $= -2 \ln(L_0/L_1)$] for an overall test with M intervals in the genome can be very well approximated by $\chi^2_{\alpha/M,v}$, where v is the number of parameters being tested (including one for QTL position). Translated to LOD scale, this is $(1/2)(\log_{10} e)\chi^2_{\alpha/M,v}$. Thus, when $\alpha = 0.05$ and $M = 15$, the critical value is 2.48 for separate mapping of each backcross ($v = 2$) and 2.98 for the joint mapping of both backcrosses ($v = 3$). For testing the QTL × backcross interaction, there appears to be no need to adjust the significance level for multiple tests as the test is assumed to be performed only at the relevant positions where putative QTL are located (JIANG and ZENG 1995). Thus, for the QTL × backcross interaction, the relevant threshold is $(1/2)(\log_{10} e)\chi^2_{0.05,1} = 0.83$.

Estimation of effects: The effect of a QTL in backcross i is estimated by the regression coefficient b_i^*. The additive effect of a QTL is then the average of the regression coefficients from each backcross. When the QTL × backcross interaction is significant, a nonadditive effect was estimated as $(b_1^* - b_2^*)/2$.

RESULTS

Quantitative descriptors of size and shape: The posterior lobe of the male genital arch provides the only reliable morphological character for distinguishing the four species, *D. melanogaster, simulans, mauritiana* and *sechellia* (ASHBURNER 1989a). The outlines in Figure 1 show, at a glance, that the trapezoidal lobe of *melanogaster,* the helmet-shaped lobe of *simulans,* the fingerlike lobe of *mauritiana* and the bootlike lobe of *sechellia* are widely divergent among species but relatively consistent among strains within species. Although this character is qualitatively distinct among species, quantitative descriptors of size and shape are required for a genetic analysis.

Because the posterior lobe has no reliable landmarks, we have chosen elliptical Fourier analysis (KUHL and GIARDINA 1982) to provide a morphometric description of shape. This procedure provides a simple mathematical reconstruction of any closed contour to an arbitrary degree of precision, depending on the number of harmonics used. The posterior lobe is not a naturally closed contour but was closed artificially as described in MATERIALS AND METHODS. The Fourier analysis with 25 harmonics results in a vector of 100 coefficients that reproduce the closed outline of a posterior lobe very well (Figure 2). A principal components analysis of the 100 coefficients was performed to obtain a smaller number of variables that encapsulate much of the information about variation in shape. In the experiments described here, the first two principal components (PC1 and PC2) account for >85% of the variation in the Fourier coefficients.

The distinction between size and shape of a morphological structure is often difficult and not always biologically meaningful. However, we have attempted to make this distinction by calculating Fourier coefficients for coordinate sets that have been rescaled so that all outlines have a unit area. The first principal component obtained from rescaled data is referred to as ADJPC1

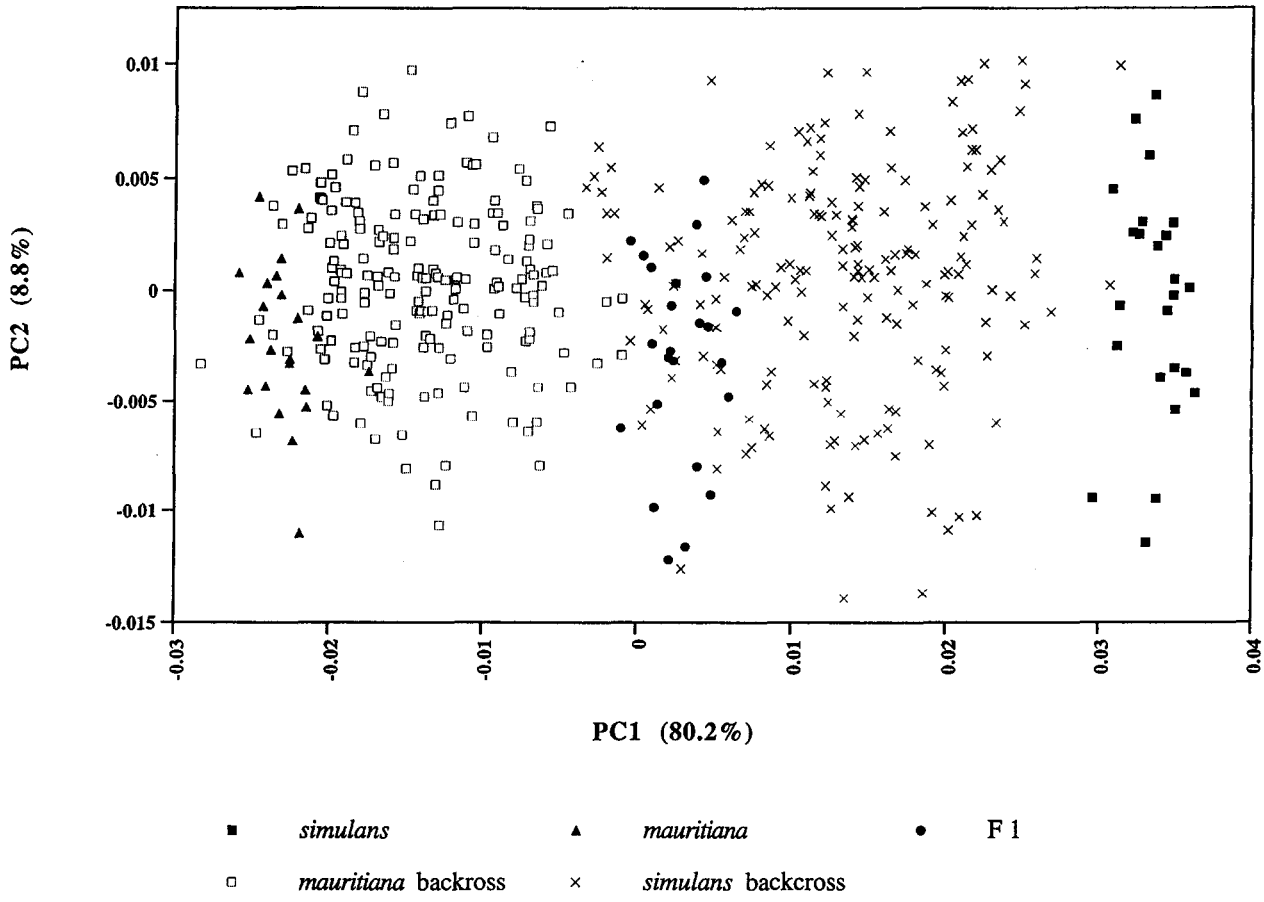

FIGURE 5.—A plot of the first two principal components of the Fourier coefficients from posterior lobe outlines. Many individuals from each of five genotypic classes are represented. Each point represents an average of scores from the left and right sides of an individual (with a few exceptions for which the score is from one side only). The percentage of variation in the Fourier coefficients accounted for by each principal component is given in parentheses.

(adjusted for size). This variable may not be completely independent of size, but it contains primarily shape information. Thus, posterior lobe area and ADJPC1 provide measures of size and shape, respectively, while PC1 contains information about both size and shape. We have also examined tibia length, which provides a measure of overall body size.

Posterior lobe variation within and among species: Figure 4 shows a plot of PC1 and PC2 for the outline data from a sample of strains from each of the four species (experiment I). All four species are well separated in this plot. *D. mauritiana, simulans* and the *melanogaster/sechellia* pair are separated mainly by PC1, which accounts for 68.9% of the variation, while *melanogaster* and *sechellia* are separated mainly by PC2, which accounts for 18.7% of the variation. This paper will focus on a genetic analysis of the difference in PC1 between *simulans* and *mauritiana.*

In another experiment (II), variation among eight strains within each of the species, *simulans* and *mauritiana,* was analyzed. Table 3 provides the mean values of three lobe traits (PC1, ADJPC1, area), as well as tibia length. Within each species, the variation among strains is highly significant for all four traits ($P < 0.005$). Species are clearly very different for the three lobe measurements, but tibia length is not significantly different between species. Thus, there is intraspecific genetic variation for both size and shape of the posterior lobe, but this variation is small compared with the species differences.

Descriptive results for *mauritiana* × *simulans* F_1 and backcrosses: Two inbred lines of *mauritiana* and *simulans* were crossed to produce F_1 females, which then were crossed to each of the parental strains to produce BM (*mauritiana* backcross) and BS (*simulans* backcross) populations for genetic analysis. Table 3 shows that the two parental lines used for this experiment (III) have species-typical values for all three posterior lobe variables.

The phenotypic variances in the parental and F_1 populations are expected to be due almost entirely to environmental effects because the parental lines are highly inbred. These three populations have homogenous variances for PC1 (Bartlett's test), which provide a pooled standard deviation of 0.00186 (referred to hereafter as the environmental standard deviation). The mean difference between the parental populations (0.0566) equals 30.4 environmental standard deviations.

TABLE 3

Strain variation in mean phenotypic values

Strain	PC1 ($\times 10^{-3}$)	ADJPC1 ($\times 10^{-2}$)	AREA ($\times 10^{-3}$ mm^2)	TIBIA (mm)
D. mauritiana				
w	−29.5	−27.5	1.75	0.448
mau 2	−29.2	−24.4	1.77	0.433
mau 7	−28.4	−29.2	1.88	0.475
mau 1	−28.3	−29.4	2.13	0.448
Rob A JJ[a]	−26.1	−18.3	2.04	0.445
mau 4	−25.7	−27.2	2.08	0.450
mau 3	−24.8	−16.8	2.12	0.470
mau 6	−24.2	−27.0	2.55	0.455
SD_{within}[b]	2.5	3.2	0.22	0.008
Species mean	−27.1	−25.0	2.04	0.453
SD_{among}[c]	5.0	11.8	0.62	0.033
D. simulans				
sim 1	25.3	22.0	12.4	0.462
sim 2	25.8	26.7	11.7	0.460
sim 6	25.8	26.2	11.7	0.465
sim 7	26.1	26.0	11.7	0.445
13w	26.1	22.6	12.4	0.445
sim 4	28.2	25.6	12.6	0.445
sim 3	28.2	26.5	12.0	0.460
13w JJ[a]	30.8	24.3	13.5	0.460
SD_{within}[b]	2.2	1.2	0.7	0.013
Species mean	27.1	25.0	12.2	0.455
SD_{among}[c]	4.6	4.4	1.5	0.021

Each number represents the mean phenotypic value of six individuals from a given strain, except for rows labeled "SD," which represents a standard deviation defined in a footnote, and "species mean," which represents the mean of the eight strain means for a given species.

[a] These two lines were used in the backcross experiment.

[b] SD_{within} = standard deviation from variance within strains.

[c] SD_{among} = standard deviation from variance among strains.

Several observations indicate that PC1 provides an excellent morphometric descriptor of posterior lobe variation, while PC2 adds relatively little useful information. (1) A plot of the first two principal components of the Fourier coefficients (Figure 5) shows that all five genotypic classes are well separated by the value of PC1, but not by PC2. (2) PC1 accounts for 80.2% of the variation, while PC2 accounts for only 8.8%. (3) The correlation of PC1 between the left and right side of individual flies is very high (0.88 and 0.94 for BM and BS, respectively), while that for PC2 is lower, but statistically significant (0.14 and 0.42 for BM and BS, respectively). The high correlation between sides for PC1 suggests a strong genetic component with little measurement error. (4) Visual inspection of a series of outlines presented in PC1 order (Figure 6) shows a relatively smooth transition from a *mauritiana*-like to a *simulans*-like lobe (with respect to both size and shape). Therefore, analyses presented here will focus on PC1.

Correlations among the variables analyzed in the two backcrosses are summarized in Table 4. All three posterior lobe variables (PC1, ADJPC1, area) are highly correlated in both backcrosses, which suggests either pleiotropy or close linkage between genes affecting size and shape (although environmental effects may also contribute to this phenotypic correlation). It is possible that most (perhaps all) of the developmental controls on posterior lobe shape operate through processes (such as cell division patterns) that also affect its size, producing a tight mechanistic coupling between these two properties. The resulting correlation may be considered a form of pleiotropy.

There is a small, but significant, difference in tibia length between the two parental lines (0.023 mm, which is 2.3 environmental standard deviations). However, tibia length is not strongly associated with posterior lobe area in the backcross populations. The correlation between area and tibia length is small (although statistically significant because of a large sample size) and of opposite sign for the two backcrosses (+0.17 for BM and −0.17 for BS). This result suggests that overall body size variation is not an important source of variation in posterior lobe size.

The distributions of PC1 shown in Figure 5 for each of the five genotypic classes suggest a polygenic mode of inheritance with largely additive gene action. However, there are some indications of partial dominance of *mauritiana* alleles. With strictly additive gene action, and assuming some effect of the *X* chromosome, the F_1 mean should be greater than the parental midpoint (*i.e.*, more like *simulans*), since all F_1 males have a *simulans X* chromosome. However, the F_1 mean (0.0028) is significantly less than the midpoint (0.0054) between *mauritiana* (−0.0230) and *simulans* (0.0337). Also, with additive gene action, each backcross mean should lie halfway between the parental midpoint and the pure species parent. Both backcross means are less than their expected value (*i.e.*, more like *mauritiana*), although neither difference is significant.

Composite interval mapping of QTL: The data set for QTL mapping consists of phenotypic values and the genotypes of 18 marker loci for 192 and 184 individuals of the *mauritiana* and *simulans* backcrosses, respectively. The marker loci are distributed throughout the genome (Figure 3) with an average distance of 22.7 cM and a range of 15.7–30.7 cM. This level of coverage should permit detection of QTL anywhere in the genome, except on the tiny fourth chromosome.

The backcross data were analyzed by a composite interval mapping procedure that combines the interval mapping concept of LANDER and BOTSTEIN (1989) with multiple regression on other markers (ZENG 1994). In this procedure, the genome is systematically scanned for evidence of a QTL by determining whether a test statistic (LOD score) exceeds a critical value. Each marker interval is divided into a number of test positions (in this case every 1.0 cM), and a LOD score is calculated for each position. If the maximum LOD score within an interval exceeds a critical value, then a

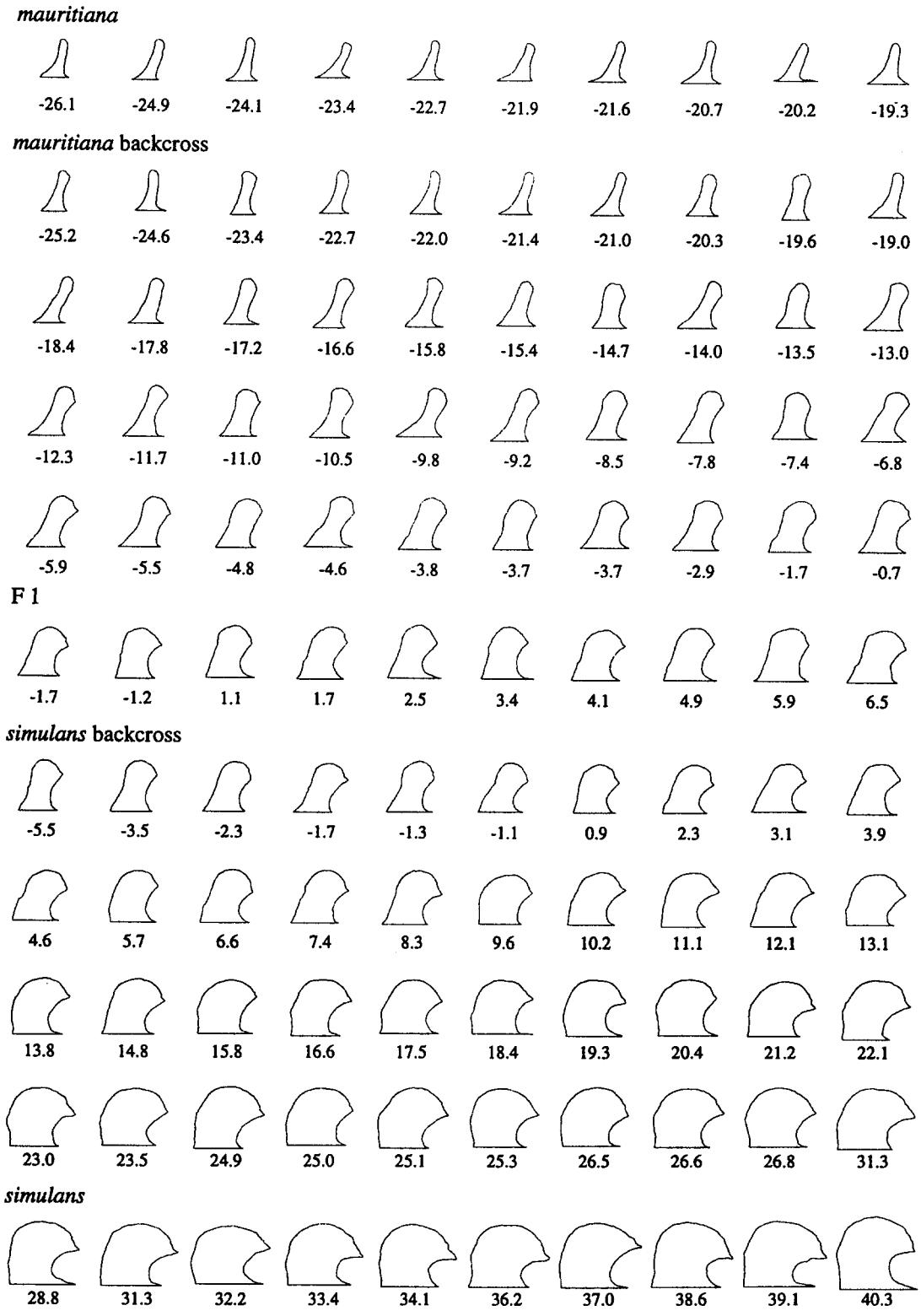

FIGURE 6.—Outlines of the posterior lobe from a sample of individuals from each of the five groups: pure *mauritiana, mauritiana* backcross, F_1, *simulans* backcross, and pure *simulans*. Within each group, the outlines are presented in order of their PC1 score (sampled at even intervals from the range of variation). The number below each specimen is its PC1 score. The outlines are drawn to scale with the origin at the centroid of each outline and with all baselines parallel.

QTL (with nonzero effect b^*) is indicated at the map position corresponding to that local maximum. In simple interval mapping, both the estimates of QTL position and effect can be influenced by other linked QTL outside of the test interval. However, in the multiple regression approach of ZENG (1994), estimates of posi-

TABLE 4

Pearson correlation coefficients (with significance levels) among the four traits in the *mauritiana* backcross (above diagonal, n = 192) and the *simulans* backcross (below diagonal, n = 186)

	PC1	ADJPC1	AREA	TIBIA
PC1	—	0.82 (0.0001)	0.99 (0.0001)	0.16 (0.0292)
ADJPC1	0.90 (0.0001)	—	0.84 (0.0001)	0.10 (0.1520)
AREA	0.99 (0.0001)	0.86 (0.0001)	—	0.17 (0.0199)
TIBIA	−0.18 (0.0159)	−0.23 (0.0014)	−0.17 (0.0216)	—

tion and effect are not affected by other linked QTL, provided there are markers that separate those other QTL from the QTL under consideration and that those markers are fitted in the model. In other words, when all markers are fitted in the model (as we have done here), estimates for putative QTL within adjacent intervals are interdependent, whereas those for other pairs of intervals are not.

The QTL mapping summary in Figure 7 for PC1 gives three types of LOD score corresponding to three types of hypothesis tests. When each backcross is analyzed separately, the null hypothesis is that a QTL at the test position has no effect in that particular backcross. When the two backcrosses are analyzed together (joint mapping), the null hypothesis is that a QTL at the test position has no effect in either backcross, in contrast to an alternative that it has a nonzero effect in both backcrosses. A third type of LOD is calculated from the combined analysis: the QTL × backcross interaction, which tests the null hypothesis that the effects in both backcrosses are equal. Further details about the model, hypothesis testing and critical values are given in MATERIALS AND METHODS.

The LOD score profiles in Figure 7 provide evidence that at least eight (and probably nine) of the 15 intervals contain one or more QTL. Some intervals (such as *v/run, ninaC/prd, Antp/Mst* and *hb/Ald*) have LOD scores that exceed the critical value, but the maximum LOD is less than that in an adjacent interval and the profile "leans" toward its more significant neighbor. Therefore, the evidence for a QTL in those intervals is not convincing. The effects of "putative" QTL have been estimated for nine of the intervals, which all show LOD scores that exceed their critical value in both the joint and single backcross analyses. The evidence for a QTL in one of those intervals, *h/tra*, is equivocal since it is flanked by two other highly significant intervals. However, none of the remaining eight intervals are adjacent to one another, so the estimates of their effects should be independent. Within the nine intervals, the map position corresponding to the local LOD score maximum is identified as a putative QTL position, although it is possible that each interval contains more than one QTL.

Within one backcross, the effect of a QTL is the expected change in phenotype due to replacing a *mauritiana* with a *simulans* allele. In the joint analysis, the effect of a QTL is partitioned into additive and nonadditive components. The LOD score for the QTL × backcross interaction tests whether the nonadditive component is significantly different from zero. In other words, if the effect of replacing genotype *mm* with *ms* (in the *mauritiana* backcross) is the same as replacing *ms* with *ss* (in the *simulans* backcross), then there is no deviation from additivity. In this experiment, a significant deviation from additivity for an autosomal locus can be due to dominance and/or epistasis.

Estimates of the effects of putative QTL are given in Figure 7. The additive effect is the average of the effects estimated from each backcross ($(b_{ms} + b_{sm})/2$). When the QTL × backcross test is significant, a nonadditive effect ($(b_{ms} - b_{sm})/2$) and separate effects for each backcross (QTLsim = b_{ms} and QTLmau = b_{sm}) are also given. The effects are expressed as a percentage of one-half the difference in mean value between the parental lines, (S − M)/2.

The estimated additive effects of putative QTL on PC1 vary in magnitude from 5.7% to 15.9%, nearly a threefold range. The sum of all estimated effects (except for equivocal QTL #6 in the *h/tra* interval) is 94%.

These additive effects are all positive, which means that replacement of a *mauritiana* allele with a *simulans* allele increases the trait value in every case. The nonadditive effects are also all positive, which means that the effect of changing from *ms* to *ss* on the *simulans* background is greater than the effect of changing from *mm* to *ms* on the *mauritiana* background. This difference is consistent with a suggestion from the means comparison that *mauritiana* alleles may be partially dominant. However, the nonadditive effects are relatively small and the general picture is clearly one of largely additive inheritance.

On the *X* chromosome, which may contain only a single QTL, the fraction of variation in PC1 explained by that putative QTL is estimated to be 5.7% for the *mauritiana* backcross and 14.5% for the *simulans* backcross, based on the R^2 of a simple interval mapping model. When there is evidence for more than one QTL per chromosome, as for both autosomes in this experiment, estimating the phenotypic variation explained by putative QTL is more difficult. In principle, a suitable strategy would be to find the maximum likelihood estimate of the residual variance in a model with multiple indicator variables (one per putative QTL position), but in practice this would be very difficult to implement. Therefore, rough estimates have been obtained through stepwise regression of PC1 on the markers. Considering each chromosome separately, ~20% of

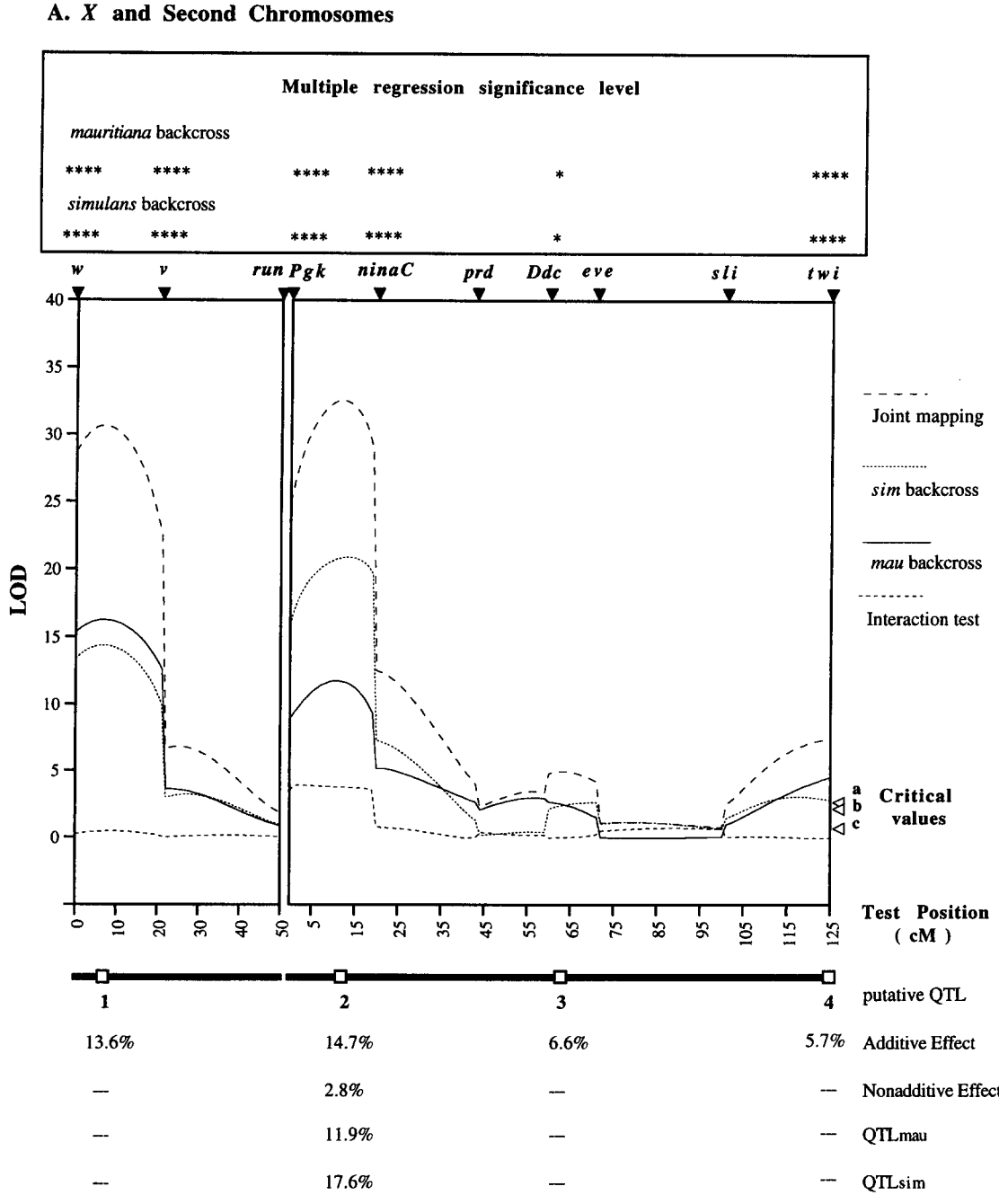

FIGURE 7.—The results of QTL mapping of trait PC1 with respect to a series of molecular markers. The top panel shows significance levels of the partial regression coefficients from a multiple regression of PC1 on all 18 markers ($*P < 0.05$, $**P < 0.01$, $***P < 0.005$, $****P < 0.001$). The middle panel shows the results of composite interval mapping. The molecular marker positions are indicated by ▼ above each panel. The critical values are 2.98 for joint mapping (a), 2.48 for separate mapping of each backcross (b), and 0.83 for the QTL × backcross interaction test (c). The bottom panel provides estimated locations of putative QTL and their estimated effects as a percentage of (S − M)/2, where S and M are mean phenotypic values of the *simulans* and *mauritiana* parental lines, respectively. The additive and nonadditive effects are from joint mapping. When the QTL × backcross interaction is significant, separate effects for each backcross are also given (QTLmau and QTLsim). A is for the *X* and second chromosomes, and B is for the third chromosome.

the variation in the *mauritiana* backcross and ~25% in the *simulans* backcross can be explained by QTL on chromosome *2*. The corresponding estimates for chromosome *3* are 48 and 57%. When all 18 markers are included in a multiple regression analysis, the model accounts for 81% of the variation in the *mauritiana* backcross and 86% of the variation in the *simulans* backcross.

The composite interval mapping results are consistent with those of the multiple regression analysis in which PC1 is regressed on all 18 of the markers. The significance levels of the partial regression coeffi-

B. Third Chromosome

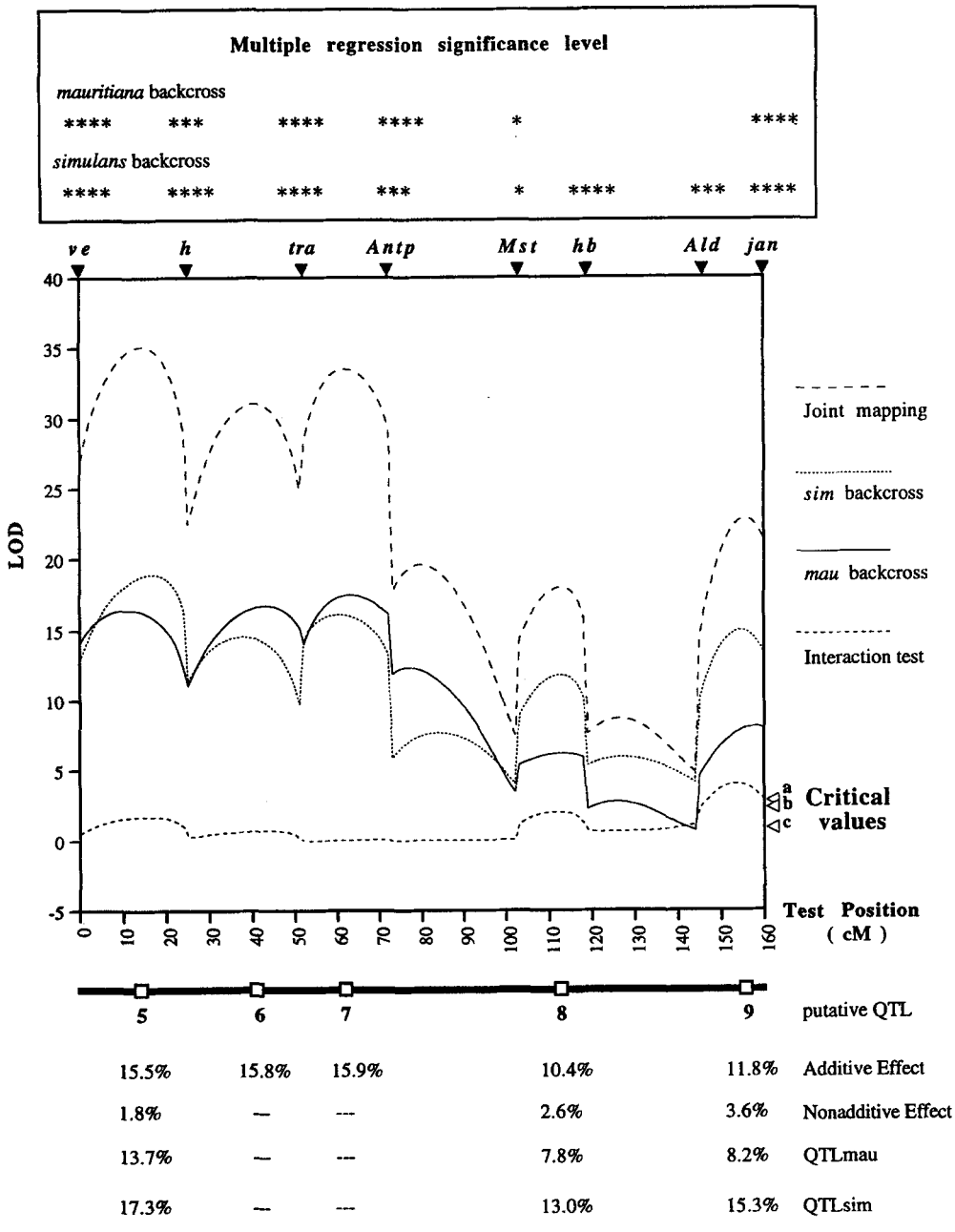

FIGURE 7.—Continued

cients are shown in the panels above the LOD plots in Figure 7.

The composite interval mapping results for ADJPC1 and lobe area (not shown) are very similar to those for PC1. The only notable difference is that the *Ddc/eve* interval shows convincing evidence of a QTL for area and PC1, but not for ADJPC1. Evidently this region contains a QTL that affects size but not shape, whereas the other significant intervals contain factor(s) that affect both size and shape. Because eight of nine significant intervals affect both size and shape, while the remaining six intervals appear not to affect either size or shape, it is likely that the association is mainly due to pleiotropy rather than close linkage. Therefore, at the level of resolution of this analysis, size and shape appear to be largely inseparable properties of the posterior lobe. This close association may be due to a mechanistic coupling, as suggested earlier.

Although the QTL mapping analysis provides some evidence for nonadditive effects, it is not clear to what extent those effects are caused by dominance *vs.* epistasis. Another approach to detect epistasis is to look for nonadditive interactions between effects associated with different markers. With 18 markers there are 153 two-way interactions, which is only somewhat less than the number of individuals in each backcross. Therefore,

each two-way interaction was tested in a separate analysis of variance in which the sources of variation are 18 main effects (one per marker) and one two-way interaction. Table 5 summarizes the number of significant tests for PC1 and ADJPC1. For both variables, considerably more than the expected number of tests were significant, especially for ADJPC1 in the *simulans* backcross, although it should be noted that the expectation is based simply on the number of tests performed and does not take into account the fact that many tests are not independent. The magnitude of epistatic effects cannot be estimated from this approach, but the lack of strong nonadditive effects in the QTL mapping analysis suggests they are relatively weak.

The results in Table 5 indicate that there are more significant interactions in the *mauritiana* than in the *simulans* backcross for PC1, whereas the opposite holds for ADJPC1. Differences in gene action between reciprocal backcrosses are frequently observed with respect to hybrid incompatibility traits (BOCK 1984), and this observation is explained readily by simple models of gene substitution in isolated populations (WU and BECKENBACH 1983). Epistatic interactions between alleles from different species might be expected more often than intraspecific interactions because the former have not been selected for combining ability (see PALOPOLI and WU 1994).

DISCUSSION

Shape variation in morphological structures provides one of the most striking aspects of animal diversity, yet very little is known about its genetic basis. When shape is under polygenic control, genetic analysis requires morphometric descriptors that encode shape information in an economical way. In this study we have used elliptical Fourier and principal components analyses to construct a single morphometric descriptor (PC1) that encapsulates, to a remarkable degree, genetic variation in size and shape of the posterior lobe in hybrid backcross populations. Two additional variables, posterior lobe area and ADJPC1 (PC1 adjusted for size), provide the possibility of distinguishing between genetic effects on size and shape.

The parental lines used for the backcross analysis show a difference in the value of PC1 equal to 30.4 environmental standard deviations, and regression analyses (of phenotypic value on genetic markers) indicate that a very high proportion of the variance in backcross populations is genetic. These are very favorable circumstances for the detection and estimation of QTL effects. In addition, the combined analysis of both backcrosses provides increased statistical power, as well as the opportunity to detect nonadditive effects due to dominance and/or epistasis. This type of analysis is particularly important when generation of an F_2 population is prevented by sterility of F_1 hybrid males (as in the *simulans* × *mauritiana* cross).

In the composite interval mapping of PC1, eight of the 15 intervals analyzed show convincing evidence for a QTL effect. This set includes one on the *X*, three on the second and four on the third chromosome. For most of these intervals, the LOD score exceeds the critical value by a very wide margin, reflecting the expected power. However, the resolution of the analysis clearly is limited by the number of markers in relation to the apparent number of QTL, particularly on 3L. Therefore, we regard the map of putative QTL and the estimates of their effects only as a rough approximation to the actual situation.

Some conclusions about the genetic architecture of the interspecific difference in PC1 can be made, in spite of the relatively low resolution of the current analysis. The results do not support an infinitesimal model of a large number of loci each contributing a small effect to the phenotype (BULMER 1980), since some intervals clearly have much larger effects than others. Rather, they are consistent with a relatively small number of loci that cause most of the phenotypic variation and contribute effects of variable magnitude. The estimated additive effects of putative QTL in the backcross experiment (*i.e.*, the difference between a homozygote and heterozygote) range from 5.7 to 15.9% of the difference between one parent and the midparent value. Because the inheritance is largely additive, we infer that the difference between homozygotes would be the same percentage of the difference between the parental lines. However, it is important to note that these estimates do not represent individual gene effects if there are multiple QTL within an interval, which is very possible, given that the interval sizes are relatively large (with an average of 23 cM). Even very small intervals (<1 cM) may contain clusters of genes with related functions. For example, three genes located within a 0.6-cM interval play related roles in imaginal disc development (GOTWALS and FRISTROM 1991), two nonhomologous genes located within a region spanned by a single small deficiency both function in programmed cell death in Drosophila (GRETHER *et al.* 1995) and multiple factors that contribute to male sterility in Drosophila hybrids can be found within a small interval (PEREZ and WU 1995).

Do the results presented here provide evidence of "major gene" effects? The answer depends on the definition of a major gene and whether each significant interval contains more than one QTL. An effect of 10% in this experiment corresponds to about three environmental standard deviations (from the variance among individuals within an inbred parental line), which means that homozygotes for alternative alleles would have essentially nonoverlapping phenotypic distributions in an isogenic genetic background. Such an effect would be regarded as "major" by most workers, provided that it is actually due to a single gene. Therefore, more work is needed to further dissect the significant intervals to determine whether they contain multiple QTL.

TABLE 5

The number of significant interactions between pairs of markers

$Chr_i \times Chr_j$[a]	Number of tests	ADJPC1 $P < 0.05$	ADJPC1 $P < 0.01$	ADJPC1 $P < 0.005$	ADJPC1 $P < 0.001$	PC1 $P < 0.05$	PC1 $P < 0.01$	PC1 $P < 0.005$	PC1 $P < 0.001$
mauritiana backcross									
$X \times X$	3	0	0	0	0	0	0	0	0
2×2	21	1	0	0	0	3	0	0	0
3×3	28	1	0	4	2	3	2	2	0
$X \times 2$	21	0	0	0	0	0	0	0	0
$X \times 3$	24	3	0	0	0	1	0	0	0
2×3	56	3	0	0	0	2	3	1	1
Sum	153	8	0	4	2	9	5	3	1
Expected[b]		7.7	1.5	0.77	0.15	7.7	1.5	0.77	0.15
simulans backcross									
$X \times X$	3	0	0	0	0	0	0	0	0
2×2	21	0	0	0	0	0	0	0	0
3×3	28	6	2	3	5	1	0	1	0
$X \times 2$	21	0	0	0	0	0	0	0	0
$X \times 3$	24	3	1	1	1	0	2	0	0
2×3	56	10	2	4	3	0	0	0	0
Sum	153	19	5	8	9	1	2	1	0
Expected[b]		7.7	1.5	0.77	0.15	7.7	1.5	0.77	0.15

The model to test the interaction between marker m and marker n:

$$Y_{ij} = \mu_i + \sum a_{ik} + c_{imn} + e_{ij},$$

where Y_{ij} = trait value of the jth individual in the ith backcross, μ_i = mean of the ith backcross, i = backcross (1 or 2), j = individual within backcross (1, 2, . . ., n_j), a_{ik} = effect of kth marker in ith backcross, c_{imn} = interaction effect of marker m with marker n in the ith backcross, and e_{ij} = residual effect. The summation is over all 18 markers (k = 1, . . ., 18).

[a] Chr_i = marker m located in the ith chromosome, Chr_j = marker n located in the jth chromosome.

[b] The expected number of significant tests based on a total number of 153 tests.

Another important aspect of genetic architecture is the type of gene action. There is some evidence for partial dominance of *mauritiana* alleles and for epistatic effects on PC1, but these appear to be relatively small in comparison with additive effects. Overall, the results indicate that inheritance of PC1 is largely additive.

An interesting question is whether different QTL affect the shape and/or size of the posterior lobe in distinctly different ways. However, with one exception, separate QTL effects on size *vs.* shape could not be distinguished at the level of resolution provided by this experiment. The exception is a single interval on the second chromosome that appears to affect size (area) but not shape (ADJPC1). The possibility that different QTL affect shape in different ways is difficult to test in this type of experiment because so many different loci are segregating simultaneously in the backcross. We have developed a set of introgression lines that will provide a better opportunity for addressing this question in the future.

Although the QTL map for PC1 presented here is relatively crude, the prospect of developing a high resolution map at the level of single genes is promising. Three different approaches to this endeavor are planned or in progress. (1) The backcross analysis can be expanded to include more markers and more individuals. The only theoretical limit to this approach is the number of markers that can be assayed from a single fly, which is at least 50. There appears to be sufficient power to improve the resolution by doubling the number of markers (particularly in high LOD intervals) without increasing the number of individuals assayed. (2) A high resolution analysis of specific regions known to contain effects of interest can be performed by repeating the backcross analysis with introgression lines that contain a relatively small segment of the *mauritiana* genome in an otherwise *simulans* genetic background. This approach would allow greater precision than the species cross, since there will be less noise due to segregation of many factors in the background. (3) Individual genes involved in species differences can be identified in a two-stage strategy: mutagenesis within *D. melanogaster* can identify candidate genes required for the normal development of the posterior lobe, and loss of function mutants can be used in a hybrid test to determine whether a candidate gene contributes to a species difference. This approach also provides an opportunity for cloning QTL that contribute to a species difference.

Previous work on the genetic architecture of quantitative traits deals with three different types of populations: (1) domesticated strains of plants and animals and their

wild relatives that differ for a trait because of an often long and varied history of artificial selection, (2) strains that differ because of a short-term and well-defined artificial selection in the laboratory, and (3) natural species or subspecies that differ because of a long and varied history of natural and/or sexual selection. Genetic architectures in these three types of populations are a function of their different histories and may provide different types of inferences about evolutionary processes.

LANDE (1983) suggested that evolution by mutations with major effects occurs most often in domesticated or artificially disturbed populations, because very strong selection over a period of several generations is required to overcome their deleterious pleiotropic effects. In addition, the protected environment and lack of competition in domesticated populations may permit a major effect mutation to survive long enough for the evolution of modifiers that ameliorate deleterious effects. This type of evolution may rarely, if ever, occur in natural populations. However, analysis of agricultural plants and animals provides important and interesting information about the historical process of domestication (see DOEBLEY and STEC 1991).

Short-term artificial selection experiments in the laboratory [such as the Drosophila bristle number study of LONG *et al.* (1995)] may provide the most useful information about standing variation in local natural populations, since the response to selection depends mainly on polymorphisms present in the base population at a given time. Long-term evolutionary responses also depend on new mutations that occur during the course of selection.

The genetic architectures of natural species and subspecies are likely to reflect the operation of processes that do not occur in the other two types of populations. For example, if mutations with large advantageous effects occur very rarely, then they are not likely to contribute to a short-term selection response from a relatively small base population. The species *D. mauritiana* and *simulans* have large population sizes and have been isolated for ~0.6–0.9 million years (HEY and KLIMAN 1993), which provides a vast number of fly gamete generations during which a rare event may have occurred. Evolution over long time periods may also involve sequential substitutions of alleles at a single locus, producing a major gene effect that is actually composed of a series of mutants with smaller individual effects [as for *Adh* alleles in Drosophila (LAURIE and STAM 1994)]. In addition, it has been suggested that special processes [like the "genetic revolutions" of MAYR (1954) and TEMPLETON (1980)] only occur during speciation events, perhaps because they require certain types of population structure or demography that would not exist for domesticated or artificially selected populations.

It has also been suggested that sexual selection and natural selection may give different types of genetic architectures because of repeated and coordinated changes in male ornamentation and female preferences (ORR and COYNE 1992). The runaway process of sexual selection described by LANDE (1981b) provides a mechanism for the rapid and unstable evolution of sexually dimorphic characters. Although it is not clear that this process will necessarily produce genetic architectures that differ consistently from the many different modes of natural selection, both sexually dimorphic characters and those associated with adaptations common to both sexes should be studied.

Divergent male genital structures, such as the posterior lobe studied here, may evolve by sexual selection due to female preference implemented through postmating mechanisms such as sperm displacement and remating frequency (EBERHARD 1985). The posterior lobe has no known mechanical function in mating and is much reduced or entirely absent in some members of the melanogaster subgroup (*e.g., D. erecta,* TSACAS and LACHAISE 1974). Therefore, it may be a male ornament or courtship device analogous to many of the rapidly evolving secondary sexual characteristics of other animals, such as the plumage patterns of birds.

Although QTL mapping information is accumulating rapidly for domesticated plant populations (TANKSLEY 1993), there is still very little information for populations subjected to short-term artificial selection and for natural species. Thus, there is clearly a need for more high resolution studies of genetic architecture using the recently developed molecular and statistical technologies.

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