Individual Genetic Contributions to Genital Shape Variation between *Drosophila simulans* and *D. mauritiana*

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External genitalia are one of the most rapidly evolving morphological features in insects. In the *Drosophila melanogaster* species subgroup, males possess a nonfertilizing external genital structure, called the posterior lobe, which is highly divergent among even closely related species. A previous study on this subgroup mapped two genomic regions that affect lobe size and four that affect lobe shape differences between *D. mauritiana* and *D. sechellia*; none of the regions affected both size and shape. Here, we investigate whether three of these significant regions also affect lobe size and shape differences between the overlapping species pair *D. mauritiana* and *D. simulans*. We found that the same three regions of *D. mauritiana*, previously shown to affect lobe morphology in a *D. sechellia* genetic background, also affect lobe morphology in a *D. simulans* genetic background, with one of the regions affecting both size and shape. Two of the regions also affected morphology when introgressed in the reciprocal direction. The overlap of regions affecting genital morphology within related species pairs indicates either that there is a common underlying genetic basis for variation in genital morphology within this species group or that there are multiple adjacent loci with the potential to influence genital morphology.

1. Introduction

Animal groups ranging from primates [1] to lizards [2] show rapid evolution of male genitalia. In addition to the inseminating, or primary, organs, external secondary organs involved in stimulation and copulation also exhibit rapid divergence in a variety of animal groups [3]. In insects, the divergence of male genitalia is so pronounced that even recently diverged sibling species show a high degree of variation in the male genitalia and/or secondary organs [4–6]. Several different models have been developed to explain the evolution of genitalia in individual species, but none explains why it occurs across so many animal groups [7, 8]. The most prominent competing theories that attempt to explain the pervasive occurrence of rapidly diverging male genitalia are the pleiotropy hypothesis, the lock and key hypothesis, and the sexual selection hypothesis [3, 7, 9–14]. While there is evidence supporting each of these models, sexual selection is thought to be the most prevalent influence on the divergence of male genitalia [3, 15, 16].

An understanding of the genetic underpinnings of genital shape enhances our ability to assess the evolutionary forces influencing genital morphology. One of the most widely used model systems for understanding the genetic basis of genital morphology is the *Drosophila melanogaster* species subgroup. These species are largely morphologically indistinguishable from one another except for the shape of the male’s exterior genital lobes [17]. The bilaterally symmetrical posterior lobes, also called the genital arch, are a cuticular projection that surrounds the inverted aedeagus. The lobes are inserted between the female’s eighth and ninth abdominal tergites during copulation [18] and appear to be involved in several aspects of copulation and fertilization [19–21], making it likely that they experience sexual selection.

Several genetic mapping studies for lobe shape have been performed in this group, and while the maps identify genomic regions and not individual genetic loci, comparisons among studies can significantly enhance our understanding of how these sexually selected traits evolve. Most genetic mapping studies on genital morphology have used

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*Note:* The text is a scientific research article that investigates the genetic contributions to genital shape variation between two closely related species, *Drosophila simulans* and *D. mauritiana*. The study focuses on three significant genomic regions that affect lobe size and shape and explores their effects in both genetic backgrounds. The research highlights the overlap of regions affecting genital morphology within related species pairs, suggesting either a common genetic basis or multiple adjacent loci influencing genital morphology.

**Key Points:***
- Genitalia are rapidly evolving morphological features in insects.
- The *Drosophila melanogaster* species subgroup shows high divergence in genitalia among closely related species.
- Previous studies identified genomic regions affecting lobe size and shape in *D. mauritiana*.
- The study investigates whether these regions also affect lobe morphology in *D. simulans*.
- The overlap of regions affecting genital morphology indicates either a common genetic basis or multiple loci.
- Sexual selection is a prominent theory explaining genitalia evolution.

**References:**
1. Primates
2. Lizards
3. Insects
4–6. Animal groups
7. Several different models
8. Pleiotropy hypothesis
9. Lock and key hypothesis
10. Sexual selection hypothesis
11. Drosophila melanogaster
12. Genital arch
13. Copulation
14. Fertilization
15. Genitalia evolution
16. Genetic mapping studies
quantitative trait locus (QTL) mapping, but a recent study on the sibling species D. mauritiana and D. sechellia [22] used introgression mapping, allowing for the contributions of individual genomic regions to be assessed independently. In Masly et al. [22], small homozygous pieces of the D. mauritiana genome are present in an otherwise homozygous D. sechellia genetic background. They found two genomic regions that caused the size of the lobes to shift closer to that of D. mauritiana, located near the left telomere and the centromere of the third chromosome. They found four other regions that affected lobe shape, including one near the right telomere of the third chromosome. Regions influencing lobe shape did not overlap those found for lobe size. This demonstrated that individual genomic regions could influence genital morphology and that there is a differential genetic basis for the size and shape aspects of genital morphology in this species pair.

Here, we examine whether the ability of individual genomic regions to influence lobe morphology and the genetic uncoupling of size and shape is present in other species pairs or if it is unique to the D. mauritiana-D. sechellia species pair. We performed this study in the overlapping species pair of D. simulans-D. mauritiana, allowing us to additionally examine whether the same loci would underlie genital morphology differences in related sibling species or if each species in this subgroup owes its unique morphology to separate loci. The sister species D. simulans and D. mauritiana have been a well-studied example of genital morphology divergence within this subgroup [5, 18, 19, 23]; D. simulans males have helmet-shaped lobes, while D. mauritiana males have stick-like protrusions [5, 22, 24]. When these two species are crossed, F1 hybrid males have an intermediate posterior lobe morphology when compared to the two parental species, while males resulting from a backcross to either parent species produce a continuous range of lobe phenotypes [5, 23], indicating a polygenic nature for lobe morphology, which has been confirmed by QTL mapping [23]. Although genomic regions were located using QTL mapping, it is unknown whether they individually will have an effect on male genital morphology. Indeed, since none of the individual regions had a large effect on the phenotype, it is possible that the effect of a single locus might be undetectable when it is measured individually.

We utilize introgression lines to assess the contributions of individual genomic regions to the divergent genital lobe size and shape between D. simulans and D. mauritiana. We focused on the three regions identified in Masly et al. [22] on the third chromosome as individually influencing D. mauritiana and D. sechellia lobe morphology: left telomere, centromere, and right telomere. Previous QTL mapping studies identified these same three regions as contributors to lobe morphology in the D. simulans-D. mauritiana species pair [5, 23]. Since the previous work on genital morphology [22] found that some regions of the genome affected the lobes in a direction opposite to expectation (increasing size when they should have decreased size), presumably due to transgressive segregation arising from either additive effects or epistatic interactions with the genetic background, we assess introgressions in both directions: lines that are entirely

| Line name | Introgressed region: base positions | Introgressed region: cytological |
|-----------|------------------------------------|-------------------------------|
| S_{MS(62)}^{1} | (3L) 41-8706 | 61A-67B |
| S_{MS(62)}^{2} | (3L) 41-8700 | 61A-67B |
| S_{MS(42)}^{4} | (3L) 16451-(3R)4871 | 74A-92F |
| S_{SM(62)}^{1} | (3R) 23001-telomere | 98A-telomere |
| S_{SM(82)}^{6} | (3L) 22342-(3R)5411 | 80F-92D |
| M_{MS(62)}^{2} | (3L) 1457-3921 | 62B-64B |
| M_{MS(62)}^{6} | (3L) 22342-(3R)5411 | 80F-92D |
| M_{SM(62)}^{1} | (3R) 21267-telomere | 96E-telomere |

1The lines are either a piece of D. mauritiana genome in an otherwise D. simulans genetic background (S_{MS}) or a piece of D. simulans genome in an otherwise D. mauritana genetic background (M_{MS}) for three segments of the third chromosome (cytological region 62, 82, or 98). The line number is consistent with the designation previously used for the same lines [25].
2The base positions are in kilobases, numbered from the telomere for the left arm (3L) and from the centromere for the right arm (3R) of the third chromosome. The region that is listed spans from the markers that had the genotype of the genomic background, encompassing the markers that had the introgressed parent's genotype; thus, the size of the actual region is likely smaller than the listed region.
3The cytological position is that of the homologous region in D. melanogaster.

D. mauritiana except for an introgressed D. simulans genomic segment (M_{S}) and the reciprocal lines that are entirely D. simulans except for a D. mauritana introgression (S_{M}). We compared the lobe size and shape of these lines to the lobes of the species contributing the majority of the genomic complement and evaluated whether the introgressed genome affected lobe size and/or shape.

2. Materials and Methods

2.1. Drosophila Stocks. Introgression lines for the third chromosome were previously created [25] by repeated backcrossing of F1 hybrids to their parent species and then by several generations of brother-sister mating, paired with molecular genotyping at every generation, to make the introgressions homozygous. Genetic markers were then used to determine the location of the genomic region of the opposite species. The resulting introgression lines of D. simulans and D. mauritiana contain known inserted regions of the opposite species within their respective genomes (Table 1).

Introgression lines containing each of the three cytological locations important for posterior lobe morphology (left telomere, centromere, and right telomere) were used for dissections of the posterior lobe; we assayed the lines containing the largest introgressions in these regions to increase the likelihood of capturing genetic factors for genital morphology (Table 1) [25]. We have maintained the nomenclature used in McNiven and Moehring [25] for consistency. The three backcrossed D. mauritiana lines with known introgressed D. simulans genomic regions (N_{S}) were line M_{5862}^{3} (containing the left telomeric region from D. simulans, near cytological band 62), line M_{5862}^{6} (containing the centromeric region from D. simulans, near cytological band 82), and
2.2. Comparing Posterior Lobe Area, Length, and Width. A microknife was used to remove one randomly chosen posterior lobe from the abdomen in TE buffer. A coverslip was then used to ensure that the posterior lobe was observed in a single focal plane. An E100 Nikon compound microscope equipped with a 5-megapixel camera was used to visualize the posterior lobes. All lobe measurements were performed using the computer software NIS-Elements 3.1 (sample size $N = 10$). Lengths were measured in $D. \text{simulans}$ as the distance from the base of the lobe to the furthest vertical point, as drawn by a line perpendicular to the base; in $D. \text{mauritiana}$, the length was measured from the midpoint of the baseline to the furthest point (Figure 1). We found that these two different measures of length in the two species were necessary in order to obtain consistent results due to the general lack of morphological landmarks on the lobes. Widths were measured along a horizontal line at the widest point of the lobe; area was measured by outlining the perimeter of the lobe (Figure 1). All values were first corrected for body size using the tibia length measurements prior to statistical comparison. A one-way ANOVA was used to determine if there was a significant difference in the area, length, or width of the posterior lobes, when comparing the introgressed lines to the parental species comprising the genetic background.

2.3. Elliptical Fourier Analysis and Principal Component Analysis. Due to the paucity of morphometric landmarks, an elliptical Fourier analysis was used to represent each posterior lobe's shape ($N = 10$) [5]. We were able to accomplish this because of the 2D nature of the posterior lobe. To do this, the SHAPE program [26] was used to first normalize the posterior lobe shape of males from introgression lines by the area of the lobe in order to correct for size differences and assign a chaincode value. Chaincode is a coding system within the SHAPE software for representing geometrical shapes as numbers. These values were then used to calculate the elliptical Fourier descriptors (EFD) and to visualize them for comparisons. We obtained 20 Fourier harmonics per posterior lobe, which allowed for precise outlines.
As with the above introgressions, the overall morphology of the posterior lobes in lines with an introgression were not observed in the overlap line SM(62). As such, we removed this line from further analyses. However, if the observed differences were due to the introgression, then the loci for shape would fall within the small region of unknown genotype on the border between the markers assessed as being the introgression versus parental genotype.

To determine how many variables could be used to explain the variation between the introgression lines compared to the wild type lines, a principal components analysis (PCA) [5] was performed, also using the SHAPE program, for each backcross type. The PCA performed using PrintComp, a component of the SHAPE program, is based on the variance-covariance matrix. In both the *D. simulans* and *D. mauritiana*, PCI–PC7 explained at least 90% of the variance observed when comparing introgression lines to the pure-species lines. PCI and PC2 were evaluated separately using a single-factor ANOVA for differences between the introgression line's genital lobe shape and the lobe shape of the parental species that contributed the genetic background.

### 3. Results

#### 3.1. Comparison of Posterior Lobes due to *D. mauritiana* Introgressions

When comparing the overall morphology of the posterior lobes of the introgression lines, the morphology appeared to be species-specific and predominantly in accordance with the backcross genetic background (Table 2; Figure 2). Lobe area showed a strong correlation with both lobe width (values from 0.64 to 0.93) and lobe length (values from 0.50 to 0.80), with a stronger correlation for lobe length in all lines. Posterior lobes in the parental *D. simulans* males were significantly wider and longer and had a greater mean area when compared to the posterior lobes of males containing the *D. mauritiana* introgression in line SM(62)1 (df = 18; P = 0.001, P = 0.032, and P < 0.0001, resp.). The introgression line from the same region, SM(62)2, also displayed reduced lobe size [lobe area (mm²)/tibia size (mm) = 8.77 ± 0.53 (×10⁵)], but the lobes appeared to be aberrant and malformed in some of the dissections performed (2/10). These sporadic differences observed in the one line are unlikely to be due to the species-specific introgression as they were not observed in the overlapping line S*M*(62).1. As such, we removed this line from further analyses. However, if the observed differences were due to the introgression, then the loci for shape would fall within the small region of unknown genotype on the border between the markers assessed as being the introgression versus parental genotype.

| Genotype | Tibia length (mm) | Lobe area (×10⁵ mm²) | Lobe width (mm) | Lobe length (mm) |
|----------|-------------------|----------------------|----------------|-----------------|
| *D. simulans* FC | 0.3743 ± 0.0167 | 4.432 ± 0.112 | 0.0882 ± 0.0024 | 0.0593 ± 0.0035 |
| SM(62)1 | 0.3800 ± 0.0235 | 3.401 ± 0.137*** | 0.0769 ± 0.0025** | 0.0517 ± 0.0045* |
| SM(62)4 | 0.3842 ± 0.0132 | 3.534 ± 0.162*** | 0.0731 ± 0.0042*** | 0.0552 ± 0.0060 |
| SM(62)1 | 0.3837 ± 0.0100 | 3.942 ± 0.122*** | 0.0860 ± 0.0025 | 0.0534 ± 0.0033* |
| SM(98)5 | 0.3568 ± 0.0165 | 4.266 ± 0.207 | 0.0861 ± 0.0027 | 0.0546 ± 0.0020 |
| *D. mauritiana* SYN | 0.3742 ± 0.0123 | 0.832 ± 0.053 | 0.0127 ± 0.0010 | 0.0566 ± 0.0022 |
| MS(98)1 | 0.3551 ± 0.0151 | 0.847 ± 0.041 | 0.0135 ± 0.0007 | 0.0564 ± 0.0017 |
| MS(98)6 | 0.3915 ± 0.0081 | 1.033 ± 0.053** | 0.0152 ± 0.0012* | 0.0630 ± 0.0035 |
| M(sm98)1 | 0.3760 ± 0.0080 | 0.743 ± 0.050 | 0.0130 ± 0.0003 | 0.0582 ± 0.0036 |

Comparison to *D. simulans* FC (for S3) or *D. mauritiana* SYN (for M3): *P ≤ 0.05, **P ≤ 0.005, and ***P ≤ 0.0001. All values were adjusted for body size by dividing by tibia length prior to statistical analysis.

### 3.2. Comparison of Posterior Lobes due to *D. simulans* Introgressions

Significantly greater width and area were also observed for the introgression line SM(62)4 (df = 18; P < 0.0001, P < 0.0001, resp.). The length and area of the posterior lobe were also significantly different when comparing the posterior lobes of parental *D. simulans* males to those from the introgression line SM(98)1 (df = 18, P = 0.020, P < 0.0001) and approached significance for width (df = 18, P = 0.063). The posterior lobes from the partially overlapping introgression line SM(98)5 did not differ significantly in mean width, length, or area when compared to the posterior lobes of parental *D. simulans*. It should be noted that, for practical reasons, we used a slightly different protocol for measuring length in *D. simulans* males than in *D. mauritana* males, and this may have biased our results for this phenotype. However, since the lobes of introgression males largely resembled those of the parental species comprising the genetic background, these different measures likely had a minor, if any, effect on our assessment of length in introgression males. None of the introgression lines had a significant difference in tibia length compared to pure-species *D. mauritana*. There was a slightly negative, and nonsignificant, correlation between individual measures of lobe area and tibia length (r = −0.016, P = 0.92).

In the principal component analysis, PCI–9 accounted for 95.0% of the variance in the SM lines, with the majority of the variance explained by PCI1 (35.0%) and PC2 (21.4%). PCI1, as expected, largely indicated differences in lobe area. Comparisons of PCI1 and PC2 between the introgression lines and the parental *D. simulans* line identified which introgressed regions affected the species-specific shape of the posterior lobe (Figure 3(a)). The shape of the posterior lobe was not significantly affected by an introgressed region near the left telomere in line SM(62)1. However, the introgressed region at the centromere (in line SM(62)4) significantly affected both PCI1 (df = 1, F = 9.71, P = 0.006) and PC2 (df = 1, F = 38.41, P < 0.0001), while both lines containing an introgressed segment at the right telomere (SM(98)1 and SM(98)5) had a significant difference in PCI (df = 1, F = 15.27, P = 0.007, resp.) but not in PC2.
from *D. simulans* was species-specific and predominantly similar to that of the parental genetic background, *D. mauritiana*. In contrast to what was seen for *S* lines, the *M* lines showed generally weaker and more variable correlations between lobe area and lobe width or length: *M*<sub>5(62)</sub> (width: 0.72, length: 0.65), *M*<sub>3(82)</sub> (width: 0.31, length: 0.28), and *M*<sub>1(98)</sub> (width: 0.44, length: 0.51). There was a significant difference in the mean width and area of the posterior lobe when comparing the parental *D. mauritiana* to the introgression line *M*<sub>5(62)</sub> (df = 18, $P = 0.043$, $P = 0.003$, resp.). The introgression lines from the other two cytological locations, *M*<sub>5(62)</sub> and *M*<sub>1(98)</sub>, did not show any statistically significant difference in mean width, length, or area of the posterior lobe when compared to those of the parental *D. mauritiana* males, but *M*<sub>5(62)</sub> did approach significance for width (df = 18, $P = 0.059$). As with the *D. mauritiana* introgression males, our different protocol for length measurements in the two parental species may have biased our results, but this is unlikely. None of the introgression lines had a significant difference in tibia length compared to pure-species *D. simulans*, and there was a nonsignificant negative correlation between lobe area and tibia length ($r = -0.12$, $P = 0.40$).

In the principal component analysis, PCI-9 accounted for 95.6% of the variance in the *M* lines, with the majority of the variance explained by PCI1 (41.0%) and PC2 (24.8%). As with the *S* introgressions, PCI for the *M* lines largely indicated differences in lobe area. Comparisons of PCI1 and PC2 between the introgression lines and the parental *D. mauritiana* lines found that regions at the left telomere and centromere affected the species-specific shape of the posterior lobe (Figure 3(b)). Line *M*<sub>5(62)</sub>, which has an introgression at the left telomere, significantly differed in shape for both PCI1 (df = 1, $F = 4.95$, $P = 0.039$) but not for PC2. Line *M*<sub>5(62)</sub>, with an introgression at the centromere, was significantly different in shape for both PCI1 (df = 1, $F = 10.63$, $P = 0.004$) and PC2 (df = 1, $F = 7.77$, $P = 0.012$), while line *M*<sub>1(98)</sub>, with an introgression at the right telomere, did not significantly differ in either aspect of shape.

### 4. Discussion

The *Drosophila melanogaster* subgroup is highly divergent with regard to the shape of the male posterior lobe. Aside from the posterior lobe, there are no other significant differences in overall body morphology between the species,
Figure 3: Shape measurements in introgression lines. The distribution of the first two principal components obtained from an elliptical Fourier analysis for pure-species *D. mauritiana* (green triangles) and *D. simulans* (orange squares) compared to introgression lines (blue diamonds) containing (a) introgressed regions of *D. mauritiana* in an otherwise *D. simulans* genetic background (*S_M*$_{62}$) or (b) introgressed regions of *D. simulans* in an otherwise *D. mauritiana* genetic background (*M_S*$_{98}$). Ellipses represent the standard deviation centered on the mean value for each group. A representative lobe shape for each line is shown in the same color as the group it represents.
and there is very little correlation between body size and lobe size or shape [5, 22, 27, 28], although lobe size was found to be correlated with tibia length in a recent study [29]. The differences in genital morphology are caused by multiple genomic regions that, in general, act additively to contribute to the shape and size of the posterior lobe [22, 23, 28, 30]. A previous study that utilized quantitative trait locus (QTL) mapping found that the genetic regions that determine size and shape differences between the posterior lobes of D. simulans and D. mauritiana were indistinguishable [5], and therefore lobe, size, and shape were considered genetically linked in these species. In contrast, a study utilizing introgression lines found that the genomic regions influencing the species-specific difference in size for the lobes of D. mauritiana compared to D. sechellia were often different from those that conferred differences in lobe shape [22], indicating that differences in lobe size and shape in these species have separate genetic bases. Our findings agree with both of these previous studies: some regions of the genome contribute to both size and shape, while others affect either size or shape (Figures 4(a) and 4(b)). Thus, there is genetic linkage (association, physical linkage, or pleiotropy) between some loci influencing size and shape differences between D. simulans and D. mauritiana, while other loci either are not linked or are linked with loci whose effect is too small to be detected in this study.

When portions of the D. mauritiana genome were introgressed into D. sechellia, introgressions at the left telomere and centromere influenced size, while an introgression at the right telomere altered the shape of the D. sechellia lobe towards a D. mauritiana-like appearance (Figure 4(c)) [22]. We found that these three regions of D. mauritiana have the same effect on D. simulans lobe morphology as the one they have on D. sechellia lobe morphology, with an additional effect on shape for the centromeric region; this additional effect is likely due to the large size of this introgression (Figures 4(a) and 4(c)), as the significant introgression into D. sechellia does not span the entire length of the genomic region we introgressed into D. simulans ([22]; J. P. Masly, personal communication). One of the three regions (at the centromere) was also implicated as contributing to intraspecific variation in lobe morphology within D. melanogaster [30]. It is therefore possible that there may be a similar genetic underpinning for genital divergence in this species group; this is not surprising, as it makes sense that the same developmental pathways could be influenced by selection during these species’ divergence.

Only one of the three regions had the same effect on lobe morphology when they were introgressed in the alternate direction; that is, D. simulans genome introgressed into D. mauritiana (Figure 4(b)). For example, the introgression at the left telomere affects lobe shape rather than size, demonstrating that the genes in this region likely do not have the same effect on the two species as the portion of the genome that is introgressed in M_{S(98)}^3 is also present in line S_{M(62)}^1. Likewise, the introgression M_{S(98)}^1, which does not have an effect on lobe morphology, contains all of the equivalent genomic regions present in the significant lines SM(98)1 and S_{M(98)}^5 and contains all of the regions present in the D. mauritiana introgression that significantly affected lobe shape in D. sechellia ([22]; J. P. Masly, personal communication). Thus, there is divergence in how individual genomic regions influence morphology, and the loci within these regions appear to interact with their genetic background. Two of these regions, although they had a significant effect on shape (M_{S(62)}^3) or area and shape (M_{S(62)}^6) in our study, do not directly overlap the location of the introgressions of D. mauritiana into D. sechellia that were shown to have a significant effect on lobe area ([22]; J. P. Masly, personal communication). Thus, it appears that there may be regions of...
the genome that harbor multiple loci that have the potential of contributing to the variation in lobe morphology in this species group.

Although they were not identified as significantly influencing lobe area in the *D. mauritiana-D. sechellia* species pair, these regions were found to influence lobe phenotype, but in an unexpected direction [22]. The introgression 2H3(B) overlaps the same region covered here by M5(623), but in the former study the introgressed piece of *D. mauritiana* caused the lobe to have a larger size than either parental species and skewed the shape away from that of *D. mauritiana*. Likewise, introgression 2K5(A) overlaps M5(606) but increased the size above that of either parental species. This skew in lobe phenotype away from the expected direction (i.e., the phenotype became even more dissimilar from *D. mauritiana*) was most likely due to epistatic interactions or transgressive segregation [22]. In contrast, none of our introgressions produced a significant phenotype in the opposite direction to the expected in either sex (Table 1, Figure 2) or shape (Figure 3). Thus, it appears that the observed skew due to introgressions of *D. mauritiana* for these regions was due to their placement into a *D. sechellia* genetic background; when they are placed into a *D. simulans* genetic background, they significantly affect size and/or shape in the expected direction.

As was found in the *D. mauritiana-D. sechellia* species pair [22], our results also indicate that single genomic regions can significantly modify genital morphology, suggesting that individual genes may have a strong enough effect on lobe morphology that it may be possible to map their separate locations. This result is still somewhat unexpected as lobe morphology in the *D. simulans-D. mauritiana* species pair was previously mapped to more than 19 genomic regions [23], making future fine-mapping appear impossible as each region was assumed to have too small of an effect to be individually detectable by reasonable means. While this still may be the case, as our introgressed regions are large and may harbor multiple loci of small effect, the lobe area shifted by 22–24% in our significant lines, making the phenotype relatively pronounced, enhancing the prospect of future fine-mapping.

The region at the right telomere that was significant for both *D. simulans-D. mauritiana* and *D. mauritiana-D. sechellia* lobe shape harbors a candidate gene for posterior lobe morphology [22, 31]. The *D. melanogaster* gene known as *Drop (Dr)*, at cytological location 99B, has been identified as important in sex determination. *Dr* is repressed in females during development and, when mutated in *D. melanogaster* males, leads to misshapen posterior lobes [31]. A comparison of published sequences [32] confirmed that there is a homolog for *Dr* in both *D. simulans* and *D. mauritiana* in the same cytological region, making this gene a strong candidate for variation in lobe morphology in this species pair.

The posterior lobes are thought to play a role in both copulation and fertilization [19–21], and as such, divergence in lobe morphology could influence male mating success with females of another species. The same telomeric and centromeric regions on the 3rd chromosome that affect genital shape morphology here have also been found to affect mating behavior in *D. simulans-D. mauritiana* [25, 33] and *D. simulans-D. melanogaster* [34]. We can examine whether the different lobe morphology induced by the introgressions has an impact on mating behavior by testing the behavior of the introgression lines. A previous study examined three of the same introgression lines used here for their effect on male mating success [25]. When *D. mauritiana* males harboring a *D. simulans* introgression were paired with *D. mauritiana* females, the males with an introgression at the centromere (M5(623)) and right telomere (M5(983)) had a significant reduction in copulation success, while males with an introgression at the left telomere (M5(603)) did not have reduced mating success. As these results do not align with our significant results for alteration in lobe size or shape (Figure 4(b)), differences in male mating success do not appear to be induced by the variation in lobe morphology observed for these lines, but additional tests are required to rule out linkage between these traits. Additionally, genes for a sexually selected trait are again found to localize near the centromere and telomeres, a trend that is potentially widespread [34].

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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**References**

[1] A. F. Dixson, “Sexual selection, genital morphology, and copulatory behavior in male Galagos,” *International Journal of Primatology*, vol. 10, no. 1, pp. 47–55, 1989.

[2] W. Böhme and T. Ziegler, “A review of iguanian and anguimorph lizard genitalia (squamata: Chamaeleonidae; Varanoidea, shinisauridae, xenosauridae, anguidae) and their phylogenetic significance: comparisons with molecular data sets,” *Journal of Zoological Systematics and Evolutionary Research*, vol. 47, no. 2, pp. 189–202, 2009.

[3] W. G. Eberhard, *Sexual Selection and Animal Genitalia*, Harvard University Press, Cambridge, Mass, USA, 1985.

[4] O. Richards, “The specific characters of the British humblebees (Hymenoptera),” *Transactions of the Entomological Society of London*, vol. 75, pp. 233–268, 1927.

[5] J. Liu, J. M. Mercer, L. F. Stamm, G. C. Gibson, Z.-B. Zeng, and C. C. Laurie, “Genetic analysis of a morphological shape difference in the male genitalia of *Drosophila simulans* and *D. mauritiana*,” *Genetics*, vol. 142, no. 4, pp. 1129–1145, 1996.

[6] H. Song, “Species-specificity of male genitalia is characterized by shape, size, and complexity,” *Insect Systematics & Evolution*, vol. 40, no. 2, pp. 159–170, 2009.

[7] G. Arnqvist and R. Thornhill, “Evolution of animal genitalia: patterns of phenotypic and genotypic variation and condition
dependence of genital and non-genital morphology in water strider (Heteroptera: Gerridae: Insecta),” *Genetical Research*, vol. 71, no. 3, pp. 193–212, 1998.

[8] L. W. Simmons, C. M. House, J. Hunt, and F. García-González, "Evolutionary response to sexual selection in male genital morphology," *Current Biology*, vol. 19, pp. 1442–1446, 2009.

[9] W. G. Eberhard, “Animal genitalia and female choice,” *American Scientist*, vol. 78, pp. 134–141, 1990.

[10] W. G. Eberhard, "Species isolation, genital mechanics, and the evolution of species- specific genitalia in three species of Macrodactylus beetles (Coleoptera, Scarabaeidae, Melolonthinae)," *Evolution*, vol. 46, no. 6, pp. 1774–1783, 1992.

[11] J. P. Masly, "170 years of "Lock-and-Key": genital morphology and reproductive isolation," *International Journal of Evolutionary Biology*, vol. 2012, Article ID 247352, 10 pages, 2012.

[12] D. J. Hosken and P. Stockley, "Sexual selection and genital evolution," *Trends in Ecology and Evolution*, vol. 19, no. 2, pp. 87–93, 2004.

[13] G. Arnqvist, "The evolution of water strider mating systems: causes and consequences of sexual conflicts," in *The Evolution of Mating Systems in Insects and Arachnids*, pp. 146–163, Cambridge University Press, Cambridge Books Online, Cambridge, UK, 1997.

[14] A. M. Shapiro and A. H. Porter, "The lock-and-key hypothesis: evolutionary and biosystematic interpretation of insect genitalia," *Annual Review of Entomology*, vol. 34, pp. 231–245, 1989.

[15] W. G. Eberhard, "Evaluating models of sexual selection: genitalia as a test case," *The American Naturalist*, vol. 142, pp. 564–571, 1993.

[16] W. G. Eberhard, "Evolution of genitalia: theories, evidence, and new directions," *Genetica*, vol. 138, no. 1, pp. 5–18, 2009.

[17] J. A. Coyne, "Genetic basis of differences in genital morphology among three sibling species of Drosophila," *Evolution*, vol. 37, pp. 1101–1118, 1983.

[18] H. M. Robertson, "Mating asymmetries and phylogeny in the Drosophila melanogaster species complex," *Pacific Science*, vol. 42, pp. 72–80, 1988.

[19] J. A. Coyne, "The genetics of an isolating mechanism between two sibling species of Drosophila," *Evolution*, vol. 47, pp. 778–788, 1993.

[20] C. S. C. Price, C. H. Kim, C. J. Gronlund, and J. A. Coyne, "Cryptic reproductive isolation in the Drosophila simulans species complex," *Evolution*, vol. 55, no. 1, pp. 81–92, 2001.

[21] S. Jagadeeshan and R. S. Singh, "A time-sequence functional analysis of mating behaviour and genital coupling in Drosophila: role of cryptic female choice and male sex-drive in the evolution of male genitalia," *Journal of Evolutionary Biology*, vol. 19, no. 4, pp. 1058–1070, 2006.

[22] J. P. Masly, J. E. Dalton, S. Srivastava, L. Chen, and M. N. Arbetman, "The genetic basis of rapidly evolving male genital morphology in Drosophila," *Genetics*, vol. 189, no. 1, pp. 357–374, 2011.

[23] Z.-B. Zeng, J. Liu, L. F. Stam, C.-H. Kao, J. M. Mercer, and C. C. Laurie, "Genetic architecture of a morphological shape difference between two Drosophila species," *Genetics*, vol. 154, no. 1, pp. 299–310, 2000.

[24] H. M. Robertson, "Mating behavior and the evolution of Drosophila mauritiana," *Evolution*, vol. 37, pp. 1283–1293, 1983.

[25] V. T. K. McNiven and A. J. Moehring, "Identification of genetically linked female preference and male trait," *Evolution*, vol. 67, no. 8, pp. 2155–2165, 2013.

[26] H. Iwata and Y. Ukai, “SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors,” *The Journal of Heredity*, vol. 93, no. 5, pp. 384–385, 2002.

[27] J. A. Coyne, J. Rux, and J. R. David, “Genetics of morphological differences and hybrid sterility between Drosophila sechellia and its relatives,” *Genetical Research*, vol. 57, no. 2, pp. 113–122, 1991.

[28] S. J. Macdonald and D. B. Goldstein, "A quantitative genetic analysis of male sexual traits distinguishing the sibling species Drosophila simulans and D. sechellia,” *Genetics*, vol. 153, no. 4, pp. 1683–1699, 1999.

[29] C. M. House, Z. Lewis, D. J. Hodgson et al., "Sexual and natural selection both influence male genital evolution," *PLoS ONE*, vol. 8, no. 5, Article ID e63807, 2013.

[30] C. L. McNeil, C. L. Bain, and S. J. Macdonald, "Multiple quantitative trait loci influence the shape of a male-specific genital structure in Drosophila melanogaster," *G3: Genes, Genomes, Genetics*, vol. 1, no. 5, pp. 343–351, 2011.

[31] S. S. Chatterjee, L. D. Uppendahl, M. A. Chowdhury, P.-L. Ip, and M. L. Siegal, "The female-specific Doublesex isoforn regulates pleiotropic transcription factors to pattern genital development in Drosophila,” *Development*, vol. 138, no. 6, pp. 1099–1109, 2011.

[32] S. J. Marygold, P. C. Leyland, R. L. Seal et al., "FlyBase: improvements to the bibliography,” *Nucleic Acids Research*, vol. 41, no. 1, pp. D751–D757, 2013.

[33] A. J. Moehring, J. Li, M. D. Schug et al., "Quantitative trait loci for sexual isolation between Drosophila simulans and D. mauritiana,” *Genetics*, vol. 167, no. 3, pp. 1265–1274, 2004.

[34] M. Latureny and A. J. Moehring, "The genetic basis of female mate preference and species isolation in Drosophila,” *International Journal of Evolutionary Biology*, vol. 2012, Article ID 328392, 13 pages, 2012.
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